UNVIERSITY OF CALIFORNIA, SAN DIEGO

Fear of spiders: role of predator and prey odors

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

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in

Biology

by

Spencer Huey

Committee in charge:

Professor James Nieh, Chair
Professor Elsa Cleland
Professor David Holway

2013

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

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Chair

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2013

DEDICATION

To my family and friends who have lovingly supported me over the years.

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

ABSTRACT OF THE THESIS

Fear of spiders: role of predator and prey odors

by

Spencer Huey

Master of Science in Biology

University of California, San Diego, 2013

Professor James Nieh, Chair

Interspecific relationships among pollinators and predators give insight to pollination and plant fitness for a given ecosystem. We focused on pollinator-predator interactions on three common native plants (*Eriogonum fasciculatum, Baccharis pilularis, Encelia californica*) in California coastal sage scrub habitat. When allowed to

forage at an array of five inflorescences (one with a crab spider), the pollinators (honey bees, Vespidae, other Diptera and other Hymenoptera) exhibited fear by spending less time on the dangerous inflorescence. There is also a spatial effect that depends upon pollinator type. Syrphid flies spent more time on safe inflorescences that were further away from the dangerous inflorescence. We then focused on honey bees (*Apis mellifera*), the most common visitor to these native plants. Honey bees exhibited fear and significantly avoided a feeder with crab spider odor. However, they did not avoid an artificial spider that did not provide spider odor. When spider odor was added to the model, they showed significant avoidance. This suggests that honey bees used olfaction as the primary cue to identify crab spiders (*Mecaphesa celer* and *Xysticus elegans*). In addition, honey bees avoided the odors of dead heterospecific pollinators (*Villa lateralis, Agapostemon texanus, Ceratina* sp. and *Vespula pensylvanica)* that are abundant in their environment. Thus, pollinator fear of predation mediated by olfactory detection can allow information about predator danger to flow in a community of pollinating insects.

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Introduction

Pollinators, such as bees, are faced with predation risk while foraging on inflorescences (Dukas, 2001b). Not only is there danger in foraging but also evidence shows that this predation may in fact lower the visitation rate of pollinators on inflorescences (Dukas, 2005; Dukas and Morse, 2003; Elliott and Elliott, 1994; Goncalves-Souza et al., 2008; Suttle, 2003). This pollinator-predator relationship is important in understanding pollination ecology. However, not much is known about how pollinators identify predators and the relative roles of olfactory and visual cues are involved in this detection. In addition, detailed field studies that examine how predators affect the spatial foraging of a broad guild of insect pollinators remain few, though such studies are important for understanding the ecology of predation and fear (Brown et al., 1999; Laundré et al., 2010) and how this may differentially affect the insect pollinator community (Schmidt et al., 2010).

Predation on pollinators may not only directly reduce population size but it also has an indirect effect, fear. Fear causes cautiousness and avoidance of the predator or areas associated with the predator (Blumstein, 2006). As understood in Behavioral Ecology, fear is functionally defined not as an emotion but rather a measure behavior of avoidance or fleeing from the predator. This fear can cause the pollinators to avoid dangerous patches, altering pollinator distributions and affecting the plant-pollinator mutualism (Brown et al., 1999). The fear of a predator can cause a lack of pollination in a given area, thereby lowering the overall plant fitness. The presence of crab spiders can

decrease pollination effectiveness, thereby lowering plant fitness and decreasing seed production (Goncalves-Souza et al., 2008; Suttle, 2003).

Crab spiders (Thomsidae) are common predators encountered by pollinators. Crab spiders are cryptic ambush predators that wait motionless on inflorescences for their insect prey (Lovell, 1915). This generalist predator preys on bumblebees (*Bombus* sp.), smaller bees (honey bees and solitary bees) and flies (Morse, 1979; Morse, 1981). Because these spiders are effective predators, visiting pollinators would benefit in distinguishing safe and dangerous inflorescences.

Previous studies show that pollinators recognize and avoid inflorescences harboring crab spiders. Patches of milkweed that contained crab spiders received significantly lower pollinator visitations than milkweed patches without crab spiders (Dukas and Morse, 2005). Bumblebees (*Bombus ternarius*) visited milkweed patches with crab spiders (*Misumena vatia*) at a lower rate than spider free patches (Dukas and Morse, 2003). Similarly, the number of insects that visited slickspot peppergrass inflorescences was significantly decreased at those inflorescences that contained a crab spider compared to equally rewarding safe inflorescences (Robertson and Maguire, 2005). Additionally, honey bees (*Apis mellifera)* significantly avoid inflorescences containing a frozen crab spider (Dukas, 2005). Foraging pollinators are able to identify the predators and avoid them.

Researchers have hypothesized that solitary bees are expected to exhibit higher anti-predatory behavior than social bees (Clark and Dukas, 1994; Dukas, 2001a; Dukas and Edelstein-Keshet, 1998). One study supports this hypothesis in which social bees (*Apis mellifera* and *Bombus* sp.) did not avoid dried spiders on inflorescences but solitary

bees and syrphid flies significantly avoided them (Brechbühl et al., 2010). This study, which utilized dried spiders, does not accurately represent the avoidance of a live crab spider in the field. Conversely, another study found that honey bees (*Apis mellifera*) do not accept inflorescences that contained crab spiders whereas solitary bees (*Eucera notata*) was not affected by the presence of crab spiders (Reader et al., 2006). This study opposes the hypothesis that was originally presented, but it focuses on only one species of solitary bee. Another opposing study found that bees (primarily *Apis mellifera* and *Bombus* sp.) did not avoid inflorescences with spiders on them (Morse, 1986). We therefore decided to explore the predator avoidance among various taxa, the presence of preyed upon heterospecifics and the importance of predator cue detection in pollinators. We tested if pollinators from various taxa would avoid dangerous inflorescences and would spend more time foraging on inflorescences further away from a dangerous inflorescence.

To our best knowledge, no studies have directly tested the hypothesis that insect pollinators will prefer to forage on inflorescences *further away* from an inflorescence with a dangerous predator. Previous studies have shown that honey bees preferred foraging at safe inflorescences compared to inflorescences harboring a spider predator (Dukas, 2001a; Reader et al., 2006; Robertson and Maguire, 2005). Similarly, foraging insects significantly avoid inflorescences containing crab spiders (Dukas and Morse, 2003; Robertson and Maguire, 2005). However, these studies examined the visitation rate and did not explicitly measure the distance at which the pollinators foraged relative to the predator inflorescence.

We also wanted to test the ability of honey bees to avoid predated pollinators, specifically testing the hypothesis that olfactory cues of predation can provide information about danger from a wide variety of pollinating heterospecifics. Conspecific avoidance has been better studied. While foraging, *A. mellifera* avoided the inflorescences containing a dead conspecific (Dukas, 2001a; Reader et al., 2006). Similarly, another study found that bumblebees (*Bombus*) visited inflorescences containing killed conspecifics at a lower frequency than equally rewarding inflorescences. Few studies have examined heterospecific avoidance of predator cues. Bumblebees and honey bees avoided inflorescences that had been recently visited by conspecifics and heterospecifics (Stout and Goulson, 2001; Stout et al., 1998). However, these inflorescences were avoided because they had been depleted of their resources. There is some evidence of heterospecific avoidance of predation cues. Bumblebees (*B. impatiens*) foragers avoided honey bee hemolymph odor at a food source, although honey bees did not avoid bumblebee hemolymph (Goodale and Nieh, 2012). We hypothesize that honey bees would avoid the crushed odors of heterospecifics pollinators that they commonly encounter and we therefore examined responses to the whole body odors of crushed heterospecifics on a food source. To our best knowledge, this is the first study that examines the response of honey bees to bodily fluids from a wide variety of dead heterospecifics pollinators.

Finally, we wanted to test how honey bees detect crab spiders, parsing out the effects of olfactory and visual cues. Bees can use visual cues to identify spiders. Goncalves-Souza et al. (2008) showed that pollinator visitation rate was higher on safe control inflorescences compared to inflorescences containing an artificial spider (with no

spider odor). Conversely, pollinators did not avoid an artificial spider (paper spider) placed on inflorescences (Brechbühl et al., 2010). Moreover, it is not known if honey bees can detect and avoid predators based upon their odor alone. Honey bees avoided inflorescences that had previously contained a crab spider, suggesting that bees use olfactory cues to detect the presence of predators (Reader et al., 2006), but this has not been directly tested.

We therefore conducted three experiments to address the following questions. First, will pollinators from various taxa avoid dangerous inflorescences and if they do, at what distance away from the predator inflorescence? Second, will honey bees be able to avoid the olfactory predation cues of heterospecifics pollinators? Third, do honey bees utilize visual cues, olfactory cues or both to detect and avoid crab spiders?

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Methods & Materials

Field sites

We collected data at three different sites that provide habitat for native pollinators and spider predators. The UCSD Biological Field Station (BFS: 32° 53' 13" N, 117° 13' 48" W) is surrounded by coastal sage scrub habitat. The 32,375-hectare Crest Canyon Open Space Park (32° 57' 30" N, 117° 15' 13" W) and 405-hectare Scripps Coastal Reserve (32° 52' 32" N, 117° 14' 52" W) are both coastal sage scrub habitats.

Experiment 1: Fear of spiders

We used two crab spider species, *Mecaphesa celer* and *Xysticus elegans* because of their abundance in San Diego County. We collected crab spiders with insect nets at different San Diego County sites at which permits are not required and reared them in 500 ml plastic containers on crickets (3 crickets per week/spider). After each trial, spiders were recaptured to avoid introducing new predators to the field sites. We used 60 adult crab spiders over 90 trials. We used one crab spider per trial unless the spider repeatedly fell off the inflorescence, then another crab spider of the same species was substituted.

We used three native angiosperm species that were highly visited by different species of insect pollinators and upon which we observed our crab spider species. We used California buckwheat (*Eriogonum fasciculatum*), coyote bush (*Baccharis pilularis),* and California bush sunflower (*Encelia californica*). To test pollinator inflorescence choices, we chose two patches per field site that were being visited by pollinators. The majority of observations were made beginning at noon when the pollinators were most active and abundant. We harvested five fresh inflorescences of the same general

condition. We placed each inflorescence into a 15 ml Falcon test tube vial filled with 7 ml of water to prevent wilting during the trial. Each vial was set into a polystyrene foam packaging tray (12.5 x 10.5 cm) such that four vials occupied the edges and one was in the center (Figure 1). Observers sat approximately 1 m away from the floral array to record data and avoid disturbing pollinators. The tray was then placed within a meter of floral patches at approximately the same height as patch inflorescences.

At the beginning of each trial, we gently placed a crab spider on one randomly selected inflorescence (out of five) and began a 3-hour trial. If the spider moved off its inflorescence, we carefully moved it back. Spiders were recaptured at the end of each trial to avoid increasing the density of spiders at our field sites. We recorded how much time a pollinator spent on each inflorescence. We did not capture pollinators and therefore visually classified them into six categories: honey bees, other Hymenoptera, Syrphidae, other Diptera, Vespidae, and Coleoptera. We observed only natural pollinators and did not train them or otherwise enhance their natural rate of visitation. The probability of the same pollinator revisiting our array was low. Although we did not capture or mark these pollinators to avoid affecting their behavior, we placed our test five-inflorescence array (identical in average quality to patch inflorescences) next to patches (on average 1x2 m) that contained roughly 200-400 inflorescences. In addition, pollinators foraged for a total of 25.5 hrs out of 270 hrs (9%). Thus, their visits were rare and widely temporally spaced. In field season 1 (August 2012), we recorded the time spent per inflorescence on safe and dangerous inflorescences. We observed a potential distance effect and thus in field season 2 (October-November 2012) and season 3 (April-May 2013), added a new measure, the distance of the safe inflorescence from the

dangerous inflorescence. We used all three field sites each season and ran trials for 30 hours per field site per season for a total of 270 observation hours over all field seasons (90 total trials, 30 at each field site).

Experiments 2 & 3: general methods

To test the choices of honey bees, we used paired feeder choice tests at the BFS apiary with honey bee (*A. mellifera* ligustica) colonies obtained from (C. F. Koehnen & Sons, Inc., Glenn, California, USA 95943). The feeder consisted of a 4 cm diameter petri dish painted pink (on its outside base), filled with 5 ml of 2.5 M unscented sucrose solution, and centered in a 9 cm diameter petri dish painted. This feeder was put on a 20 cm white circular platform atop a 1 m high tripod. We trained bees by presenting the feeder at the colony entrance until bees began to feed and then moved it further away until we were approximately 10 m away from the focal colony. We then set out two identical, clean test feeders (without sucrose solution) on separate tripods, spaced 30 cm apart and equidistant from the focal colony. We then covered training feeder and began the 30 min trial. We swapped feeder locations each 5 min to avoid potential site bias. We used a small plastic vial to immediately capture all bees that landed on the feeder. At the end of each trial, captured bees were chilled, marked with acrylic paint on their thoraces, and released so that their choices could not be recounted. The choice of each bee was counted only in the absence of all other bees on the array to avoid social facilitation effects and to ensure choice independence.

Experiment 2: heterospecific olfactory cues of predation

Using eight honey bee colonies, we tested if honey bees would show fear (avoidance) of odors from bees damaged by predators. The native pollinators used for the whole body extracts were commonly found pollinators in San Diego County that share floral resources with honey bees and are also, generally, prey to crab spiders (Morse, 1979). We captured these pollinators with an insect net, placed them into plastic vials, weighed them (as a measure of size), and then froze them until use. We used the green sweat bee (*Agapostemon texanus;* average weight 23 mg), the small carpenter bee (*Ceratina* sp.*;* average weight 5 mg), the bee fly (*Villa lateralis;* average weight 48 mg) and the western yellow jacket (*Vespula pensylvanica;* average weight 100 mg). Immediately before a trial, we allowed the insect to defrost and then crushed it with a clean Teflon pestle between the sides of a 2.5 cm Whatman number 2 filter paper circle folded in half. We placed this paper, extract side down so that only the white side without body parts was visible inside the pink feeder dish and used a small metal weight to hold it down. The control was identical, but we did not crush a pollinator. Trials were conducted November-December 2011 and October-November 2012. We used Alconox laboratory detergent and 100% ethyl alcohol to carefully clean all equipment between trials and wore gloves and used tweezers to handle the test apparatus.

Experiment 3: detecting crab spiders

Using nine different honey bee colonies, we tested if the honeybees would avoid the visual and olfactory cues of crab spiders. We prepared an extract of crab spider odor by gently agitating placing 32 individual spiders of both species (0.2552 g total weight,

50% of each species because there was no effect of spider species, see results) for 72 hrs in 32 ml reagent-grade hexane (1 spider/ 1ml of hexane). At the beginning of a trial, we pipetted 1 ml of extract onto a 2.5 cm circle of filter paper held down with a metal weight in the inner feeder dish. The control was identical, but we used 1 ml of pure hexane. To test the visual cues, we placed a 1.27 cm plastic model crab spider (painted brown to match their native counterparts) onto the outer petri dish of the feeder. The control was identical except for the spider model. Lastly, to test combined olfactory and visual cues, 1 ml of spider extract was placed onto a 2.5 cm circle of filter paper inside the inner feeder dish and the 1.27 cm spider model was set onto the outer petri. The control was identical, but with pure hexane. We ran trials from November-December 2012 and April-May 2013.

Statistics

We started with a parameter rich model including all factors and interactions. We then used stepwise eliminations and report the minimum adequate model comprised of only significant factors and interactions. For experiment 1 (field season 1-3), we calculated the proportion of time that pollinators spent on the dangerous inflorescence in the array in of five inflorescences and used Wilcoxon Signed Ranks tests (2-tailed) to determine if the mean was significantly different from the null expectation (20%). Because we observed a potential trend of bees spending more time on inflorescences further away from dangerous inflorescence, we recorded the distance of bee-visited safe inflorescences from the dangerous inflorescence in the second and third field seasons. We log-transformed time spent on each inflorescence and used linear regression to analyze

the relationship between distance from the dangerous inflorescence and pollinator foraging time. In cases where a pollinator visited multiple flowers within the array, we calculated the average visit time for the average distance at which it foraged from the dangerous inflorescence. For experiments 2 and 3, we used the Chi-Square test to analyze bee responses to each treatment. To compare between treatments, we used Tukey HSD tests. We apply the Bonferroni Sequential correction as appropriate, indicating tests that pass the correction as SB*. All analyses were conducted with JMP v10.

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Results

Experiment 1: fear of spiders

There is no significant effect of crab spider species ($F_{1,519}$=2.59, P=0.11) or plant species ($F_{1,519}$= 0.038, P=0.54) on the time that pollinators spent on safe or dangerous inflorescences (Figure 1). We therefore did not test for predator or plant species effects in our subsequent analyses. We compared the time pollinators spent on safe and dangerous inflorescences (Table 1). Our null hypothesis is that pollinators would spend 20% of their time on each inflorescence (five inflorescences in each array). Other Diptera, Vespidae, honey bees and other Hymenoptera spent significantly less time (17%, 16%, 16% and 13% respectively) on the predator inflorescence than safe inflorescences (Wilcoxon Signed Ranks tests: other Diptera T=221, N=222, P<0.0001; Vespidae T=24, N=25, P<0.01; honey bees T=246, N=247, P<0.0001; other Hymenoptera T=101, N=102, P<0.0001, Figure 2). There was no significant effect for Coleoptera (Wilcoxon Signed Ranks tests T=31, N=32, P=0.58). In our trials, we had only one crab spider (*X. elegans*) successfully catch a fly (other Diptera).

In our first season, we observed some pollinators appearing to prefer inflorescences further away from the dangerous inflorescence. In the next two field seasons, we therefore recorded pollinator distance choices. Although, other Diptera, Vespidae, honey bees and other Hymenoptera, avoided the dangerous inflorescence, only one of the pollinator groups, the Syrphidae, spent more time foraging at inflorescences significantly further away from the dangerous inflorescence ($F_{1,5}$=26.2, P=0.0037, SB*), with distance accounting for 84% of variation in feeding time (Figure 3). After Sequential Bonferroni correction, there was no significant effect of distance for Vespidae

($F_{1,26}$=4.58, P=0.04, not significant after SB). There is no significant effect of distance for any of the other pollinators: honey bees ($F_{1,245}$=2.84, P=0.09), other Hymenoptera ($F_{1,89}$=0.007, P=0.93), Coleoptera ($F_{1,28}$=0.69, P=0.41), or Diptera ($F_{1,120}$=0.002, P=0.96).

Experiment 2: heterospecific olfactory cues of predation

Honey bees significantly avoided the odors of all crushed heterospecifics tested: *V. lateralis* (N=538, χ^2_1=48.82, P<0.0001) *A. texanus* (N=398, χ^2_1=9.65, P<0.01), *Ceratina* sp. (N=417, χ^2_1=35.11, P<0.0001) and *V. pensylvanica* (N=370, χ^2_1=112.48, P<0.0001). Honey bees avoided the presence of crushed heterospecifics pollinators while foraging. Bees showed significantly greater avoidance of *V. pensylvanica* odors as compared to all other crushed pollinator odors (Tukey HSD Q=2.57155, P<0.05). This avoidance may arise from larger pollinators releasing more odors. *Vespula pensylvanica* was significantly heavier than all other species, followed by *V. lateralis*. The species *A. texanus* and *Ceratina* sp. were the lightest and not significantly different in mass from each other (Tukey HSD Q=2.7008, P<0.05, Figure 4).

Experiment 3: detecting crab spiders

Honey bees significantly avoided spider odor (N=391, χ^2_1=241.05, P<0.0001) as well as the spider odor applied onto a spider model (N=193, χ^2_1=78.39, P<0.0001). Honey bees showed no avoidance of the spider model without spider odor (N=301, P=0.057). There are significant differences between responses to the model spider (visual cues only) and spider odor alone, but no difference between odor alone and odor plus

model spider (Tukey HSD, Q=2.3477, P=0.05, Figure 5). Thus, honey bees did not significantly avoid the visual presence of a predator, but they significantly avoided crab spider odor.

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Discussion

We found that an assemblage of pollinators will exhibit fear and avoid crab spiders on inflorescences of three different plant species. When pollinators were allowed to forage on an array of inflorescence with one inflorescence containing a crab spider, they spent less time on the dangerous inflorescence. Similarly, foraging pollinators (specifically Syrphidae) spent more time on inflorescences that were furthest away from the predated, dangerous inflorescence instead of inflorescences that were closer to the predator inflorescence. Honey bees avoided the whole body extracts (a product of simulated predation) of heterospecific pollinators (*V. lateralis, A. texanus*, *Ceratina* sp. and *V. pensylvanica)* while foraging. Honey bees also avoided the crab spider odors as well as a spider model paired with the crab spider odor. However, honey bees did not avoid the spider model without spider odor.

Experiment 1: fear of spiders

Foraging pollinators avoided dangerous inflorescences and spent more time at inflorescences further away from the predator inflorescence. Some previous studies show that crab spiders decrease the visitation rate of pollinators (Dukas and Morse, 2003; Dukas and Morse, 2005; Robertson and Maguire, 2005). However, other studies found no effect of crab spiders on pollinator visits (Morse, 1986). We found that crab spiders induce fear (avoidance) in a wide variety of pollinators visiting natural resources in California coastal sage scrub habitats (Figure 2). However, pollinating beetles (Coleoptera) exhibited no fear of spiders.

Our study provides the first detailed data on the effect of predator presence on pollinator foraging distance preferences (albeit within a range of 120 mm of the dangerous flower). We found that only hover flies (Syrphidae) foraged for a longer time on the inflorescences that were further away from the dangerous inflorescence with a crab spider on it (Figure 3). This makes sense given the greater vulnerability of pollinating flies to crab spider attack. Morse (1979) showed that crab spiders (*Misumena calycina*) had a significantly higher success rate preying upon syrphid flies and other small flies than upon bumblebees. In our case, with the crab spiders *Mecaphesa celer* and *Xysticus elegans*, we observed this distance effect only with syrphid flies, not with other pollinating Diptera. In addition, we had only one instance of a successfully caught a prey item; one of the crab spiders preyed upon a fly (other Diptera). However, given the general success rates of crab spiders (Morse, 1979), this is not unexpected. Moreover, it highlights the general finding that the non-consumptive effects of fear can outweigh actual consumption (Lima, 1998).

Experiment 2: heterospecific olfactory cues of predation

Many studies focus on the effect of predators on pollinators (Goncalves-Souza et al., 2008; Suttle, 2003). However, few studies examine use of heterospecific information about predation among pollinators. Goodale and Nieh (2012) showed *B. impatiens* avoided honey bee and its own hemolymph. Honey bees avoided its own hemolymph but not those of *B. impatiens* and *B. vosnesenskii*. While the previous study focused on social bees (*A. mellifera* and *Bombus* sp.) and we therefore examined the reactions of honey bees to various pollinators, including solitary bees. We chose tested odor extracts

obtained from simulated predation (crushing) of *Villa lateralis*, *Agapostemon texanus*, *Ceratina* sp. and *Vespula pensylvanica*, because they are commonly found, along with honey bees, foraging on the native plants that we used. This increases the likelihood that information about predation could flow between these pollinators. Additionally, we used whole body extracts, which simulated predation rather than strictly hemolymph or sting glands. Thus, this is the first study to examine the response of honey bees to overall bodily fluids of a variety of dead heterospecific pollinators. Honey bees avoided inflorescences containing the odors of crushed heterospecific pollinators (*Villa lateralis*, *Agapostemon texanus*, *Ceratina* sp. and *Vespula pensylvanica*, Figure 4). They should enable honey bees to avoid dangerous foraging sites. We found significantly greater avoidance of *V. pensylvanica* odors compared to all other tested species odors. This may have arisen from the greater volume of bodily fluids produced by the larger insect, producing more conspicuous odors.

Experiment 3: detecting crab spiders

Honey bees avoided crab spider odor but not the visual-only cues provided by our model crab spider.

Goodale and Nieh (2012) showed that honey bees did not avoid haemolymph from two species of bumblebees (*Bombus impatiens* and *Bombus vosnesenskii).* This *Bombus* hemolymph study was done using similar methods and the same honey bee colonies as our study. Thus, we were able to show the honey bee avoidance of predatory spider odor and not a simply response to a new odor. We used crab spider extract composed of both crab spider species (*X. elegans* and *M. celer*) because we did not find an effect of species

on avoidance. It is unclear if bees recognize the odors of specific crab spider species or generalize, recognizing the general olfactory cues of crab spiders. It is possible that combining the odors of both crab spider species may have elicited a stronger aversion response than using only one species alone. However, it is clear that honey bees could detect and avoid the general odors of crab spiders (Figure 5).

The lack of visual recognition may be due to our spider model not being sufficiently realistic. However, a finely detailed model is evidently not necessary to elicit avoidance. Goncalves-Souza et al. (2008) used simple sphere-shaped models to simulate a spider's body with extended legs (metal staples) and elicited bee avoidance. They reported that the model forelimbs were key to recognition (Goncalves-Souza et al., 2008). Our spider models did not have forelimbs that were as pronounced. This may have contributed to a lack of recognition by bees. However, even though the spider model alone did not elicit fear, it shows that placement of a foreign object alone on the food source does not elicit neophobia (fear of unfamiliar objects) (Barnett, 1958; Bolbroe et al., 2000) by honey bees.

In summary, the presence of crab spider predators and evidence of successful predation (odors of injured heterospecifics) leads to pollinator avoidance (fear) of dangerous inflorescences (Figure 2). Not all pollinators exhibit such fear and interestingly, beetles did not avoid the spiders, perhaps because they have hard exoskeletons (elytra) that give them better protection. Within the small patch presented by our experimental array, only syrphid flies spent more time foraging at more distant inflorescences (Figure 3), perhaps because they are more vulnerable to spider predation.

Morse (1979) found that crab spider capture rate for syrphid flies was 61% of the total prey compared to 8% for honey bees. Thus, vulnerability to predators may play a major role in determining how prey respond. A guild of pollinating insects provides a variety of olfactory cues when preyed upon, and we found that honey bees recognized and avoided the odors of killed bees, flies and wasps at a food source (Figure 4). The flow of olfactory information occurs among pollinators and between pollinators and prey. Honey bees likewise recognized and avoided the odor of crab spiders. Thus, olfaction may play a major role structuring how prey recognize predation: the results of successful predation (rare) and predator presence (more common). Previous research has investigated how bee pollinators learn to avoid nectar-depleted flowers by recognizing the cuticular hydrocarbons deposited by previous visitors (Goulson D. et al., 2000; Leadbeater and Chittka, 2007; Witjes, 2009; Yokoi, 2008). However, our results suggest that we should also consider a broader olfactory landscape that includes detection of predation and predators, all of these cues weighing in to influence pollination and, ultimately, pollinator and plant fitness.

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Figures and Tables

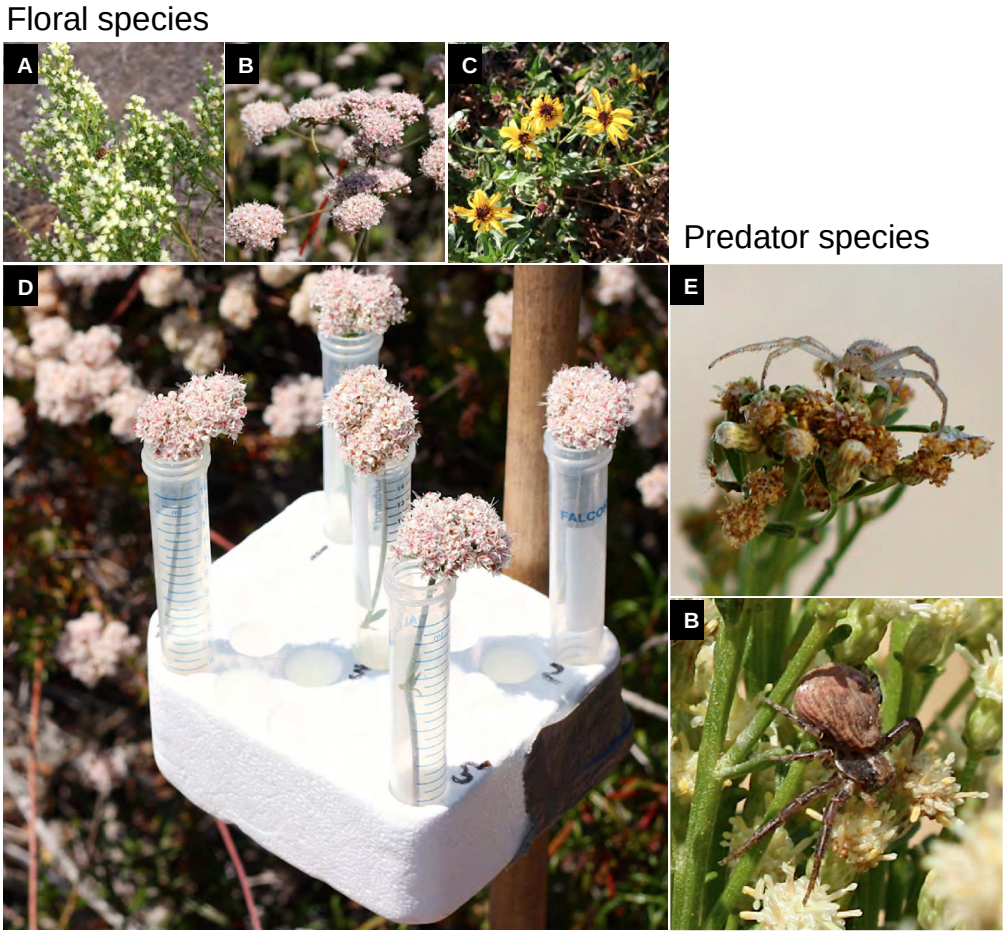

Figure 1. The native plants (A) *Baccharis pilularis*, (B) *Eriogonum fasciculatum* and (C) *Encelia californica* used in the (D) choice assays for experiment 1. We used two species of crab spiders: (E) *Mechaphesa celer* and (F) *Xysticus elegans*.

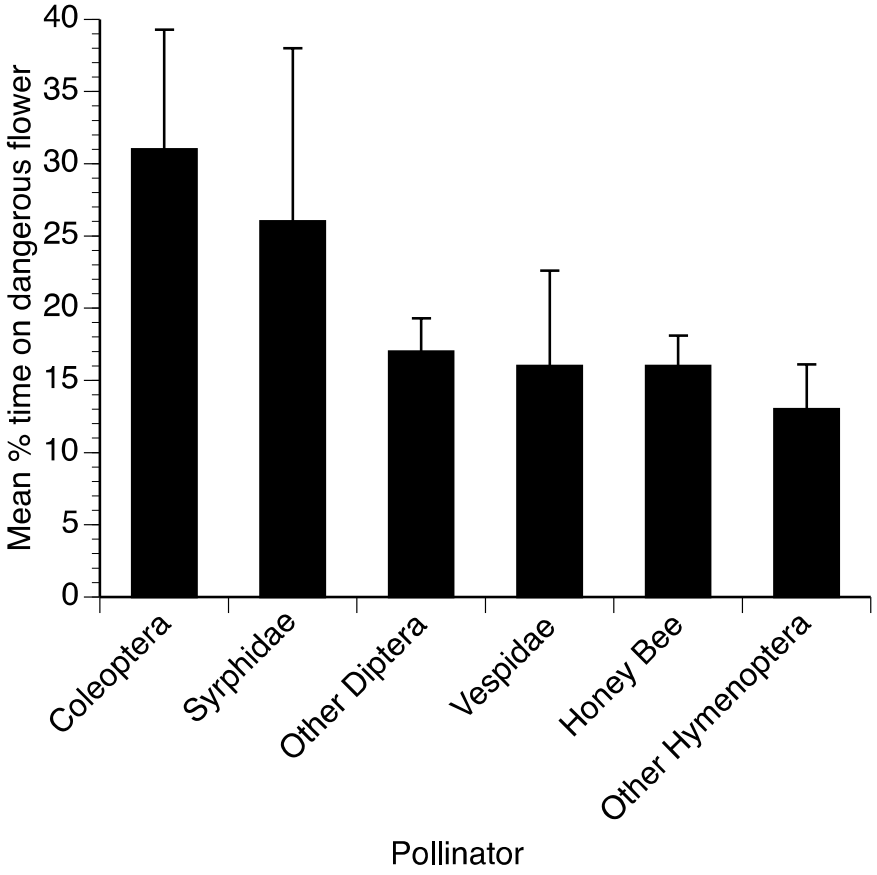

Figure 2. The mean percentage (+SE) of pollinator visitations on dangerous inflorescences for experiment 1. In experiment 1, pollinators visited an array of 5 inflorescences (one containing the crab spider). Thus, the null expectation is that a pollinator will spend, on average, 20% of its time on each inflorescence. Other Diptera, Vespidae, honey bees, and other Hymenoptera spent significantly less time on the dangerous inflorescences than safe inflorescences (Sample sizes show in table 1).

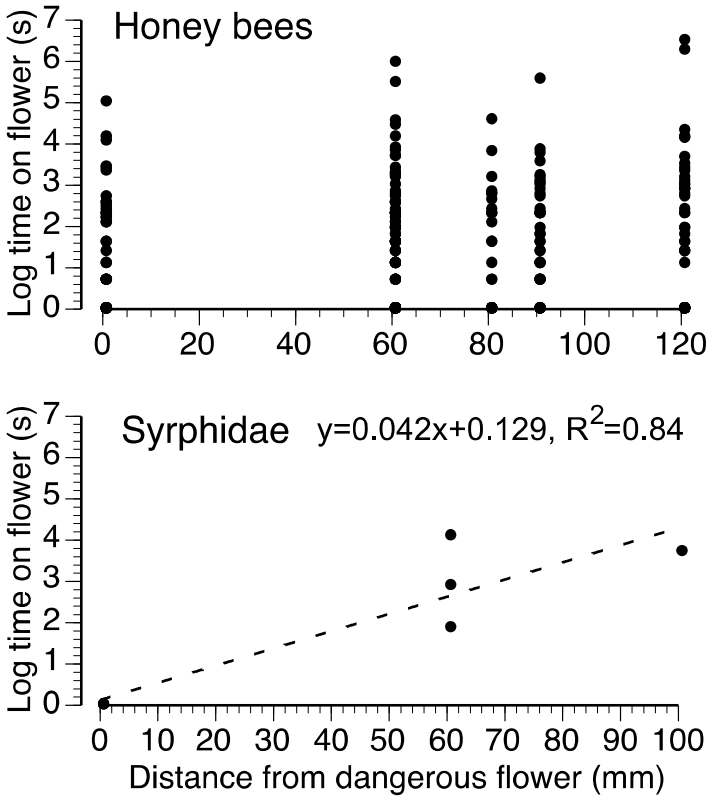

Figure 3. Effect of crab spider presence on pollinator foraging time at different distances from the dangerous inflorescence. Only Syrphidae spent significantly more time at inflorescences farther away from the dangerous inflorescence. We show the linear regression lines and equations for these groups. Honey bees are an example of a group in which fear did not alter distance choices.

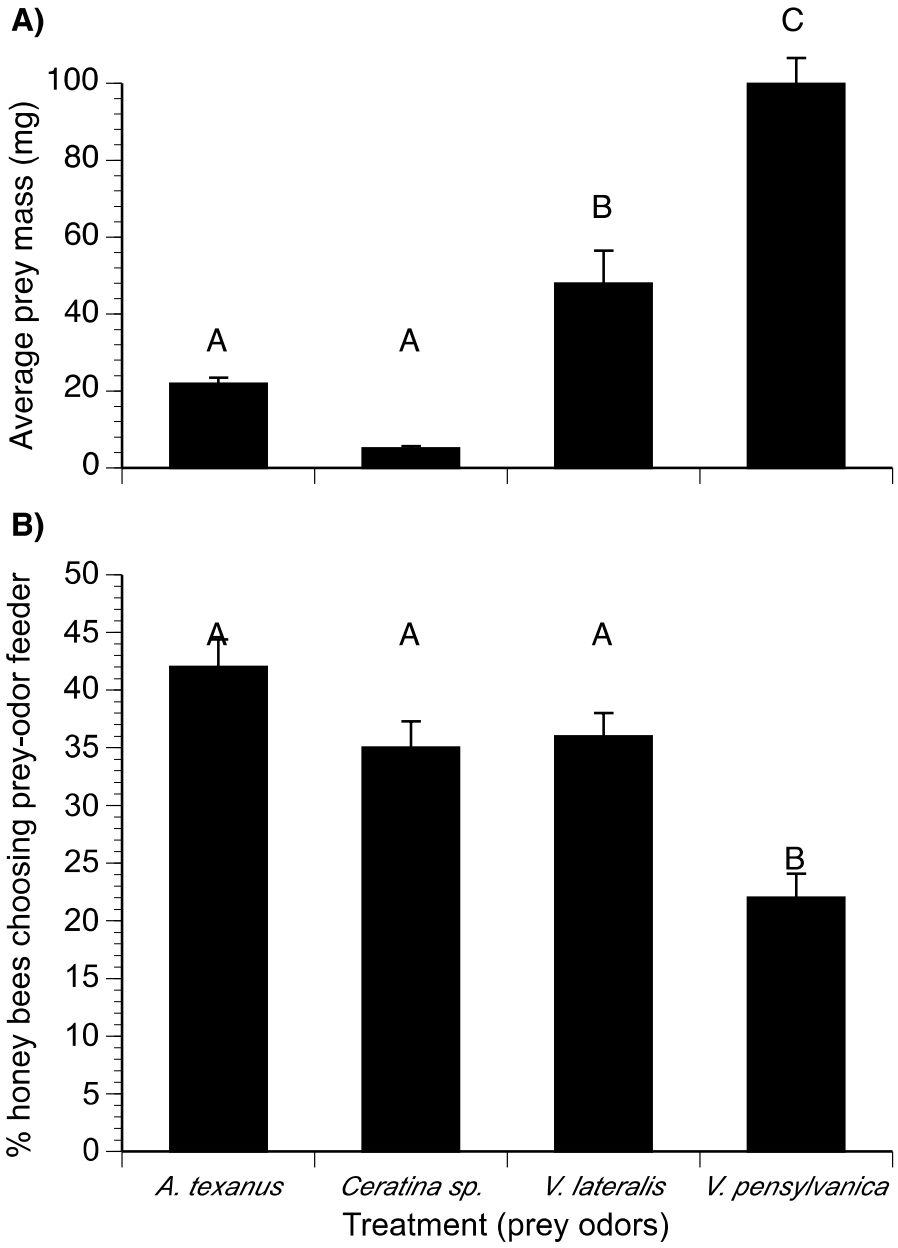

Figure 4. (A) The mean mass (+SE) of pollinators used for the odor extracts and (B) the percentage (+SE) of honey bees (*Apis mellifera*) that chose the dangerous inflorescence (experiment 2). Honey bees were given a choice between a inflorescence containing heterospecific pollinator extract or an equally rewarding safe inflorescence. Different letters show significant differences.

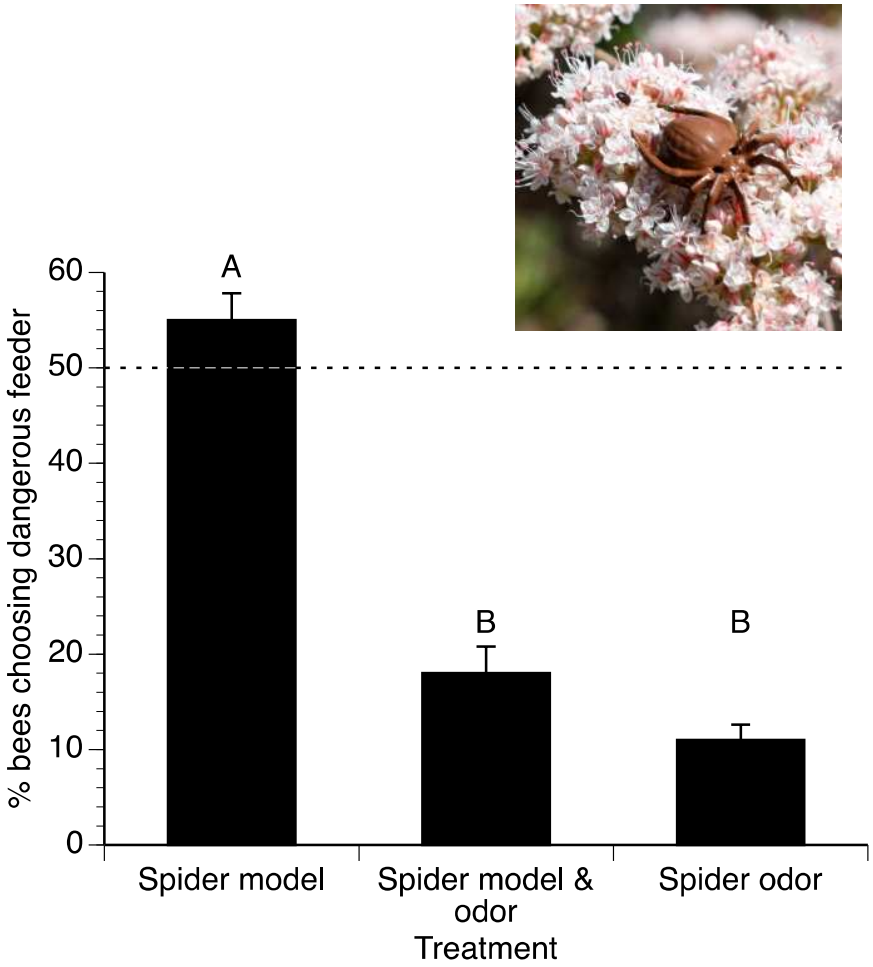

Figure 5. The percentage (+SE) of honey bees (*Apis mellifera*) that choose the dangerous feeder (experiment 3). Different letters indicate significantly different treatments. The dashed line shows the null expectation of bees equally choosing both feeders. Image of the spider model on inflorescence (*Eriogonum fasciculatum*) is shown above.

Table 1. The mean visitation time pollinators exhibited on safe inflorescences and dangerous inflorescences from all three field seasons. We observed inflorescences for 270 hours.

Pollinator	Number of Pollinators	% total visits	Mean(±1SD) time on safe inflorescence (s)	Mean(+1SD) time on dangerous inflorescence (s)	Number of visits per hour	Mean(±1SD) foraging time per inflorescence (s)	Mean(±1SD) foraging time on *Baccharis pilularis*	Mean(±1SD) foraging time on *Encelia californica*	Mean(±1SD) foraging time on *Eriogonum fasciculatum*
Apis mellifera	247	38.5%	23.6±70.2	2.6±11.7	0.91	5.2±14.2	5.2±15.9	6.5±12.1	4.4±11.7
Other Diptera	222	34.6%	93.7±207.5	13.2±46.9	0.82	21.4±42.5	21.3±43.1	23.1±58.8	20.8±34.9
Other Hymenoptera	102	15.9%	111.7±213.3	5.8±17.0	0.38	23.5±42.4	38.0±55.1	15.4±27.6	11.0±24.5
Coleoptera	32	5.0%	774.2±1503.4	704.2±1848.3	0.12	295.7±249.1	-	313.5±453.9	170.7±156.3
Vespidae	25	3.9%	77.32±152.8	3.2±10.5	0.09	16.1±30.8	17.9±34.0	-	9.0±10.6
Syrphidae	13	2.0%	17.8±25.6	0.4±0.5	0.05	3.6±5.1	5.1±6.6	-	2.0±2.2

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

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