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Predator presence elicits vigilance and decreases honey bee recruitment dancing /

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Predator presence elicits vigilance and decreases honey bee recruitment dancing

A Thesis submitted in partial satisfaction of the requirements for the degree of

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in

Biology

by

Allison Bray

Committee in charge:

Professor James Nieh, Chair
Professor Elsa Cleland
Professor David Holway

2013

The Thesis of Allison Bray is approved and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2013

DEDICATION

To my friends and family - always there when I need them.

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Sections 1-6 are currently being prepared for submission for publication. Allison Bray; James Nieh, 2013. This thesis author was the primary investigator and author of this paper.

ABSTRACT OF THE THESIS

Predator presence elicits vigilance and decreases honey bee recruitment dancing

by

Allison Bray

Master of Science in Biology

University of California, San Diego, 2013

Professor James Nieh, Chair

Predators can reduce bee pollination and decrease plant fitness through fear: prey avoiding predators. However, the effects of predator-induced fear on bee communication are poorly understood. For bees that are mass foragers, such as honey bees, the effects of fear alter individual foraging choices and colony foraging allocation through recruitment communication. Honey bees (*Apis mellifera*) can recruit nestmates to visit food sources, thereby amplifying the effects of their individual foraging. It was not known if honey bees would alter their foraging communication, factoring in the risk of predation, when confronted with live predators on floral-type resources. We show that the potential risk of predation from live predators, not just the evidence of recent predation, alters the dance language in an adaptive way. In this study, honey bees were given a choice of safe and dangerous feeders to determine if they would avoid praying mantises (*Tenodera sinensis*), a predator that bees were not previously known to avoid. Bees exhibited fear (avoidance) and significantly avoided the live mantis (at all instar sizes ≥ 4cm) and avoided mantis odor (cuticular hydrocarbon extract) and visual cues (plastic mantis). Both olfactory and visual cues were equally effective deterrents. Honey bees decreased the number of waggle dance circuits (a measure of food source quality) for a rich food patch with a live mantis, but not for a mantis-free control, which is the first demonstration that wariness of a live predator alters bee communication inside the nest and suggests that information flow within a colony plays a role in the ecology of fear.

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Introduction

The ecological importance of non-consumptive effects of predation and the effects of fear (functionally defined as increasing wariness or avoidance of predators, Blumstein, 2006) on prey species has become increasingly evident (Brown et al., 1999; Laundré et al., 2010). In addition to prey changing their behavior after a predator attack, animals will also change their behavior in apprehension of a possible attack (Laundré et al., 2010), affecting prey spatio-temporal use of the environment (Laundré et al., 2010), often at a cost to the prey. Thus, fear of predators can have a far greater effect on a species than direct consumption. For example, by altering herbivory, fear of predation can have broader, cascading ecosystem effects (Preisser et al., 2005). Elk avoid riskier habitats even at the cost of reduced diet quality (Hernández and Laundré, 2005), chickadees reduce foraging efficiency by carrying food to safer areas when predators are detected (Lima, 1985), and cucumber beetles reduce feeding when wolf spiders were present (Snyder and Wise, 2000).

Fear can reduce pollination, a key ecosystem service with ecological and economic importance (Costanza et al., 1997). Several studies show that predation upon pollinators can reduce plant fitness. Western monkshood, which is pollinated by bumble bees, had significantly reduced fruit set where and when there was strong predatory wasp (beewolf) activity (Dukas, 2005). Presence of a lizard predator was shown to decrease visitation and visit duration of butterfly and fly pollinators, leading to decreased seed output in *Chuquiraga oppositifolia* (Muñoz and Arroyo, 2004). Insect pollinators reduced visitation frequency and visit durations in response to crab spiders, and this led to decreased seed production in *Leucanthemum vulgare* (Suttle, 2003). Artificial crab spider models reduced pollinator visits (Hymenoptera, Diptera, and Lepidoptera) to *Rubus rosifolius* flowers and decreased fruit mass by 50% and seed set by 42% (Goncalves-Souza et al., 2008). However, some studies show no effects of pollinator predators on plant fitness, possibly due to diversity of pollinators (Brechbühl et al., 2010), a high pollinator abundance (Dukas and Morse, 2005), or plants that are not prone to pollen limitation.

Previously, it was thought that the cascading effects of predation on pollinating insects, such as honey bees, would be relatively low because successful predation occurs at a low rate (2% over 9 days, Dukas and Morse, 2005), and the cost of losing a forager would be comparatively low to the colony (Morse, 1986). However, even in the absence of high mortality, fear can play an important role in pollinator foraging patterns (Romero et al., 2011) by altering prey preferences and behavior. Honey bees and bumble bees will avoid predation risk as indicated by dead bees (Abbott, 2006; Dukas, 2001) and bee hemolymph (Goodale and Nieh, 2012) and avoid predator presence: dead crab spiders (Brechbühl et al., 2010; Dukas, 2001), live crab spiders (Dukas and Morse, 2003; Robertson and Maguire, 2005), and live ambush bugs (Elliott and Elliott, 1998). Bees will also avoid flowers where they have experienced an unsuccessful predation attempt (Dukas, 2001; Jones and Dornhaus, 2011) and can generalize, avoiding similar-appearing flowers after predation attempts (Ings and Chittka, 2009). In addition to altering their space use, predators will also cause bees to increase the time spent inspecting flowers for predators (Ings and Chittka, 2008; Lenz et al., 2012) and decrease time spent foraging on flowers (Elliott and Elliott, 1998; Romero et al., 2011).

While previous studies on honey bee predators have focused mainly on crab spiders, we used praying mantises. Unlike crab spiders, mantises markedly increase in size throughout their life cycle, thereby providing the opportunity to study effects of how the same predator species, at multiple developmental sizes, affects bee fear. Praying mantids are widespread generalist predators, and prey upon foraging honey bees, bumble bees, and wasps (Barrows, 1984; Beckman and Hurd, 2003) that, in the summer, evidently comprise a vital part of the mantis diet (Hurd, 1989). The mantis, *Tenodera sinensis*, originally from eastern Asia and introduced to the United States in 1896 (Laurent, 1898), can now be found across temperate areas of North America (Gurney, 1950), is available commercially as a biological control agent (Warner and Getz, 2008), and is known to prey upon honey bees visiting flowers (Barrows, 1984; Bromley, 1948). In field experiments, they have been shown to reduce density of hymenopterans (Fagan et al., 2002; Moran and Hurd, 1994; Moran and Hurd, 1998), although it is unclear if this was due to consumptive or non-consumptive effects. We therefore used *T. sinensis* as the honey bee predator. It is unknown if honey bees will exhibit wariness (fear) and avoid mantises or alter their recruitment communication as a result of mantis presence. We therefore tested if honey bees (*A. mellifera*), given a choice between a food source with a praying mantis and one without, would exhibit fear and avoid mantises.

Another aspect of bee-predator interactions that is not resolved is how bees detect predators, which are often cryptic. Bees can use visual cues and increase inspection time and avoid dangerous flowers when predators are cryptic and visual cues are difficult to detect (Ings and Chittka, 2008; Ings and Chittka, 2009). Bees will avoid spider models that only provide visual cues (no spider odor) (Goncalves-Souza et al., 2008). Olfactory cues are also important. Honey bees will avoid a food source where a spider was previously present (Reader et al., 2006). Stingless bees avoid flowers with spiders but will approach them if predator odor (but not spider visual appearance) is excluded (Heiling and Herberstein, 2004). We therefore tested two detection modalities, vision (using a plastic mantis model without mantis odor) and odor (an extract of mantis odor provided on filter paper) to determine which cues are most important to foraging bees.

Finally, we were also interested in how the presence of a predator could alter bee recruitment communication. The waggle dance is a bee signal used to recruit additional foragers to a food source, by communicating information, such as its direction and distance (von Frisch, 1967). As a food source increases in quality (such as higher sugar concentration), bees will perform more dance circuits (Seeley, 1994). Abbott and Dukas (2009) showed that honey bees exposed to a recently killed bee will dance less than those returning from safe flowers. Bees attacked at a feeder by conspecifics will also reduce waggle dancing (Nieh, 2010). However, it is unknown if bees factor potential danger, a live predator, not just the evidence of predation (presence of a dead bee) or experience with an attack, into their dancing. The ability of honey bees to detect predators and include this information in their evaluation of food quality through the waggle dance is a potentially much more common phenomenon because encounters with unsuccessful predators are far more common than with successful predators (Morse, 1986). Thus, the non-consumptive effects of fear induced by predators might have a strong role in how honey bee colonies recruit and thereby allocate foraging labor.

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Materials and Methods

Colonies and Study Site

We used fourteen colonies of European honey bees, A. mellifera ligustica, kept at the UCSD Biological Field Station (La Jolla, California, USA) between July 2011 and June 2013. Full colonies were housed in standard 10 frame Langstroth bee hives. Observation colonies (design of von Frisch, 1967) contained three combs and were kept in insulated observation hives placed inside a lab room with a 3 cm inner diameter vinyl tube (20 cm long) to allow bees to enter and exit. We used 12 colonies in the foraging preference experiments and two observation colonies in the dance behavior experiments. We trained bees by presenting approximately 5 ml of unscented 2.5 M sucrose solution (65% sucrose w/w) in a 4 cm diameter yellow plastic petri dish. We used this very rich solution to provide a reward that would consistently elicit interest in foragers even when natural food sources were abundant and to trigger waggle dancing (Seeley, 1995). We placed this feeder at the center of a foraging platform, a 20 cm diameter white plastic disk, atop a 1 m high tripod near the nest entrance and then progressively moved it away from the hive once bees began to feed (methods of von Frisch 1967). We did not use any training odors and thoroughly cleaned all equipment with Alconox laboratory detergent after each trial to remove potential odors. We used different colors of acrylic paint to mark trained bees and verified that visiting bees were from the focal colony by visually confirming their entry into the focal nest.

Effect of predator size

To test the hypothesis that foraging honey bees would exhibit wariness and avoid a food source with a mantis or mantis cues, we ran foraging preference trials between July 2011 and September 2012, between 12pm and 3pm, on twelve bee colonies. We reared mantises from egg cases following standard methods (McMonigle and Lasebny, 2008) and tested bee responses to different instar sizes because a mantis's ability to catch prey depends on its size relative to the prey (Reitze and Nentwig, 1991). Larger mantises should present a greater threat. Each mantis was measured from the tip of the head to the end of the abdomen, or for adults, the end of the wings, as the wings extended past the abdomen. In three separate experiments, we tested avoidance of (1) a living mantis, (2) mantis odor, and (3) mantis appearance (plastic model with no mantis odor). We worked with one focal colony at a time and trained bees to a feeder (see above) placed approximately 10 m away. Once the bees were trained (approximately 5 visits), we did not replenish the sucrose solution and allowed the feeder to become empty. At the training site, we presented bees with two identical clean feeders, each containing approximately 1 ml of 2.5M sucrose, on separate foraging platforms spaced 20 cm apart and equidistant from the focal nest. At the experimental feeder, we tethered a praying mantis (Tenodera sinensis) using an 8 cm long piece of black embroidery thread tied carefully around the thorax of the mantis. The other end of the tether was attached to the center of the feeder, which placed mantises within striking distance of the feeder. Each trial lasted 15 minutes, and we recorded the individual choices (control or experimental feeder) of bees in the absence of other bees near the feeder array to ensure the independence of choices and to eliminate potential social facilitation effects. All bees that landed on either feeder were immediately captured with a manual aspirator (20 cm long x

2.5 cm inner diameter clear polycarbonate tube with a flexible 15 cm long suction tube and screened at the mouthpiece). Each five minutes, we swapped the two feeders to avoid potential site bias. At the end of each trial, all captured bees were marked on their thoraces with acrylic paint (to ensure that their choice would only be counted once) and released. In some cases, the mantis caught and began to eat a bee during a trial. We noted this and tested for an effect of such successful predation because the release of bee alarm pheromone and hemolymph repels foragers (Goodale and Nieh, 2012). Each mantis was used for an average of 5 trials. Mantises that killed bees were not reused until several days later when odors associated with their successful predation would have dissipated.

Predator recognition cues

To determine if the bees would respond only to the visual appearance of a mantis, we used a model plastic mantis. We originally planned to use a lacquer-sealed dead mantis, but the mantises retained a detectable odor (to the researchers) even after seven days of being buried in silicone desiccant inside a sealed dessicator under continuous vacuum suction. Several coats of clear nitrocellulose lacquer on the dried specimens also did not eliminate the odor. The plastic mantis (Safari Ltd. USA, Miami Gardens, Florida, 22169, USA) had the appearance of an adult mantis, and was 6.7cm long, consistent with adult T. sinensis (see results). To test bee avoidance of mantis odor, we conducted trials with a hexane extract obtained from mantis exoskeletons. The mantis extract was prepared by adding 0.111 g of dead mantises and mantis exuviae to 10 ml of hexane in a sealed clean glass bottle, which was then agitated at 400 rpm for 3 hours at room temperature (21°C). Afterwards, we separated the hexane out into a sealed clean bottle stored at 4°C until use. During the trials, we used a micropipette to pipette out 500 µl of the mantis extract (corresponding to approximately half of one adult mantis) on the experimental filter paper, a 2.5 cm diameter circle of Whatman #2 filter paper placed under the experimental feeder and an identical volume of pure hexane on an identical clean filter paper placed under the control feeder.

Live predator effects on bee recruitment communication

To test the hypothesis that foragers would decrease dancing if the feeder had a live predator, we ran trials between August 2012 and June 2013, between 12pm and 4pm, with two observation colonies. We trained bees from the focal colony to a feeder approximately 1 meter from the hive entrance. The short distance to the feeder reduced the cost of food collection for the bees, thereby making the feeder a highly desirable food source, encouraging dancing. For such short distances, recruiting bees perform what is classically called the “round dance”(von Frisch, 1967), but which recent studies suggest is actually part of a continuum of behaviors to which the term “waggle dance” should be applied (Gardner et al., 2008). Following this terminology we focused on the number of dance circuits, which are positively correlated with recruitment for both round and waggle dances (von Frisch, 1967).

Each bee was painted with a unique combination of color marks on its thorax so that it could be easily located and identified in the observation hive. During trials, the feeder monitor used a 2-way radio to inform the nest monitor what bees experienced at the feeder and when bees left the feeder for the nest. We measured the number of waggle dance circuits performed by the same individuals before and after exposure to a mantis or a control (no mantis). We measured all waggle circuits performed inside the nest during a return from the feeder. To control for the effect of time elapsed between the before and after phases of the experiment, we also performed trials in which no predator was added, and each bee was recorded dancing twice: once initially, and once following a time interval of 15-60 minutes (similar to the time intervals in the live mantis trials).

After training a bee, we recorded the before phase data on her next return to the nest: the time of day, the time the bee waited to unload her collected food (unloading wait time) and the number of dance circuits completed. Once we collected data for all trained bees, we began the after phase, and placed a live tethered mantis on the foraging platform (treatment) or did not place a mantis (separate controls) and made the same measurements on the bees' next return to the nest. In cases where the mantis caught and killed a bee during a trial, we grouped this data separately and tested for a specific effect of such successful mantis predation.

Statistics

We used a one-way ANOVA to determine if there was an effect of mantis instar on mantis length, and a Tukey HSD test to determine which instars were significantly different in length. For the foraging preference experiments, the number of bees choosing the control feeder was compared to the number of bees visiting the experimental feeder using binomial 2-tailed tests (with the null expectation that bees have an equal probability of choosing either feeder). We used a Generalized Linear Model (GLM with binomial distribution, logit link, maximum likelihood estimation) with planned contrasts to determine if there was an effect of mantis size (small mantises vs large mantises), an effect of a successful mantis kill (adult mantis vs. successful mantis), and an effect of different cues (adult mantis vs. mantis visual cues, adult mantis vs. mantis odor, and mantis visual cues vs. mantis odors). Because of these multiple tests, we apply the Bonferroni Sequential test, indicating tests that pass as SB*.

For the dancing experiments, we used a repeated-measures Analysis of Variance (ANOVA) and tested the following fixed effects: treatment, bee identity (nested within treatment), and experimental phase on the unloading wait time (log transformed) and the number of dance circuits. We also used regression analysis to test for an effect of elapsed time (time between successive measurements of forager waggle dance behavior) and unloading wait time on the number of dance circuits. These data met parametric assumptions as determined through residuals analysis. All statistical analyses were conducted with JMP v9.

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Results

Effect of predator size

Average length for each instar was: 4th instar, 2.64 cm, 5th instar, 3.4 cm, 6th instar, 4.45 cm, 7th instar, 5.25 cm, and 8th (adult), 6.56 cm (Fig. 1A). There is a significant effect of instar on mantis length (ANOVA, F=250.76, d.f.=21, $p<0.0001$), and all instars are significantly different from each other (Tukey HSD, Q=3.04248, $P<0.05$). The majority of predation attempts came from larger mantises, and the only successful predation attempts were from adult mantises (Fig. 1B). Overall, there is a significant effect of mantis size (GLM $c^2=63.7$, d.f.=7, $p<0.0001^{SB*}$) on the proportion of bees choosing the control vs. the experimental feeder (our GLM model is a good fit for the data: Pearson Goodness of Fit test, $c^2=1458.0$, d.f.=1450, $p=0.43$). Bees did not avoid the smaller 4th instar (51% chose the mantis-free feeder, n=296, binomial $p=0.68$) and 5th instar (53%, n=252, binomial $p=0.41$) mantises, but they did significantly avoid the larger 6th instar (73% chose the mantis-free feeder, n=172, binomial $p=0.001^{SB*}$), 7th instar (63%, n=149, binomial $p=0.001^{SB*}$), and 8th instar or the adult stage (67%, n=161, binomial $p=0.001^{SB*}$, Fig. 2). Bees avoided the larger mantises (instars 6-8) significantly more than the smaller mantises (instars 4-5, GLM contrast $c^2=22.32$, d.f.=1, $p<0.0001^{SB*}$).

Predator recognition cues

Bees significantly avoided the plastic mantis (Fig. 2, 67% chose the mantis-free feeder, n=262, binomial $p<0.001^{SB*}$). Bees also significantly avoided the mantis extract (Fig. 2, 74%, n=121, binomial $p<0.001^{SB*}$). The contrast tests for adult mantis vs plastic mantis (GLM contrast $c^2=0.09$, d.f.=1, p=0.76), plastic mantis vs mantis extract (GLM contrast $c^2=2.21$, d.f.=1, p=0.13), and adult mantis vs mantis extract (GLM contrast $c^2=2.68$, d.f.=1, p=0.101) were non-significant.

In the smaller number of cases where a mantis (always an adult) killed and ate a bee during the trial, the vast majority of the bees avoided the experimental feeder (Fig. 2, 92% chose the mantis-free feeder, n=51, binomial $p<0.001^{SB*}$). The proportion of bees visiting the control feeder after a mantis successfully killed a bee was significantly higher compared to the proportion for trials with an unsuccessful adult mantis (GLM contrast $c^2=18.29$, df=1, $p<0.001^{SB*}$).

Effect on bee recruitment communication

There was no significant interaction of treatment*phase ($F_{2,61}=0.74$, P=0.48). There is a significant effect of overall treatment ($F_{2,63}=5.49$, P=0.006) such that bees waited significantly longer to unload their food after encountering a mantis at the feeder as compared to the control no-mantis treatment (Tukey HSD, Q=2.40223, P<0.05). Whether a mantis killed a bee at the feeder (successful mantis) or did not (unsuccessful mantis) did not alter unloading wait time. There was no significant individual variation among bees in unloading wait times ($F_{69,63}=0.90$, P=0.66). There is a significant effect of phase: unloading wait times were longer in the after phase ($F_{2,63}=8.71$, P=0.004) than in the before phase, although they were still longest for the bees that encountered the mantis as compared to bees that did not. There is no significant correlation between time elapsed between the before and after phases and change in the unloading wait time ($F_{1,63}=0.127$, P=0.13, $R^2=0.002$).

Exposure to a mantis significantly altered the number of dance circuits performed. There is a significant effect of overall treatment ($F_{2,73}$=5.48, P=0.0061) such that bees produced the most dance circuits after the control treatment (no mantis) and significantly fewer dance circuits after exposure to a live mantis that did not kill a bee (unsuccessful mantis) or mantis that had killed a bee (successful mantis, Tukey HSD, Q=2.39245, P<0.05). There is no significant difference between the effects of the successful and unsuccessful mantis treatments. Bees responded to the treatments by altering their dancing to different degrees (significant effect of bee: $F_{71,73}$=1.96, P=0.002). There is also a significant effect of phase such bees in all treatment groups tended to produce fewer dances in the after phase as compared to the before phase. However, bees that encountered a mantis decreased dancing more compared to bees that experienced no mantis. There is also no significant correlation between time elapsed between the before and after phases and change in number of dance circuits ($F_{1,71}$=2.35, P=0.13, R^2=0.03). There is no significant correlation between unloading wait time and the number of dance circuits produced ($F_{1,134}$=2.99, P=0.086, R^2=0.021).

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Discussion

The goal of our study was to determine if the threat of a mantis predator would induce changes in foraging behavior: increasing avoidance (fear) of dangerous food sources or by foragers reducing their dance recruitment communication. Previous studies have shown that predator presence can cause avoidance of dangerous flowers, and that evidence of predation can alter dancing in the nest. However, it was not known what cues bees are using in order to determine predator risk, or whether predator presence could also affect recruitment within the hive. We show that bees were about twice as likely to visit a safe feeder rather than one with a large mantis, plastic mantis, or mantis extract. No such effect was found for the smaller mantises. After the killing of a bee by a mantis, more than 90% of bees chose to avoid the mantis feeder in favor of the safe feeder, significantly more than avoided an adult mantis alone. Visual cues and olfactory cues both elicited the same degree of wariness in foraging bees. Mantis presence also caused a decrease in dance circuits for bees returning to the hive and increased the amount of time that bees waited before unloading their food back inside the nest.

Both the Chinese mantis and the European honey bee are introduced to North America, and therefore have not evolved together. However honey bees are preyed upon by similar mantis species in their native habitat, such as the European mantis (*Mantis religiosa*) which is of a similar size to the Chinese mantises, lives in similar climates, and also is known to consume honey bees (Slingerland, 1900), and the mantises are generalist predators, which would encounter similar prey in their own native habitats. Therefore, while this combination of species may be relatively novel, the behaviors involved should not be.

We demonstrate that foragers will avoid a praying mantis, but only if it is beyond a certain size threshold (instar 6, ≥ 4cm in body length). Size plays a role in how bees assess predation risk; large bumblebees are less likely to avoid dangerous food sources compared to smaller bumblebees or honeybees because their size makes them less likely to be captured by spiders (Dukas, 2001; Dukas and Morse, 2005). The ability of a mantis to catch prey depends on its size relative to its prey (Reitze and Nentwig, 1991), therefore, smaller mantises would be unlikely to capture a bee successfully, and so the bees may discount them as a threat. This fits with our observations, because only the largest mantises (adults, ≥ 6cm in body length) caught bees (Fig. 1). Larger mantises should also provide stronger visual and olfactory cues for bees.

The avoidance effect we observed is likely due to an assessment of risk, rather than simply neophobia, the fear of unfamiliar objects (Barnett, 1958; Bolbroe et al., 2000). Similar studies on bumblebees have failed to show a neophobic response. For example, bumble bees avoided frozen spider, but not a white cylinder of approximately the same size (Abbott, 2006). Therefore it is likely that the bees are indeed avoiding the praying mantises because they recognize them as a predation threat, not just because they are unfamiliar.

Honey bees avoided both the plastic mantis (visual cues but no olfactory cues) and the hexane extract of mantis exuviae (olfactory cues but no visual cues), and there was no significant difference in visitation to either cue, bees likely use both types of cues when trying to recognize a predation threat. This redundancy could be especially useful when the threat is an ambush predator such as a crab spider or a praying mantis that, in some foraging environments, could be difficult for a bee to recognize visually. Wolf spiders also reduced their foraging after detecting mantis odors (*T. sinensis* excreta) (Wilder and Rypstra, 2004).

The visual appearance of predators is important. Goncalves-Souza et al. (2008) showed that bees recognized visual traits of a spider, and will avoid a spider model consisting of a sphere with forelimbs, but will not avoid the sphere without legs. In our experiment, it is possible that our mantis model did not exactly simulate all the visual characteristics of a praying mantis to a bee. However, the mantis model was sufficient to elicit a strong avoidance response in bee foragers. Other studies have also used artificial model predators in predation experiments (Dukas, 2001; Ings and Chittka, 2008) and elicited appropriate biological responses. Moreover, honey bees will avoid these model crab spiders (Goncalves-Souza et al., 2008). Thus, the visual appearance of the mantis alone was evidently sufficient to elicit forager fear (Fig. 2).

Previous studies showing possible use by bees of olfactory cues left by predators have not been conclusive. Heiling and Herbertstein (2004) showed that native Australian bees approached flowers with spiders when odor had been excluded, however, they suggest that this effect may have been an artifact of using plastic wrap to keep the odor contained. Reader et al. (2006) showed that bees would avoid a flower that previously contained a spider, although this result was possibly due to the presence of spider silk, and also not consistent between experiments; bees were less likely to visit flowers exposed to a spider than a control flowers in one experiment, but equally likely to visit flowers exposed to a spider, flowers with silk added, and control flowers in another experiment. Our study therefore is the first study to demonstrate that bees are capable of using solely predator scent cues to determine the riskiness of a flower, and will avoid food sources that smell like a predator, even in the absence of any visual cues.

The strong avoidance response to feeders on which a mantis killed a honey bee (Fig. 2) is probably due to the release of alarm pheromone or internal fluids as the mantis tears the bee apart. Honey bees and bumble bees are known to avoid the presence of dead conspecifics (Abbott, 2006; Dukas, 2001), especially those that had been crush killed, likely releasing alarm pheromone, hemolymph, and possibly other fluids in the process. Goodale and Nieh (2012) showed that honey bees would avoid the odor of honey bee alarm pheromone and the odor of honey bee hemolymph at a food source. It may seem counterintuitive that bees avoid cues that could signal a satiated, and therefore unthreatening predator. However, just because one bee has been killed does not guarantee predator satiation. We observed several instances where a praying mantis would eat two or even three bees within minutes of each other. Alarm pheromone and hemolymph could also signal that a bee has been attacked, but has escaped, leaving behind a still-hungry predator. Thus, unmistakable evidence of a lethal predator (mantis consuming a freshly killed bee) was more effective at eliciting fear than an unsuccessful mantis (Fig. 2).

Following a live predator encounter, individual bees decreased their unloading wait times. Unloading wait times are usually thought of as a colony effect (an indicator of the colony’s nutritional status), the result of foragers waiting for the next food unloading to become available and take the nectar load. When a colony experiences a sudden influx of nectar, unloading wait time can sharply increase and this results in waggle dancers tremble dancing (Seeley, 1992). Alternatively, if food unloading bees are removed, the unloading wait time also increases and triggers tremble dancing (Seeley, 1989). However the behavior of a returning forager may affect how rapidly she is unloaded (Fig. 3A). Foragers may not immediately seek to unload food and therefore have some control over their unloading wait time: 68% and 78% of waggle and tremble dancers, respectively began to dance before they unloaded their food (Thom, 2003). Receiving bees prefer to unload nectar from bees with familiar scents (Farina et al., 2012; Grüter and Farina, 2009), so food unloaders may be deterred if foragers bring back predator odors collected while foraging. Thus, foragers evidently wait longer to unload their food if they have been exposed to predator. However, the reason for this is unclear and deserves further study.

After encountering a live mantis at the feeder, bees decreased the number of recruitment dance circuits that they performed (Fig. 3B), the first demonstration, to our knowledge, that the presence of a live predator, and not evidence of predation (such as a dead bee), will alter waggle dancing. Although the number of dance circuits decreased in the after phase as compared to the before phase, the decrease was significantly greater when bees encountered a live mantis while foraging. This overall decrease in the number dance circuits may reflect changes in bee motivation to recruit for a relatively new food source as compared to one that the colony has been foraging on for some time. However, there was no significant effect of time elapsed between the before and after phases on the change in the number of dance circuits performed. Abbott and Dukas (2009) showed that bees visiting a flower with a dead bee on it would dance less than bees visiting a “safe” flower. Our study shows that bees will also take other cues of predator presence into account in their dances. Unlike the foraging experiments, successful and unsuccessful mantises equally reduced recruitment dancing, suggesting that foragers individually discriminated between finer degrees of risk in their foraging, but found both predator treatments to be equally risky in terms of what they communicated to the colony.

Honey bees alter their foraging and recruitment behavior according to risk, not simply in response to predator attacks, but also to the possible threat of an attack. Increased wariness, or fear, can cause changes in behavior and resource utilization, ultimately affecting the way prey species interact with their environment, a concept known as the ecology of fear (Brown et al., 1999). These changes in behavior could affect the way honeybees utilize their landscape, leading to changes in pollination patterns, and decreasing plant reproductive fitness. These effects will likely be complex because predators who repel pollinators may also decrease herbivore damage to plants (Louda, 1982; Romero and Vasconcellos-Neto, 2004). However, prey that can avoid predators should clearly benefit: prey that acquire information that enables them to avoid predators can sustain higher populations (Schmidt et al., 2010). We show that danger from live predators alters bee spatial foraging and is translated into recruitment communication, affecting how the colony will ultimately allocate its labor. This should translate the effects to fear to a new level, amplifying it by affecting how the colony decreases its emergent allocation of foragers for dangerous food patches.

Sections 1-6 are currently being prepared for submission for publication. Allison Bray; James Nieh, 2013. This thesis author was the primary investigator and author of this paper.

Figures

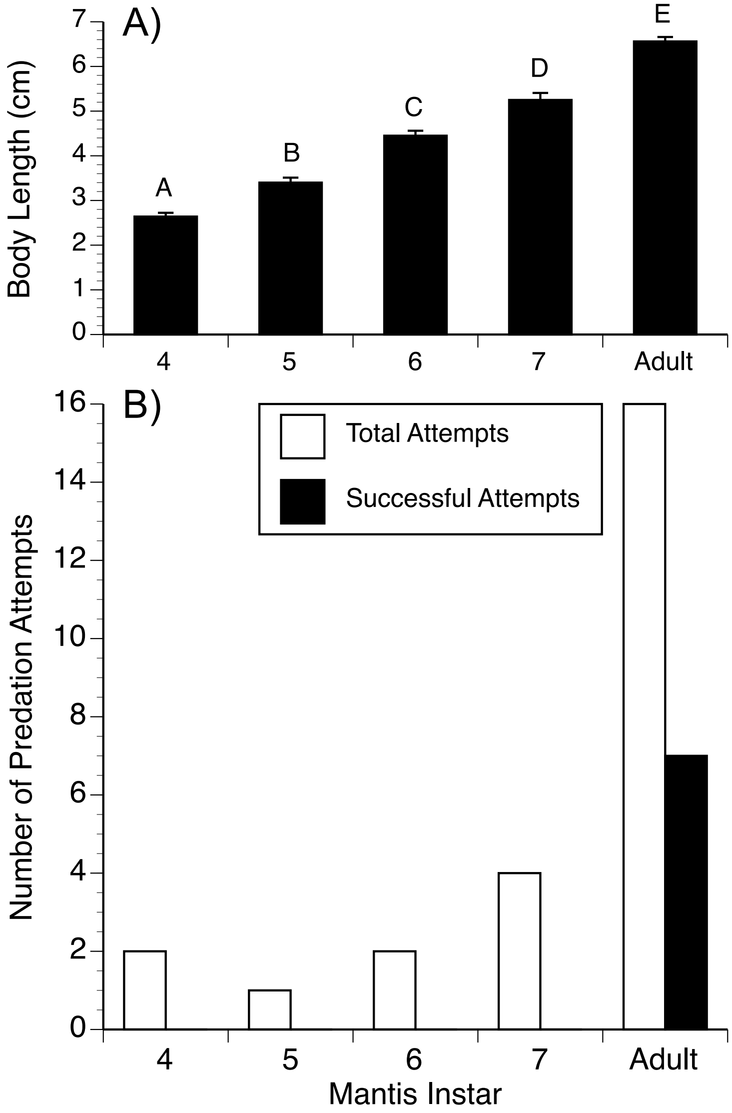

Figure 2: Proportion of bees visiting the safe feeder for each mantis size class, as well as for the plastic mantis and mantis extract treatments. The dashed line shows the null expectation of equal visitation to both feeders.

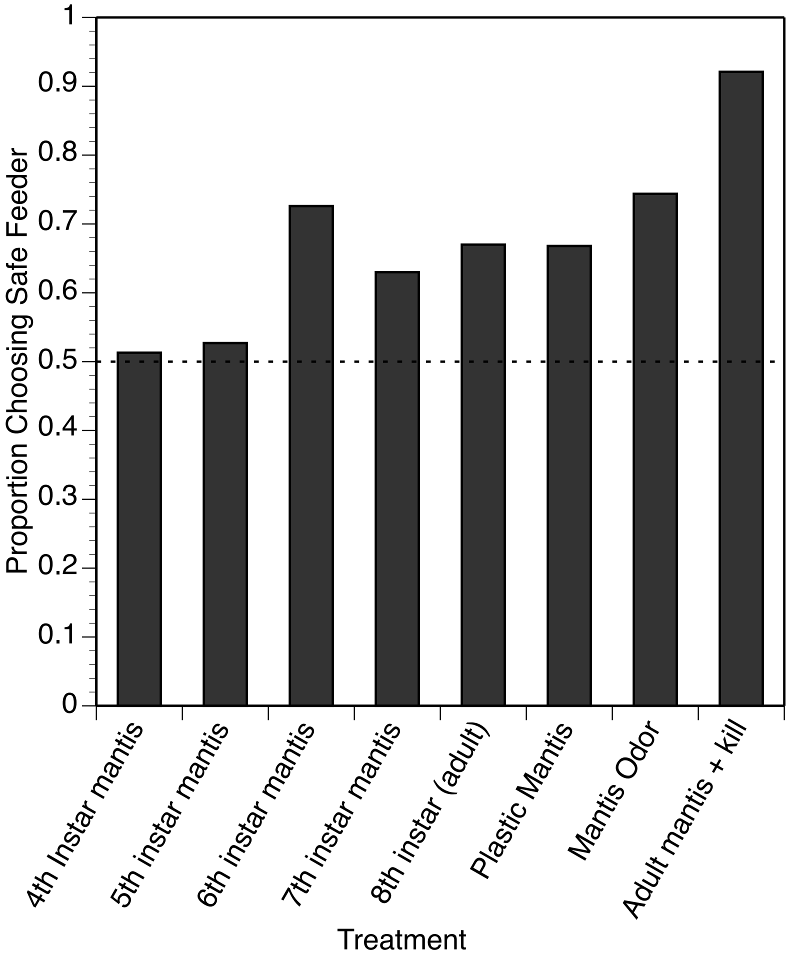

Figure 2: Proportion of bees visiting the safe feeder for each mantis size class, as well as for the plastic mantis and mantis extract treatments. The dashed line shows the null expectation of equal visitation to both feeders.

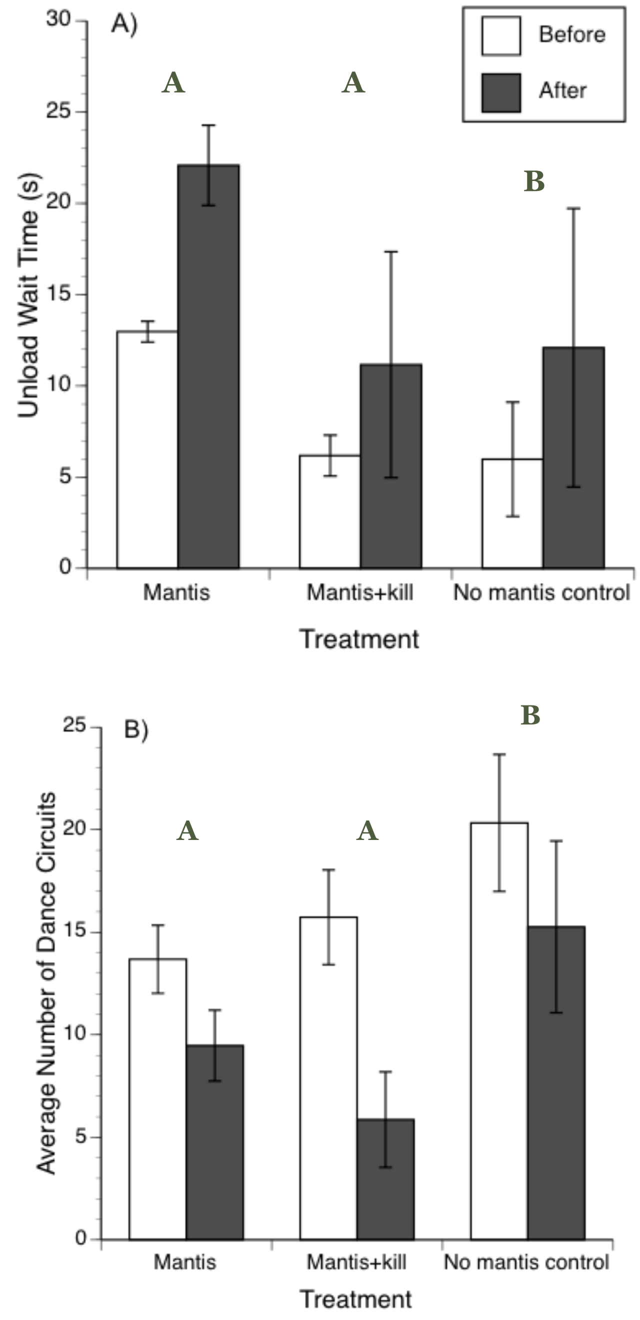

Figure 3: Difference in unloading wait time (A) and number of waggle dance circuits (B) between the before and after phases of each experiment.

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