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Approved by:

Elizabeth M. Jakob

AVERSION LEARNING IN WOLF SPIDERS: THE PERSISTENCE OF MEMORY AND THE ROLE OF SENSORY CUES

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ABSTRACT

Learning can benefit animals by enabling them to more efficiently cope with environmental variability, thus increasing survival and fitness. For example, learning discrimination against prey with toxic defenses would reduce unprofitable handling and pursuit time, and ingestion of potentially harmful chemicals. This research was conducted to examine the ability of wolf spiders (*Schizocosa ocreata*) to recognize and avoid toxic prey after experience, the persistence of the learned aversion, and the sensory cues necessary for the aversion to occur.

Milkweed bugs (*Oncopeltus fasciatus*) were used as prey in aversion learning experiments. Spiders were assigned to one of three feeding treatments: 1) toxic (milkweed-fed) milkweed bugs; 2) cardenolide-free (sunflower-fed) milkweed bugs; or 3) crickets (*Acheta domesticus*). No neophobic (fear of novel) reactions to aposematic prey were observed; initial pursuit responses were the same for all prey types. Subsequent feeding behavior varied with prey type; spiders accepted crickets and sunflower-fed milkweed bugs, but rejected cardenolide-containing milkweed bugs. Spiders were offered toxic prey until avoidance was exhibited, then offered alternative prey. Spiders acquired aversion in 1-4 trials and refused cardenolide-free milkweed bugs thereafter, but accepted crickets. Additional aversion learning experiments examined the duration of spiders' learned aversion to *O. fasciatus*. Trained spiders were re-exposed to toxic prey with differing presentation and/or feeding schedules. Overall, the learned aversion was retained for less than a day, but with repeated exposure, fewer trials were required for renewal. In experimental studies, the order of prey presentation impacted the persistence of the learned aversion more than spider hunger level (i.e., spiders given palatable cricket prey between milkweed bug trials did not retain learned aversion). We conclude spiders' predatory behavior may be influenced by recent, as well as past, foraging experiences. The specific sensory cues (chemical, visual, tactile) involved in recognition and subsequent avoidance of potentially toxic prey was also observed. Studies with video playback and painted milkweed bugs eliminated visual cues as recognition criteria, but tactile and chemical cues from *O. fasciatus* were significant factors in recognition and avoidance behavior. Results from these experiments indicate that *Schizocosa ocreata* can learn to recognize and avoid distasteful prey using primarily tactile and chemical cues; making modification of their predation patterns possible to increase foraging efficiency and possibly individual survival and fitness.

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INTRODUCTION

For many years the ability of animals to learn (alter behavior following experience with a stimulus) has been studied primarily by psychologists (Drickamer et al. 1996; Hawkins et al. 1998). However, behavioral ecologists are also interested in the subject of learning, especially with regard to the benefits of learned behaviors for survival and individual fitness (Krebs & Kacelnik 1991). By offering an alternative to a fixed response, learning can benefit an animal by enabling it to more efficiently cope with environmental variability (Drickamer et al. 1996; Alcock 2001).

Many learning studies have involved vertebrates, particularly birds (Brower & Brower 1964; Wiklund & Järvi 1982; Vos 1994; Gamberale & Tullberg 1996; Davies & Faure 2000), amphibians (Brower et al. 1960), and mammals (Garcia et al. 1961), but there is mounting evidence for the ability of invertebrates to learn as well. Historically, invertebrate responses were considered to be fairly automatic and fixed (Tinbergen 1951; Papaj 1993). Recently, however, many studies have showed that invertebrates are capable of changing their behavioral response after experience with a stimulus (Thompson & McConnell 1955; Fiorito & Scotto 1992; Bernays 1993; Papaj 1993; Dukas 1999; Punzo 2002).

A number of invertebrates, including arthropods such as praying mantids (Berenbaum & Miliczky 1984; Paradise & Stamp 1991; Bowdish & Bultman 1993) and polyphagous grasshoppers (Bernays & Chapman 2000), learn to avoid distasteful food items. This type of learning, passive avoidance learning, involves altering an existing response (feeding) after experiencing a noxious stimulus (Drickamer et al. 1996; Alcock 2001). Developing aversions to distasteful food items may have a protective value for generalist predators that encounter a variety of potential prey (Toft 1997). Juvenile praying mantids grew more slowly and gained less biomass when fed toxic prey (Paradise and Stamp 1993). Learning to discriminate against prey items containing toxins would decrease unprofitable pursuit and handling times and reduce ingestion of potentially harmful chemicals. Therefore, learned aversions may increase an individual's survival and/or fitness (Brower et al. 1968).

Although some animals may be capable of quickly learning aversions to distasteful prey (Roper & Redston 1987), others may require sufficient feeding experience with a particular toxic prey item before the association of prey consumption and unpalatability occurs. Furthermore, learned prey aversions may be only temporary, especially in invertebrates (Toft 1997; Toft & Wise 1999). The duration of a learned aversion to harmful prey may be influenced by predator memory and/or hunger level. Short-term learning may be advantageous to species with changing environments, as learned prey aversions may be unsuitable during other foraging periods or at other locations (Bernays 1993; Mangel 1993).

If an association between unpalatability and the prey's physical, behavioral and/or chemical characteristics are formed, predators may construct a recognition image, which can result in avoidance of prey types resembling the template in memory (Eisner & Grant 1980). Many prey species that are toxic or unpalatable are conspicuous in color or pattern as well (aposematic or warning coloration) (Cott 1940). This is often considered to be self-advertisement of an anti-predator defense (Cott 1940; Huheey 1984). Furthermore, brightly colored species may also possess additional chemical or behavioral properties that may serve as indicators of distastefulness. These combined characters may function to evoke neophobic responses (rejection of novel food items), or influence the acquisition and persistence of learned aversions (Guliford 1990; Endler 1991; Speed 2000). Aposematic coloration, behaviors, and aversive odors may act as multi-modal signals and function in defense to ultimately deter consumption by reminding experienced predators of the unpleasant taste or toxicity of prey (Huheey 1984).

As generalist predatory arthropods, spiders might also be expected to learn to avoid toxic prey (Vasconcellos-Neto & Lewinsohn 1984; Toft 1997; Toft & Wise 1999). Spiders of various species are capable of learning spatial tasks (Punzo & Bottrell 2001; Punzo 2002), heat avoidance (in association with colored papers) (Nakamura & Yamashita 2000), likely food locations (Persons & Uetz 1999), and predator avoidance (Punzo 1997). Spiders may therefore be ideal to use for examining the effects of memory and hunger upon foraging decisions, as these factors can be manipulated in laboratory spider populations with minimal difficulty. Additionally, spiders perceive multiple modalities of sensory cues (visual, vibratory, chemical) (see reviews in Uetz 2000; Uetz & Roberts 2002), and isolation of the multimodal cues emitted from prey types that spiders have learned to avoid could reveal the sensory elements necessary to evoke an avoidance response. Using spiders as a model organism may benefit our understanding of invertebrate learning, and the influence various conditions and sensory cues have upon learned aversion and generalist predator foraging.

SPECIFIC RESEARCH OBJECTIVES

The goal of this research was to explore aversion learning in wolf spiders, a generalist predator. All investigations were performed in the laboratory, as the predator-prey model system I used does not naturally occur. Aposematically colored and chemically defended, *Oncopeltus fasciatus* (Dallas) [Hemiptera: Lygaeidae] were used to provide the necessary noxious stimulus to wolf spiders, *Schizocosa ocreata* (Hentz) [Araneae: Lycosidae], in aversion learning experiments. This predator-prey model system was considered a conservative test of predator learning, as these two species of arthropod are not likely to co-occur in nature. The milkweed bugs are found feeding upon milkweed plants (*Asclepias sp.)*, which are often found along roadsides or in old fields. The wolf spider *S. ocreata* however, is not observed in these types of environments, but is found in complex leaf litter habitats on the floor of Eastern deciduous forests (Cady 1984). Additionally, these wolf spiders do not climb atop plants to search for prey, but rather are non-web-building, sit-and-wait, ground-foraging predators (Wise & Wagner 1992). Therefore, there is little potential for contact (in ecological or evolutionary time) between the two species and consequently, any previously learned or adaptive recognition, as well as any behavioral co-evolution was highly unlikely.

I examined the capability for aversion learning by *S. ocreata* and the possible duration of a learned aversion in this species. I also gained insight into the role of particular sensory cues in a learned aversion. My research had three primary objectives:

Objective 1) To investigate whether *S. ocreata* learn to avoid toxic prey items: Toxic prey items were repeatedly presented to spiders to determine if they were capable of learned aversion. I examined the behavioral responses of spiders (naïve to and experienced with toxic prey) to various prey types, both palatable and unpalatable. The wolf spiders were predicted to be more likely to recognize, reject and avoid toxic milkweed bugs *(O. fasciatus)* after aversive experience.

Objective 2) To examine the duration of the spider's learned aversion to toxic prey, and the effects of memory and hunger on subsequent predatory behavior: In these experiments, spiders that had acquired a learned aversion to toxic prey were subjected to various re-exposure (to toxic prey) and feeding (of palatable prey) schedules to investigate the impact of these factors upon the persistence of the learned aversion. Learned aversion was expected to increase with repeated exposure to toxic *O. fasciatus* and decrease with increasing hunger and/or time without exposure.

Objective 3) To determine the specific sensory cue(s) most critical to *S. ocreata* for recognition and subsequent avoidance of potentially toxic prey: Multimodal cues (chemical, visual, and tactile) of toxic prey were experimentally separated and presented independently to wolf spiders experienced with and naïve to the toxic prey in these experiments. It was predicted that significant differences in behavior between naïve and experienced groups would occur when spiders were presented with the sensory cue that induced an aversive response.

GENERAL METHODS

Study Organisms

The predator

Adult female *Schizocosa ocreata* were collected from the Cincinnati Nature Center, Rowe Woods, Clermont County, Ohio, during the summer of 2002. Spiderlings from eggsacs produced by these females were raised to maturity under controlled laboratory conditions, including a 13:11 light: dark cycle, constant temperature at approximately 25°C, and relative humidity between 60-80%. Each spider was held in its own opaque, cylindrical plastic "deli dish" container (10.5 cm diameter; 6 cm height) covered with a clear lid. Water was available *ad libitum* through a dental wick immersed in a water reservoir located below each spider's container. After being separated from their mothers, spiders received a diet of springtails (Collembola [mixed species]) and hatchling crickets (*Acheta domesticus*) once a week. As the spiders' size increased, they were fed early instar crickets and/or houseflies (*Musca domestica)* twice weekly. Spiders were thus naïve toward other prey types. Sexually mature adult female *Schizocosa ocreata* were used in the learning and predation trials.

The prey

Cultures of *Oncopeltus fasciatus*, the large milkweed bug, were reared in glass aquaria (50L x 26W x 29H cm) under the ambient controlled conditions described above. Water was available *ad libitum* through a dental wick immersed in water held in a 2 oz plastic container located within the insects' rearing containers. Four insect cultures were reared separately and fed two different food types. Two cultures consisted of large milkweed bugs fed a monotypic diet of milkweed seeds (*Asclepias* sp.). From the seeds, the insects sequester toxic cardenolides (cardiac glycosides) within their bodies and secrete them as a defense (Duffey 1970; Scudder & Duffey 1972; Scudder et al. 1986). Milkweed bugs in the remaining two cultures were fed a diet of sunflower seeds (*Helianthus annuus*) and were consequently free of toxic cardenolides (Scudder & Duffey 1972). These rearing methods and portions of the experimental designs were based upon those used by Berenbaum & Miliczky (1984), Paradise & Stamp (1991), and Bowdish & Bultman (1993). Nymphs (3rd –5th instar) from both feeding treatments were used as potential prey in all experiments.

Objective 1: To determine the capability for aversion learning in *S. ocreata.*

Like most wolf spiders, *S. ocreata* are generalist predators. In natural conditions, these wandering spiders potentially encounter a wide variety of prey. Novelty of prey type may influence a predator's decision to pursue or avoid prey (Coppinger 1970). Behavioral changes in prey capture and handling techniques may occur when spiders repeatedly experience prey of various types, depending on prey defenses, morphology, and size (Givens 1978; Li et al. 1996). The ability to learn to avoid well-defended or potentially harmful species could benefit the predator by reducing the handling time and energy expended on pursuit or processing of unprofitable prey.

If *S. ocreata* wolf spiders are capable of learned aversion, it would be predicted that after aversive experience with toxic milkweed bugs, spiders would be more likely to recognize, reject and/or avoid *O. fasciatus*. I tested two null hypotheses (H_{o1}: pattern of predatory behavior is independent of prey type; H_{o2}: pattern of predatory behavior is independent of predator experience with prey) and the alternative hypotheses (H_{a1}: pattern of predatory behavior is not independent of prey type; H_{a2}: pattern of predatory behavior is not independent of predator experience with prey). This was accomplished by recording and analyzing the initial and subsequent sequences of predatory behavior exhibited by naïve and experienced *S. ocreata* when presented with prey of differing type and palatability (toxic [cardenolide-containing] *O. fasciatus*, cardenolide-free *O. fasciatus*, and non-toxic *A. domesticus*).

Obj. 1 Methods:

Experiment 1.) Influence of prey type

Naïve spiders, without previous knowledge of the encountered prey, may still selectively pursue or avoid prey items based on certain prey characteristics or neophobic (fear of novel) factors. Two sets of experimental studies examined spider responses to different prey types and the role of experience in predatory behavior.

Experiment 1a.) This experiment investigated initial spider behavior toward prey items of various morphology, size, and palatability. Spiders were not fed for two weeks prior to the trial day. Individual naïve spiders were placed in a small (9cm diameter, 1.5cm high), clear, plastic petri dish and immediately enclosed within the chamber. Spiders were allowed two minutes to explore and familiarize themselves with the test arena. The floor of the test arena was covered with a clean piece of white filter paper that was replaced following each trial. The testing arenas were cleaned with alcohol between trials.

Spiders were assigned at random to one of three experimental feeding treatments: (1.) one group was fed a toxic (milkweed fed) *O. fasciatus* (N=27); (2.) another was given a cardenolide-free (sunflower fed) *O. fasciatus* (N=30); and (3.) the remaining group was given a cricket (N=40). Caution was taken to handle the insects as little as possible before introduction into the testing arena. During each five-minute trial, behaviors were videotaped using two RCA VHS video camcorders (model # CC4352 and CC4392): one mounted above, and one at the arena level. Differences in behavior due to prey type were noted and further analyzed in subsequent review of videotapes.

Trials were scored either as positive for initial attack and touch (pursuit) responses or negatively for initial avoid/ignore behaviors. Spider behaviors immediately following the initial decision to pursue the prey were noted as well. Behaviors that were scored when analyzing the videotaped trials included: 1. attack/feed—orienting to, approaching, lunging at, touching and biting the prey item and continuing to hold prey in mouthparts in order to feed upon it, often resulting in serious injury to or death of prey; 2. attack/reject—orienting to, approaching, lunging, touching and biting the prey item, then pushing it away from the mouthparts with the forelegs and often moving away from the prey (the prey usually survive); 3. touch—spider approaches prey item and places its forelegs and /or pedipalps in contact with the prey as in the attack behavior sequence, however no biting of the prey occurs (prey always survive); 4. avoid/ignore—spider maneuvers in order to limit contact with prey item, performs defensive behaviors, or makes no response, behaviors usually result from contact that is a consequence of prey activity (prey always survive). Initial and subsequent behaviors were analyzed using Chi-square contingency tests of independence with Bonferroni corrections for multiple comparisons, or McNemar's test of significant changes (for repeated measures) with prey type as a factor.

Experiment 1b.) In an effort to eliminate the potential influence of neophobia (hesitancy to attack and/or consume novel prey) upon the attack responses of wolf spiders, *S. ocreata* (N=20) were fed cardenolide-free *O. fasciatus* milkweed bugs, in addition to their control diet of *A. domesticus* crickets. Upon completing their sixth molt, spiders were alternatively given palatable, sunflower-fed *O. fasciatus,* or crickets during feedings that occurred twice weekly. Prey items remained in the spiders' home container until fed upon. Following their final molt into maturity and after being deprived of food for four days, female wolf spiders were presented with a toxic cardenolide-containing milkweed bug while enclosed in the aforementioned testing arena. Their behaviors were observed for five minutes. Toxic milkweed bugs were repeatedly offered as prey to spiders in successive trials (each separated by 15 min rest in home container) until the spider exhibited only non-predatory behaviors in two consecutive trials. Learned aversion was evidenced by avoidance of a cardenolide-free milkweed bug presented in a subsequent trial. To test for hunger level, spiders were presented with a cricket following a further 15 min resting period.

Initial responses were scored as positive (for attack behaviors) or negative (for touching or avoidance behaviors). Subsequent trial actions were observed and scored as: attack/feed, attack/reject, touch or avoid/ignore (as above). Initial and subsequent behaviors were compared to those of the group of naïve spiders (N=27) from the previous experiment using separate contingency analyses. The mean number of exposures necessary to evoke an avoidance response toward toxic milkweed bugs was also observed. Using a two-sample t-test, this mean was then compared to the average number of experiences required by completely naïve spiders (N=64) to attain aversion to toxic *O. fasciatus*.

Experiment 2.) Influence of spider experience

Repeated exposure to a particular prey item may result in behavioral changes if an association between a previous experience involving unpalatability and a particular prey item is formed. In these two experiments, spiders were presented with either a milkweed-fed [Experiment 2a] or sunflower-fed (cardenolide-free) [Experiment 2b] *O. fasciatus* following an initial experience with a toxic (cardenolide-containing) milkweed bug.

Experiment 2a.) This experiment was conducted to examine if experienced spiders respond to toxic *O. fasciatus* differently than naïve predators--which may indicate recollection of the previous encounter. After being deprived of food for four days, naïve spiders (N=63) were placed in the testing arena (as described above) and allowed two minutes to explore. They were then presented with a toxic cardenolide-containing milkweed bug and their behaviors were observed for five minutes. Spiders were then allowed to return to their own housing container to rest for at least 15 minutes. Because spiders had exposure to a toxic *O. fasciatus*, they were considered to be experienced with this prey item. The same spiders were then transferred back into the testing arena for a subsequent trial. Toxic bugs were offered in a second five-minute trial. Differences in behavior due to predator experience with toxic milkweed bugs (naïve vs. experienced) were noted and further analyzed by reviewing the trials recorded upon mini-digital videotapes. Trial behavior was scored as: attack/feed, attack/reject, touch, or avoid/ignore. A McNemar's test was used to determine if there was significant difference in the overall trial behavior of *S. ocreata* (naïve) during their first exposure to toxic *O. fasciatus* when compared to their second encounter as experienced spiders. This test is appropriate to use for paired, nominal and dichotomous data (Zar 1999).

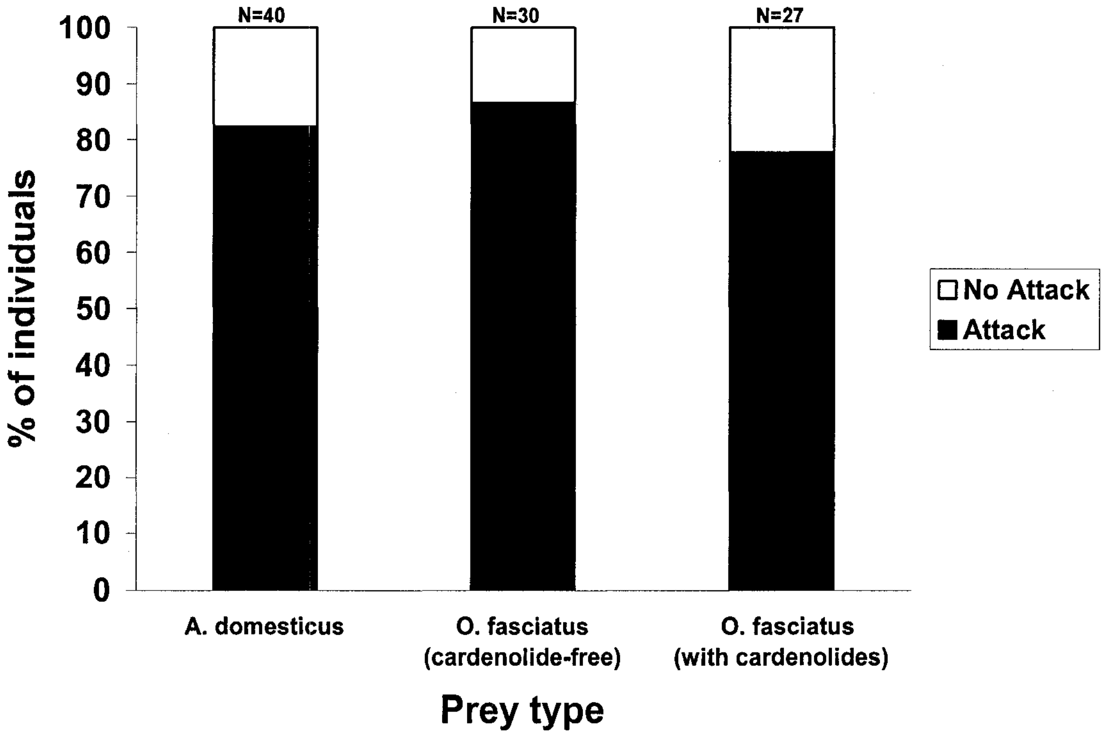
Experiment 2b.) Following exposure to toxic *O. fasciatus*, spiders may also discriminate against all types of prey. Alternatively, spiders could selectively avoid only certain prey types (ones that resemble toxic prey items) while continuing to feed on non-toxic prey. In this group of trials, each naïve spider (N=12) was given a toxic milkweed bug, followed by a cardenolide-free milkweed bug, and then a cricket in sequential five-minute trials to examine whether spiders can distinguish different prey types and consequently prey upon them in different proportions. Spiders were kept from food for four days prior to the experiment and tested in aforementioned testing arenas. All trials were separated by a fifteen-minute rest period. The overall trial behaviors that were scored (as above) were: attack/feed, attack/reject, touch, or avoid/ignore responses. To examine if the relative frequencies of attacking behaviors were the same for each spider for all three prey types, a Cochran's Q repeated measures test, followed by a test for multiple comparisons of repeated-measures data, was used.

Obj. 1 Results:

Experiment 1.) Influence of prey type

Experiment 1a.) Naïve spiders initially responded to all prey types (toxic milkweed bugs, cardenolide-free milkweed bugs and non-toxic crickets) with equal frequency (X^2=0.778, df=2, p=0.678) (Figure 1.1). Most spiders attacked or touched, rather than avoided, the three prey types. The majority of spiders pursued cardenolide-free milkweed bugs (N=26/30) and crickets (N=33/40). When presented with toxic *O. fasciatus*, most of the spiders (N=21/27) showed predatory behaviors.

Figure 1.1: Initial attack responses of naïve *Schizocosa ocreata* when presented with *Acheta domesticus,* cardenolide-free (sunflower fed) *Oncopeltus fasciatus* or cardenolide-containing (milkweed-fed) *O. fasciatus* as prey.

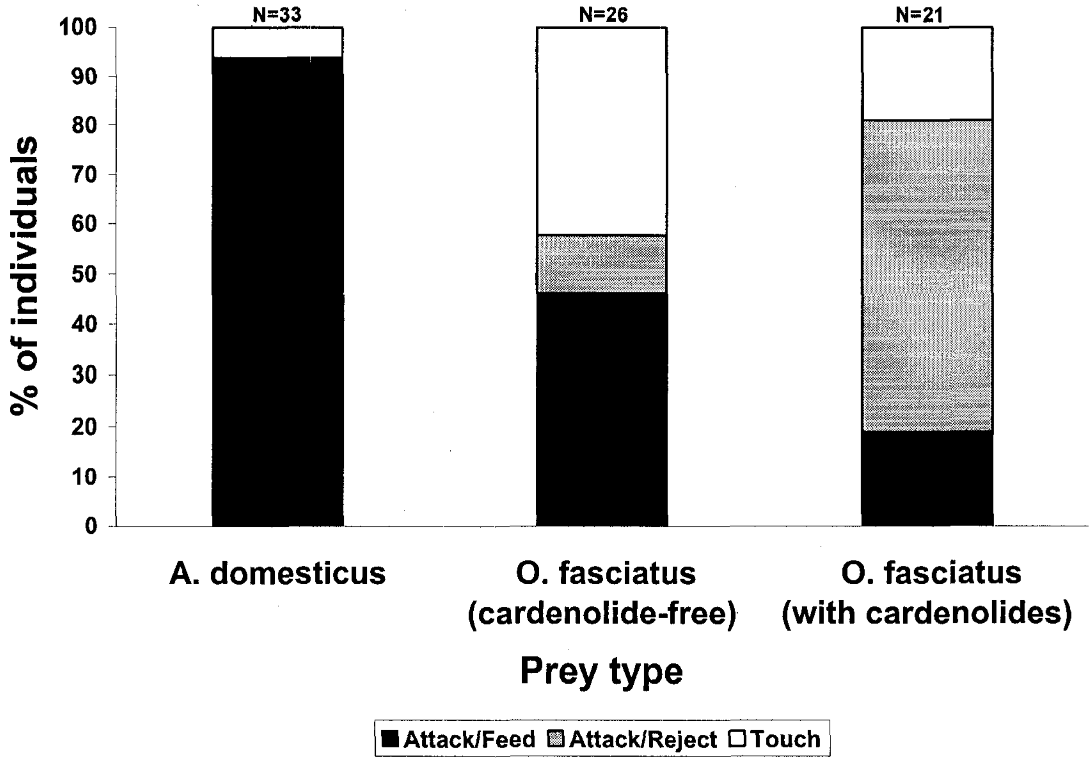

N=40
N=30
N=27
100
90
80
70
60
50
40
30
20
10
0
% of individuals
A. domesticus
O. fasciatus (cardenolide-free)
O. fasciatus (with cardenolides)
Prey type
□ No Attack
■ Attack

Spiders that initially avoided the prey items were eliminated from further analysis. Spider behaviors immediately following the initial pursuit of prey included: feeding or rejecting following an attack, touching, or avoiding the prey item. Feeding was most often observed in trials where spiders were presented with palatable crickets (94%, N=33) and cardenolide-free *O. fasciatus* (46.2%, N=26). Only 19% (N=21) of spiders fed on toxic milkweed bugs. Sixty-two percent of toxic *O. fasciatus* were rejected after being attacked (N=21). A contingency test revealed that behavior was not independent of prey type (X^2=48.317, df =4, p<0.0003 with Bonferroni correction) (Figure 1.2). There was also a significant difference between spiders presented with the two types of milkweed bugs (cardenolide- containing and cardenolide-free) (X^2=13.133, df=2, p=0.0014).

Experiment 1b.) Initially, 70% of the spiders that had previously consumed cardenolide-free *O. fasciatus* attacked toxic milkweed bugs (N=14/20). This did not significantly differ from naïve spiders (X^2=0.255, df =1, p=0.6136). Experienced spiders' subsequent behaviors were also similar to naïve spiders during their first exposure to toxic prey (X^2=3.338, df =3, p=0.3425).

Naïve spiders (N=64) took longer to attain a learned aversion to toxic *O. fasciatus* (two-sample t-test, unequal variances; t=2.08, p=0.0424). The mean number of exposures required by experienced spiders to induce an avoidance response toward toxic milkweed bugs (1.45± 0.256) were significantly fewer than the mean number of experiences required (2.19± 0.246) by naïve spiders. When presented with a cricket following the

Figure 1.2: Behaviors (subsequent to initial attack) of naïve *Schizocosa ocreata* when presented with three different prey items.


N=33
N=26
N=21
100
90
80
70
60
50
40
30
20
10
0
% of individuals
A. domesticus
O. fasciatus (cardenolide-free)
O. fasciatus (with cardenolides)
Prey type
■ Attack/Feed ▨ Attack/Reject ☐ Touch

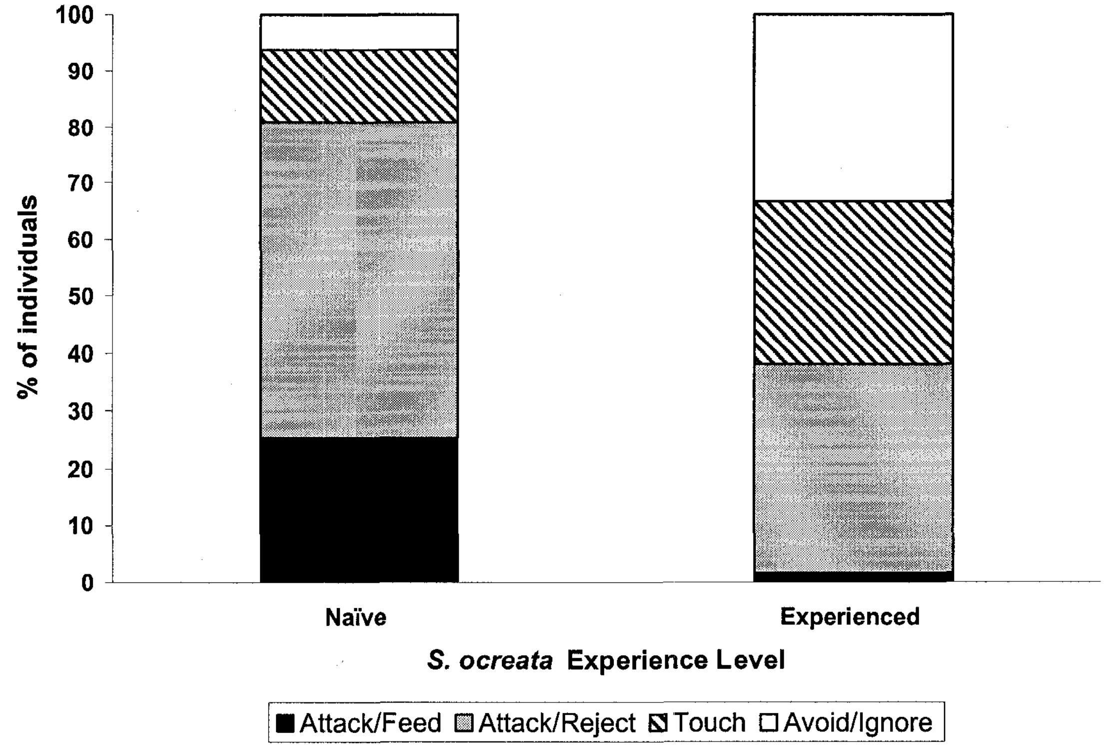
acquisition period of the learned aversion, the majority were preyed upon (95%), indicating hunger and the ability to feed on palatable prey following aversive experience.

Experiment 2.) Influence of spider experience

Experiment 2a.) During the naïve spiders' first exposure to toxic cardenolide containing milkweed bugs, 25.4% and 55.56% showed attack/feed or attack/reject behaviors, respectively (N=63). The remaining spiders touched (12.7%) or avoided (6.34%) the insects during the initial trials. The same spiders, considered to be experienced during the subsequent trial, showed a significant difference in their behaviors toward toxic milkweed bugs (McNemar's $X^2 = 36.41$, df = 6, $p < 0.001$) (Figure 1.3). Overall, fewer milkweed bugs were attacked by the spiders throughout their second encounter with the insects; only 1.59% of *S. ocreata* exhibited attack/feed behaviors. Similar to the first exposure trials, the most common behavior observed was attack/reject (36.51%). Occurrences of touching and avoidance behavior increased to 28.57% and 33.33%, respectively. In total more milkweed bugs were avoided or only touched (61.9%) than were attacked and fed upon or attacked and rejected (38.1%) during the spiders' second exposure.

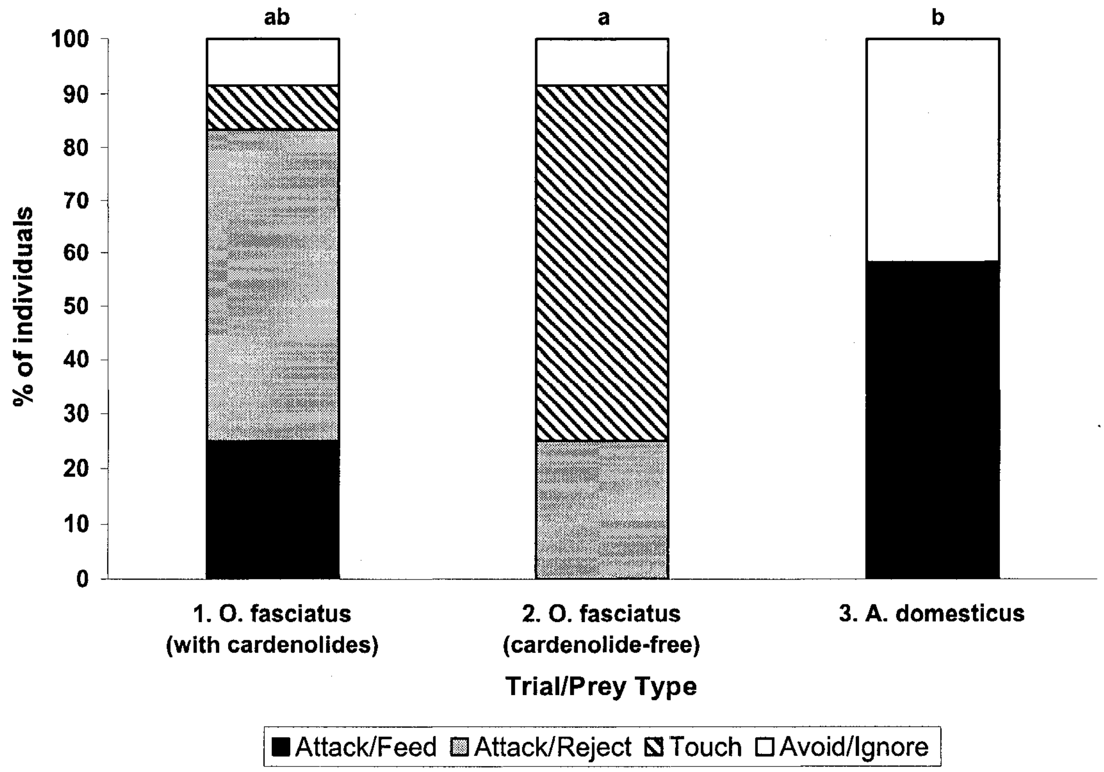
Experiment 2b.) When naïve spiders (N=12) were presented with toxic *O. fasciatus*, 58.3% of the insects were attacked, but rejected, and survived. Twenty-five percent of the toxic insects were attacked and fed upon. Upon being presented with a cardenolide-free milkweed bug in a subsequent trial, the majority of the experienced spiders (8/12) exhibited touching behaviors and only one-third (4/12) displayed

Figure 1.3: Behavioral responses of *S. ocreata* (N=63) when presented with a toxic cardenolide-containing *O. fasciatus* in two consecutive trials (separated by a fifteen minute rest period). The naïve column designates spider response during the first trial with toxic milkweed bugs, while the experienced column indicates behaviors that occurred in spiders' second exposure to the toxic prey type.


100
90
80
70
60
50
40
30
20
10
0
% of individuals
Naïve
Experienced
S. ocreata Experience Level
■ Attack/Feed ▨ Attack/Reject ▧ Touch ☐ Avoid/Ignore

attack/feed or attack/reject behaviors. The majority of spiders (58.33%) consumed crickets when they were presented in the third trial (Figure 1.4). The proportion of attacking (combined attack/feed and attack/reject) behaviors exhibited by the spiders was not the same for all three prey types when presented in sequence (Cochran's $X^2 = 7.4$, $df=2$, $p<0.025$). Further testing of pairwise comparisons (Marascuilo & McSweeney 1967) for the combined attacking behaviors based on prey type indicated significant difference between toxic *O. fasciatus* and cardenolide-free *O. fasciatus* ($S=2.71$, $p<0.05$). The proportion of attack/feed behavior only was also not the same for all three prey types presented in sequence (Cochran's $X^2 = 14.80$, $df=2$, $p<0.001$). Significant difference between the feeding behaviors observed when spiders were presented cardenolide-free *O. fasciatus* and crickets ($S=3.24$, $p<0.05$) was detected using post hoc analysis of pairwise comparisons (Marascuilo & McSweeney 1967).

Figure 1.4: Behavioral response of *S. ocreata* (N=12) when presented with a toxic cardenolide containing *O. fasciatus*, a cardenolide-free *O. fasciatus*, and an *A. domesticus* in sequential trials (each separated by a fifteen minute rest period). Shared letters indicate no significant difference in feeding behavior for prey type (nonparametric post hoc comparisons, $p<0.05$).


ab
a
b
100
90
80
70
60
50
40
30
20
10
0
% of individuals
1. O. fasciatus (with cardenolides)
2. O. fasciatus (cardenolide-free)
3. A. domesticus
Trial/Prey Type
■ Attack/Feed ▨ Attack/Reject ▧ Touch ☐ Avoid/Ignore

Obj. 1: Discussion

The wolf spider, *Schizocosa ocreata*, (Araneae: Lycosidae) was more likely to recognize, reject and avoid toxic milkweed bugs *(O. fasciatus)* after aversive experience, indicating that *S. ocreata* are capable of learned aversion. Although the milkweed bugs, regardless of their diet, may have discharged allomones from their scent glands (Games & Staddon 1973; Blum 1992), these substances did not seem to be effective in immediately deterring *S. ocreata.* No neophobic behavioral responses to milkweed bug prey were observed. This indicated that a specific inherited bias for *O. fasciatus* avoidance behavior does not occur in this wolf spider species. Furthermore, spiders that were naïve to *O. fasciatus* initially responded and subsequently behaved in the same manner as spiders that had been given cardenolide-free milkweed bugs as part of their diet prior to adulthood, which reinforces the idea that neophobic behaviors did not impact the experiments. Interestingly, spiders that had previous experience with cardenolide-free *O. fasciatus* required significantly fewer trials to acquire aversion to the toxic cardenolide-containing milkweed bugs. Due to the fact that these spiders were familiar with the cardenolide-free *O. fasciatus* and their physical and chemical attributes, the spiders may have previously formed a recognition image of the *O. fasciatus* and were therefore able to change their association of this prey type with acceptable palatability to unacceptable palatability faster than completely naïve spiders. These spiders may have also been more readily able to detect the presence of novel toxic cardenolides when presented with insects reared on milkweed seeds.

Initial predatory behaviors were found to be random with respect to prey type, but subsequent actions were not. Crickets were consumed by almost all of the spiders, likely due to the fact that they were a palatable and familiar prey item. Cardenolide-free *O. fasciatus* were fed upon as well, although at a lower frequency (46%). Spiders likely came into contact with unfamiliar or slightly distasteful cuticular substances or odors, which may have reduced their desire to feed. Cardenolide-containing *O. fasciatus* elicited spiders' rejection and avoidance behaviors, suggesting an aversive experience with the toxic prey. The cardenolide chemicals are considered to be cardiac glycosides and have emetic and toxic effects upon vertebrate predators (Brower & Brower 1964). Although the effects of the cardenolides on invertebrates are not thoroughly understood, the spiders in this study appeared to have adverse reactions to the presence of the chemicals as they would repeatedly groom and wipe their chelicerae following an attack upon toxic *O. fasciatus*. The high rate of prey rejection following an initial attack can be attributed to the effects of prey and odor unfamiliarity, combined with, most importantly, the presence of toxins.

Predatory behavior of experienced spiders was influenced by prey type. *S. ocreata* that were repeatedly presented with toxic cardenolide-containing *O. fasciatus* significantly decreased attack behaviors after their second exposure to the insect. Spiders' responses to toxic prey during the second exposure suggest recognition and learned aversion behavior. The change in behavior was not likely due to predator illness or inability to feed (as a consequence of initial attack) because the first and second encounters were separated by a fifteen-minute rest period. This allowed adequate time for the spiders to recover from any ingested toxic chemicals, as most spiders (in other experiments) were capable of consuming palatable crickets within five minutes following an attack upon cardenolide-containing *O. fasciatus*. More touching behaviors were also observed during the second exposure, suggesting that spiders might have used tactile or chemical cues as a means of identifying potentially harmful prey.

When naive wolf spiders were presented with a cardenolide-containing *O. fasciatus,* followed by a cardenolide-free *O. fasciatus,* and finally an *A. domesticus*, attack and feeding behaviors differed based upon prey type. In the first trial with toxic *O. fasciatus,* 25% of the naïve spiders fed, while the majority attacked and rejected the prey. This can be attributed to their inexperience with toxic prey and the predators' hunger level, as in the second trial the majority of the cardenolide-free *O. fasciatus* were touched, but none were fed upon. Attacks (attack/feed and attack/reject behaviors) upon the two milkweed bug types were significantly different, which indicated that after experience with the noxious cardenolides, spiders were capable of recognition and avoidance of the milkweed bug itself (possibly through the use of tactile or chemical cues), irrespective of the presence of cardenolides. Feeding (attack/feed only) behaviors were observed to be significantly different when cardenolide-free *O. fasciatus* and palatable crickets were compared. Feeding behaviors when spiders were presented with crickets returned to levels higher than those observed in the initial toxic *O. fasciatus* trial. This was likely to be largely a function of prey familiarity and palatability. This indicated that wolf spiders were capable of recognizing and selectively avoiding prey types after initial aversive experience-- while continuing to consume those that were non-toxic.

Objective 2: To determine the possible duration of learned prey aversion.

The avoidance of potentially noxious prey is likely to occur when a predator associates a previous experience involving unpalatability with a particular prey item. Although the ability to recognize and avoid well-defended or potentially harmful prey could benefit the predator, this learned aversion might not be permanent. Some studies show that learned aversions are temporary in invertebrates, lasting only a few hours or days (Toft 1997; Toft & Wise 1999). Reduced avoidance of potentially noxious prey may be a result of increased hunger or decreased persistence of memory concerning adverse encounters. There may be advantages to the waning of an aversion to potentially harmful prey, as it may be to the predator's benefit to re-sample prey in an unpredictable environment (Bernays 1993; Mangel 1993). I examined the duration of a spider's learned aversion to toxic prey with regard to the impact memory and hunger may have on predatory behavior.

I tested the null hypothesis that when presented with a cardenolide-containing *O. fasciatus,* predatory behaviors of spiders that had previously acquired the learned aversion to toxic milkweed bugs will show no change over time. I also tested three alternative hypotheses: (H_{a1}) predatory behaviors toward toxic cardenolide-containing *O. fasciatus* change with time from last exposure to unpalatable prey owing to lack of reinforcement, i.e., spiders' acceptance of toxic prey will increase over time with decreased memory of prey's noxiousness; (H_{a2}) predatory behaviors toward toxic *O. fasciatus* change with time since last exposure to palatable prey owing to hunger, i.e., hesitancy to accept toxic prey will decrease with increased predator hunger level; and (H_{a3}) spider's acceptance of *O. fasciatus* will decrease as a consequence of reinforcement, i.e., hesitancy to accept toxic prey will decrease with repeated exposure to toxic prey. I tested *S. ocreata* with toxic *O. fasciatus* after a period of one hour and/or several days following initial aversion training with a variety of re-exposure (to cardenolide-containing milkweed bugs) and feeding (of *A. domesticus*) schedules. I predicted that learned aversion would increase with repeated exposure to toxic *O. fasciatus* and decrease with increasing hunger and/or time without exposure to toxic prey.

Obj. 2 Methods:

These experiments examined the length of time learned prey aversion persists when schedules of re-exposure to toxic prey and hunger levels were varied. In all of the following experiments, spiders were subjected to a four-day period of starvation then trained to avoid *O. fasciatus*. In order to train the spiders, individuals were placed in a small (9cm diameter; 1.5cm high), clear, plastic petri dish and immediately enclosed within the chamber. The floor of the test arena was covered with a clean piece of white filter paper that was replaced following each trial. The arena was also cleaned with alcohol after each trial to remove any silk and/or chemical secretions. Spiders were allowed 2 min to explore the arena. The size of the arena allowed the spider to adequately maneuver while preventing the prey's escape or complete avoidance of the spider predator. Toxic milkweed bugs were offered to spiders in successive trials (separated by 15 min rest in the home container) until the spider no longer exhibited predatory behaviors in two consecutive trials. To test for learned aversion, a cardenolide-free milkweed bug was then presented in an additional trial. Learned avoidance was confirmed by the spiders' refusal to attack the cardenolide-free milkweed bug prey. In Experiments 1 and 3 below, spiders were presented with a cricket following an additional 15 min resting period to test hunger level. Spiders that consistently avoided *O. fasciatus*, were considered to be trained and to have acquired learned aversion of milkweed bugs. Spiders usually acquired aversion in 1-4 trials and refused cardenolide-free milkweed bugs thereafter, but still accepted crickets, indicating motivation to feed. Spiders that had never been previously exposed to *O. fasciatus* or any other type of potentially toxic prey were classified as naïve. Different naïve individuals were used for each of the initial learning trials, and randomly selected (trained) individuals were used in later testing trials.

Experiment 1.) Influence of increasing hunger and lack of re-exposure

Increased hunger and time without re-exposure to potentially harmful prey may result in greater willingness of a predator to attack. Trained *S. ocreata* spiders (N=60) were randomly assigned to one of six testing groups. Between the training day and testing trial day, spiders received no palatable or toxic prey, so therefore hunger level and time without re-exposure increased. The first group (N=10) was re-exposed to a toxic milkweed bug one hour following training in a five-minute testing trial. Other separate spider groups (N=10 per day) were tested at 24 hours, on 2-4 days or 10 days post-training. All spiders were given a cricket following the testing trial to assess hunger. Attack latency and number of bites was obtained from each testing trial. Data from these interactions was analyzed for differences in persistence of avoidance by group. Attack latency (seconds) and number of bites were used as dependent variables in one-way

ANOVA tests of the null hypothesis of no difference as a function of testing day. A Tukey post hoc test was used for each of the ANOVAs to test for significant differences between groups. It was predicted that as time without re-exposure to milkweed bugs and hunger increased, spiders would attack earlier in the trial and exhibit more biting.

Experiment 2.) Influence of daily exposure and hunger

In this experiment, hunger increased daily, as naïve spiders were starved three days prior to their first presentation with a milkweed bug and were then kept from palatable prey (*A. domesticus*) throughout the experiment. To examine the impact of daily exposure on learned aversion behaviors, naïve spiders (N=10) were presented with a toxic milkweed bug during a five-minute trial on four sequential days. Each day, toxic insects were presented (multiple trials on the same individual were separated by a fifteen-minute rest period) until spiders exhibited avoidance behaviors. Each day's first trial interactions were scored and assigned to one of several categories, depending on overall trial outcome (attack/feed, attack/reject, touch, or avoid, as previously described) and analyzed using a Cochran's Q test for independence of trial behavior and day of trial. The number of trials required for reinforcement of aversion behaviors (square-root transformed due to frequent counts of zero) over four days of trials was examined using a repeated-measures ANOVA. Latency to attack (log transformed) as a function of day was also analyzed using a repeated-measures ANOVA. The null hypothesis that the mean number of bites during each day's first trial is the same on all four testing days was also assessed using a repeated-measures ANOVA. Nonparametric and Tukey post hoc tests were used to compare differences between days.

Experiment 3.) Influence of daily exposure with control for increasing hunger

This experiment was conducted to examine the persistence of learned aversion when hunger was controlled and spiders were exposed to toxic prey daily. Spiders (N=10) were presented with one toxic *O. fasciatus* one hour following training on Day 0, and on Days 1-4. Trials were five minutes in length and took place in a standard testing arena. Behavior exhibited (attack/feed, attack/reject, touch, or avoid) with the toxic *O. fasciatus* and the number of bites in each day's trial was observed. Every day following testing trials, spiders were given a cricket to control for hunger. The cricket remained within the spider's home container, allowing the spider time--up until the next day's testing trial--to feed upon the palatable prey if hungry. Videotapes of the spider/milkweed bug interactions were scored and data were analyzed, using a Cochran's Q test, for independence of the dependant variable of attack as a function of time. A repeated-measures ANOVA was used to test the hypothesis that the mean number of bites (square-root transformed) by spiders was the same at all five testing times. Mean attack latency data (log transformed) was examined for significant differences over days using a repeated-measures ANOVA. To compare differences between days when overall significance was detected, nonparametric and Tukey post hoc tests were used.

Obj. 2 Results:

Experiment 1.) Influence of increasing hunger and lack of re-exposure

In this experiment, the effects of increased hunger level and time without re-exposure to toxic prey were explored. Spiders in the first testing group had the shortest time (one hour) between aversion training and testing, while other groups were kept isolated for longer periods (one to ten days). Upon testing with a toxic milkweed bug, mean attack latencies were longest for the group tested one-hour after training (300 ± 0s), as these spiders did not attack prey in the five minute trial period. Spiders in the group tested ten days after training showed the shortest mean attack latency (41.7 ± 29.12s). Overall, attack latencies by group were not equal for the six groups tested (one-way ANOVA, $F_{5,60}$=7.065, p<0.0001). Only spiders tested one hour post-training had attack latencies significantly different from all other groups (Tukey post-hoc analysis). The remaining testing groups' mean attack latencies were not significantly different from one another (Figure 2.1).

When re-exposed to cardenolide-containing milkweed bugs in testing trials, the mean number of bites increased as time following training and hunger increased (One-way ANOVA, square-root transformed; $F_{5,60}$=4.542, p=0.0015) (Figure 2.2). Spiders tested one hour after training had the lowest mean number of bites (0.0 ± 0), and exhibited a significant difference from spiders re-exposed on Days 3 and 10 (Tukey post-hoc test).

Experiment 2.) Influence of daily exposure and hunger

Throughout this experiment, spiders (N=10) were subjected to increasing hunger, but received daily exposure to toxic milkweed bugs until exhibiting avoidance behaviors. The mean number of trials needed to induce avoidance behaviors decreased to zero by Day 4 (Figure 2.3). On this day, no trials were needed to bring about aversion behaviors as all spiders displayed avoidance during their first testing trial. The mean number of trials needed to develop aversion to a toxic milkweed bug was not significantly different for all four days (repeated-measures ANOVA, square- root transformed; $F_{3,36}$=2.1726, p=0.1082).

During the initial exposure, 50% of the spiders attacked the toxic *O. fasciatus* (5/10). After the first day, fewer milkweed bugs were attacked. On days 2 and 3, only 30% of the spiders attacked the toxic prey in the first testing trial. By the fourth day of testing, none of the spiders attacked toxic *O. fasciatus* (Figure 2.4). The null hypothesis that attack (combined attack/feed and attack/reject) behavior and day of trial (Days 1-4) are independent was rejected (X^2= 8.053, df = 3, p <0.05) based on a Cochran's Q test. Upon further pairwise comparisons of the data using the post-hoc procedure for data analyzed with a Cochran's Q test (Marascuilo & McSweeney 1967), significant difference in attacking behavior was detected between Day 1 and Day 4. Mean attack latency across the four testing days was not significantly different (repeated-measures ANOVA, log transformed; $F_{3,36}$=2.408, p=0.0831, power=0.55, CL=0.05, 0.997). However, the mean number of bites during each day's first trial decreased daily and diminished to zero on day 4. The mean number of bites over four testing days was significantly different (Repeated-measures ANOVA: $F_{3,36}$=4.0229, p=0.0144) and a Tukey post hoc test showed significant difference between testing Days 1 and 4 (α=0.05) (Figure 2.5).

Experiment 3.) Influence of daily exposure with control for increasing hunger

During this experiment, trained spiders (N=10) received daily exposure to a cardenolide-containing milkweed bug and then to a palatable *A. domesticus*. A Cochran's Q test showed that the proportion of milkweed bugs attacked by spiders did not significantly differ across all five testing times (X^2=8.0, df=4, 0.10>p>0.05).

Figure 2.1: Mean attack latencies (bars represent standard error) of trained *S. ocreata* (N=10 per testing time) when presented with a toxic cardenolide containing *O. fasciatus* at various times following training (* significant differences at $p<0.05$; Tukey post-hoc test). Value of 300 s. indicates no predatory behaviors throughout the five-minute trial period.

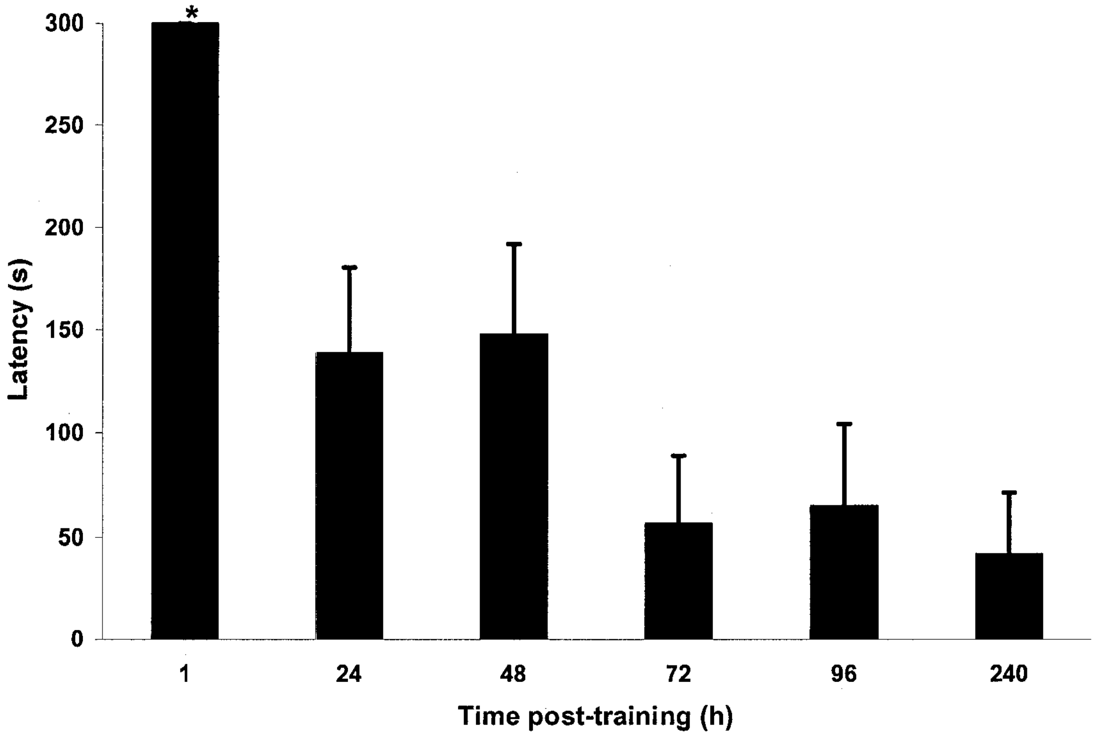

300
250
200
150
100
50
0
Latency (s)
*
1
24
48
72
96
240
Time post-training (h)

Figure 2.2: Mean number of bites (+ standard error) on toxic cardenolide containing *O. fasciatus* when trained *S. ocreata* (N=10 per testing time) were re-exposed at various times following training (shared letters indicate no significant difference ($p<0.05$) of testing time [Tukey post-hoc test]). Value of zero indicates no predatory behaviors throughout five-minute trial period.

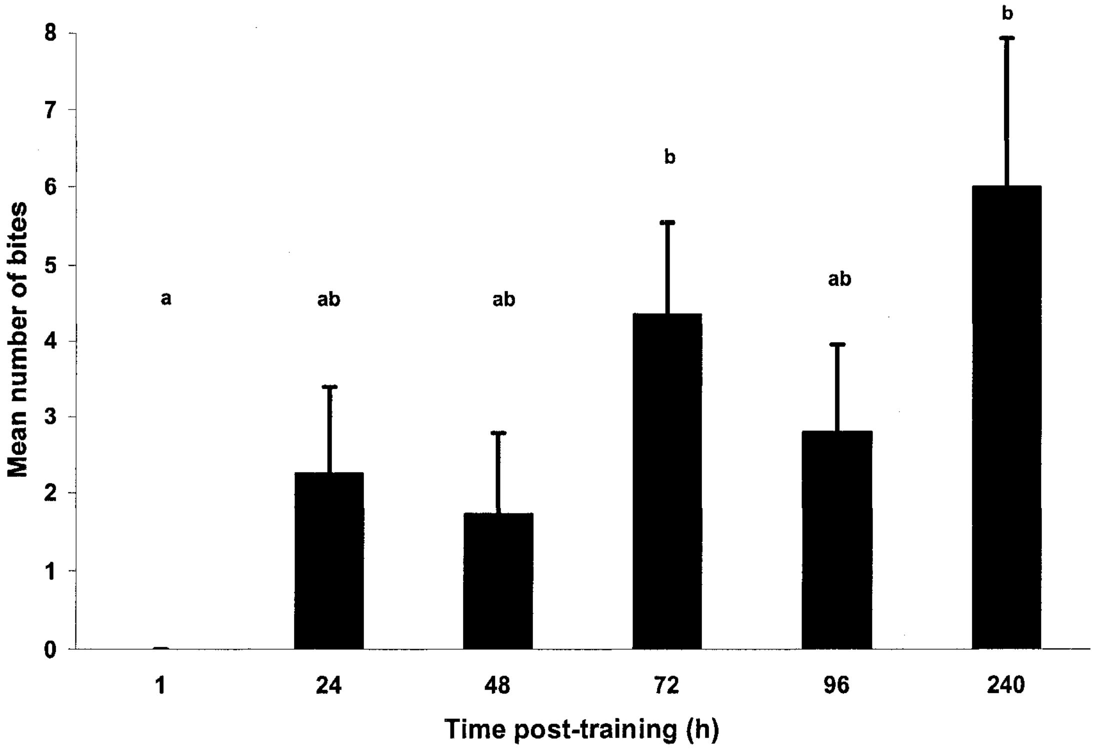

8
7
6
5
4
3
2
1
0
Mean number of bites
a
ab
ab
b
ab
b
1
24
48
72
96
240
Time post-training (h)

Figure 2.3: Mean number of trials (± standard error) required by *S. ocreata* (N=10) to acquire avoidance behavior over a period of four days with toxic cardenolide containing *O. fasciatus* (NS).

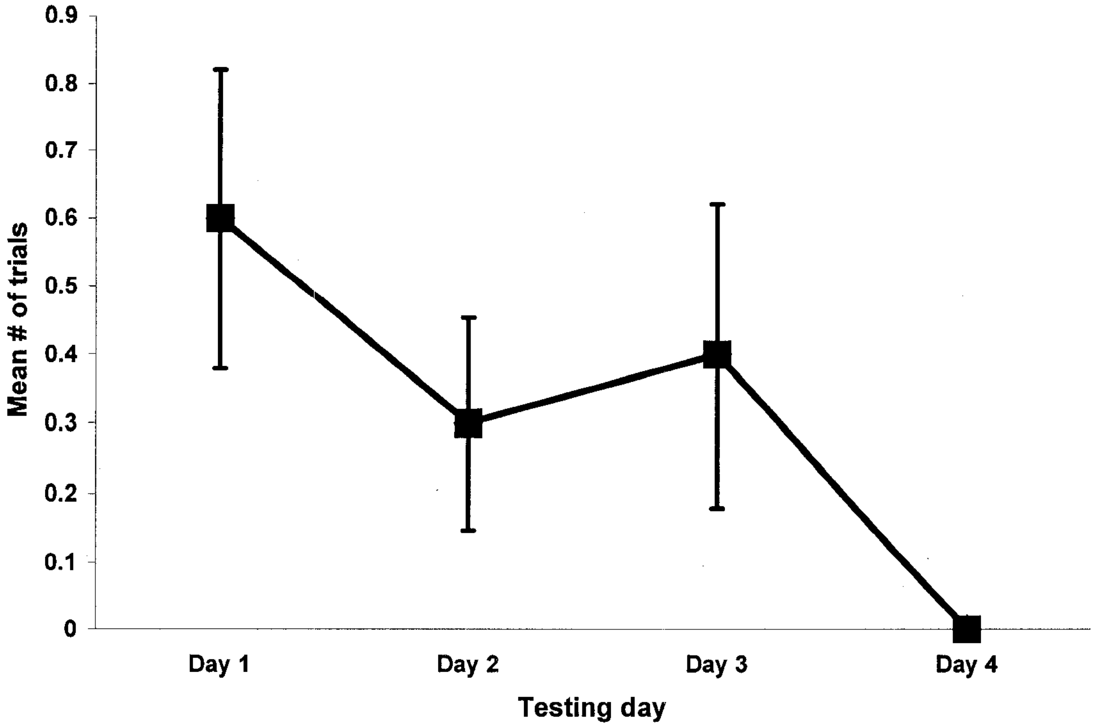

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mean # of trials
Day 1
Day 2
Day 3
Day 4
Testing day

Figure 2.4: Behaviors of *S. ocreata* (N=10) when exposed to toxic cardenolide containing *O. fasciatus* over a period of four days (shared letters indicate no significant difference in attack behavior for testing day [nonparametric post hoc comparisons, $p<0.05$]).

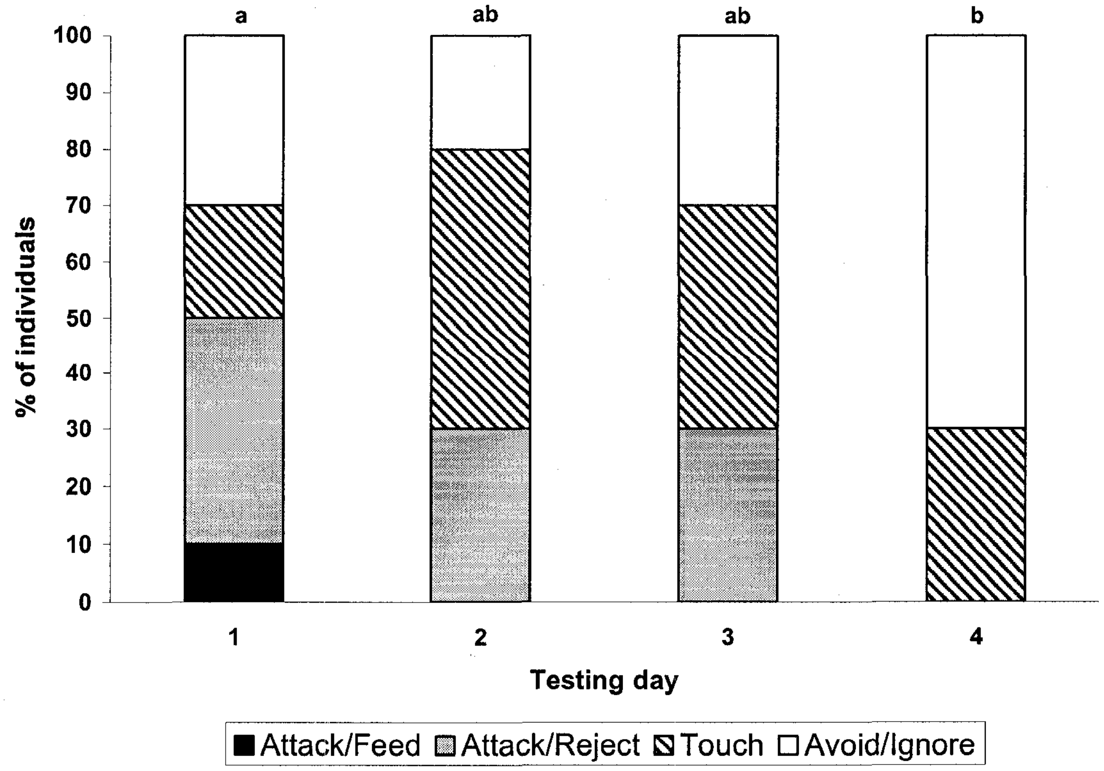
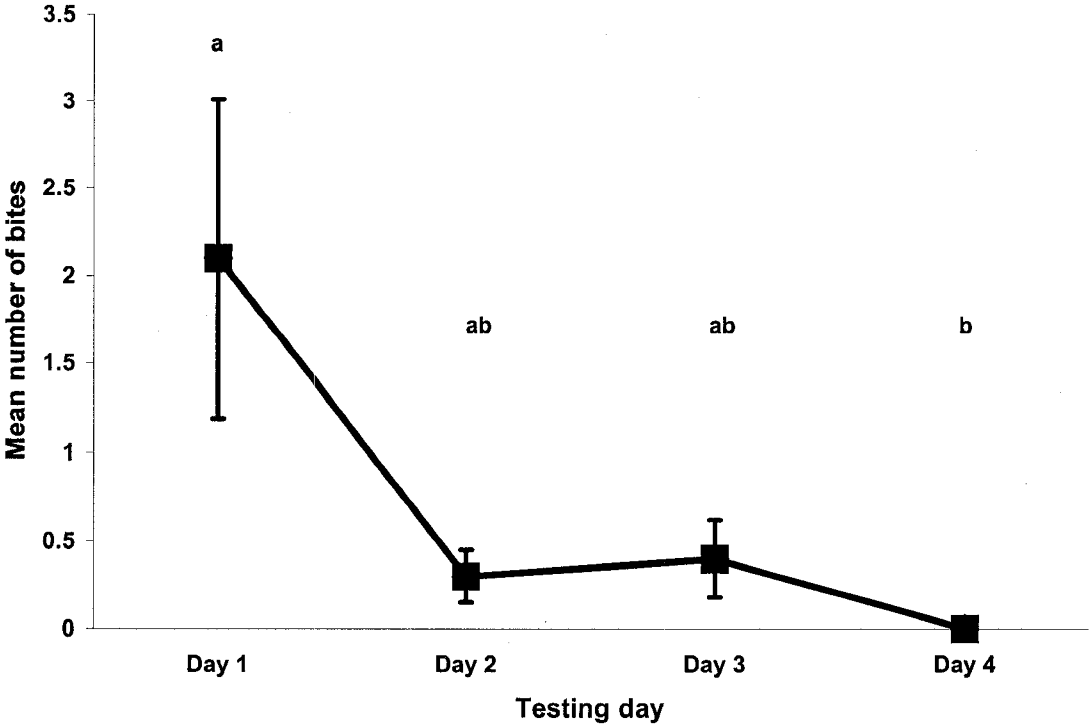

a
ab
ab
b
100
90
80
70
60
50
40
30
20
10
0
% of individuals
1
2
3
4
Testing day
■ Attack/Feed ▨ Attack/Reject ▧ Touch ☐ Avoid/Ignore

Figure 2.5: Mean number of bites (± standard error) on toxic cardenolide containing *O. fasciatus* when starved *S. ocreata* (N=10) were exposed daily to toxic prey over a four day period (shared letters indicate no significant difference in mean number of bites for testing day [Tukey post hoc comparison, $p<0.05$]).


3.5
3
2.5
2
1.5
1
0.5
0
Mean number of bites
a
ab
ab
b
Day 1
Day 2
Day 3
Day 4
Testing day

A single spider attacked the toxic *O. fasciatus* in trials one hour following training, while the others (9/10) demonstrated learned aversion behavior. Conversely, fifty-percent of the spiders displayed predatory behaviors on Days 1, 2, and 4. On Day 3, seventy-percent (7/10) of spiders attacked toxic milkweed bugs (Figure 2.6). A repeated-measures ANOVA with square-root transformed data revealed that the mean number of bites delivered to the toxic milkweed bugs by spiders as a function of day was significant ($F_{4,45}$=2.6394, p=0.046) (Figure 2.7). Only one bite occurred during the testing trial one-hour following training, but the mean number of bites increased in the subsequent testing trials. Mean number of bites one hour after training was significantly different to the mean number occurring on Day 3. Mean attack latencies were also found to be significantly different as a function of day with the longest latency occurring one-hour after training, while the shortest was on Day 3 (Repeated-measures ANOVA, log transformed; $F_{4,45}$=2.9982, p=0.0282) (Figure 2.8). Significant differences in attack latencies for tests occurring one hour and 72 hours after testing were indicated by a Tukey post hoc test (α=0.05).

Figure 2.6: Behavioral responses of trained *S. ocreata* (N=10) when repeatedly presented with toxic milkweed bugs (NS). Spiders were also given a cricket following training and each testing trial in an attempt to control for hunger.

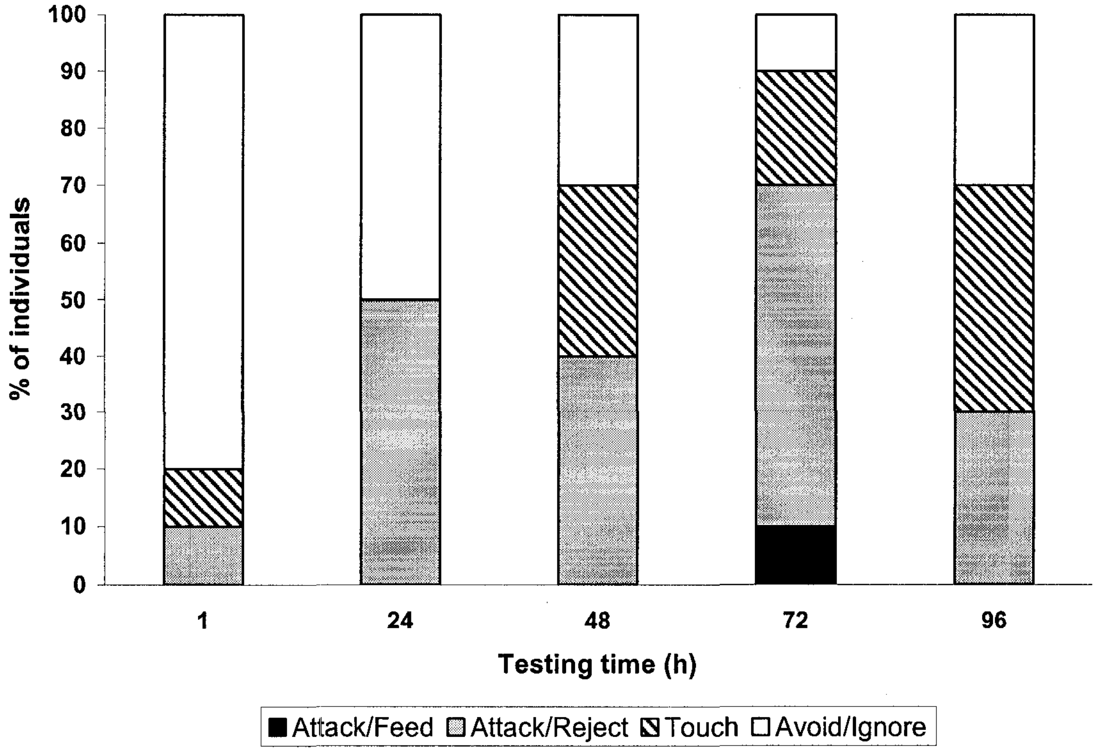

100
90
80
70
60
50
40
30
20
10
0
% of individuals
1
24
48
72
96
Testing time (h)
■ Attack/Feed ▨ Attack/Reject ▧ Touch ☐ Avoid/Ignore

Figure 2.7: Mean number of bites (± standard error) by trained *S. ocreata* (N=10) when presented with toxic milkweed bugs over a series of testing times (shared letters indicate no significant difference in mean number of bites for testing day [Tukey post hoc test (α=0.05)]). In this experiment hunger was controlled for by giving spiders a palatable cricket following training and each testing trial.

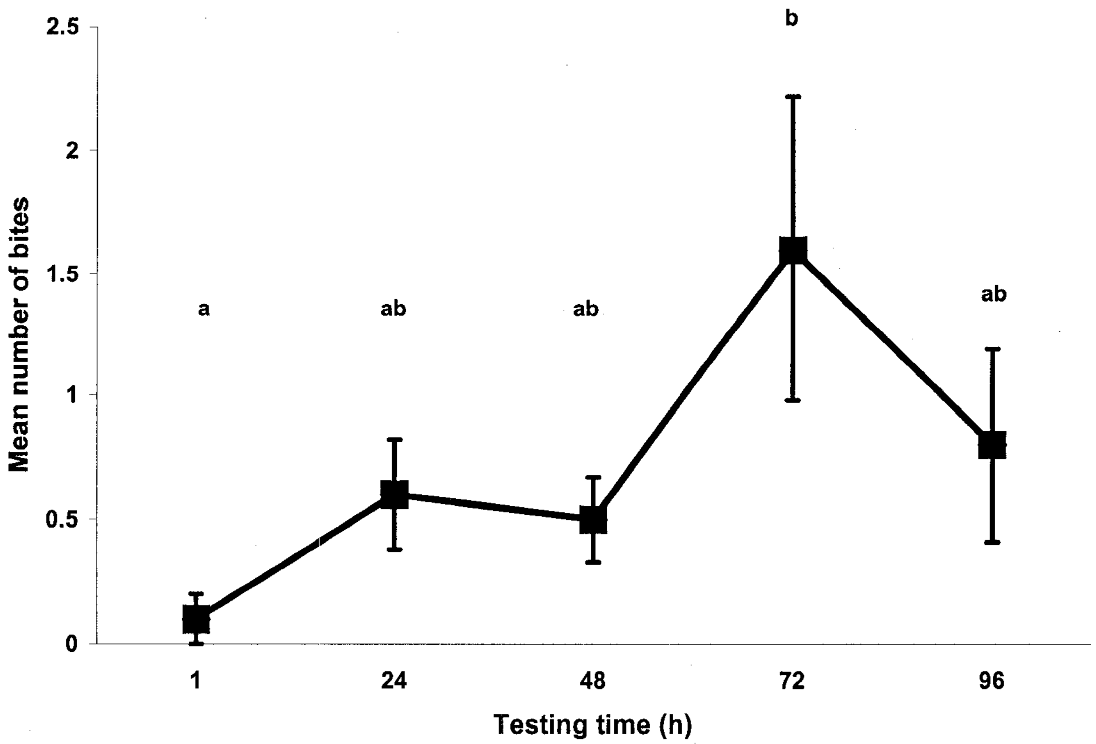
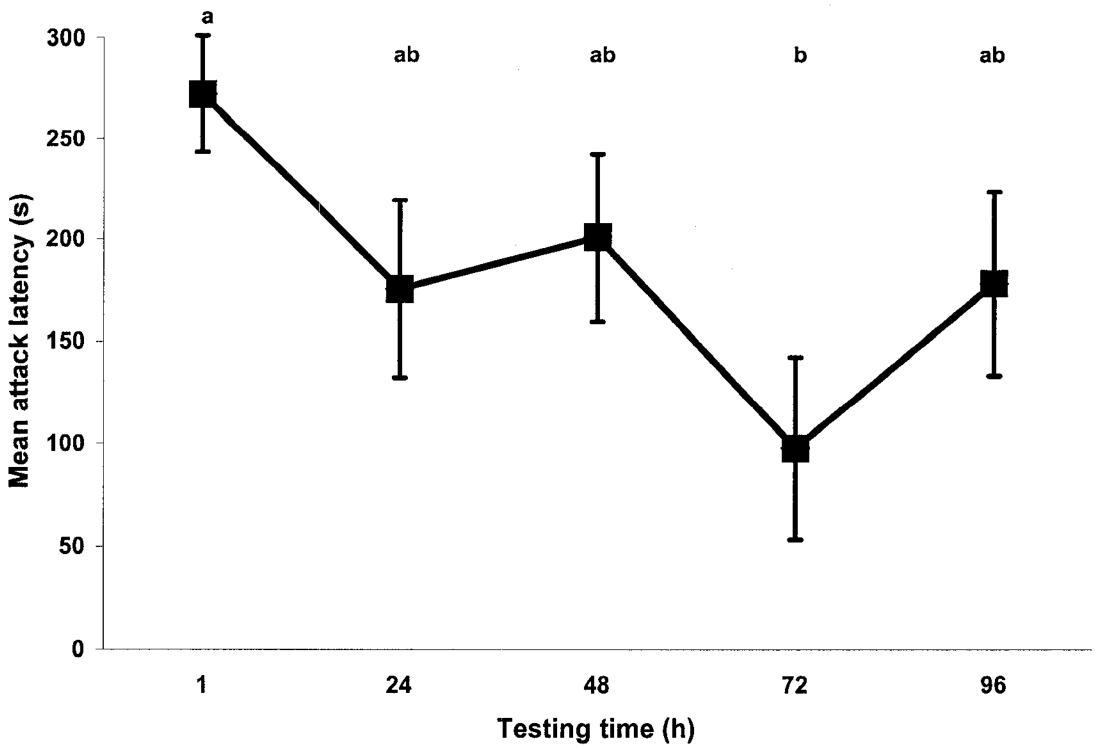

2.5
2
1.5
1
0.5
0
Mean number of bites
a
ab
ab
b
ab
1
24
48
72
96
Testing time (h)

Figure 2.8: Mean attack latencies (± standard error) when trained *S. ocreata* (N=10) were presented with toxic milkweed bugs over a series of testing times (shared letters indicate no significant difference in mean attack latency for testing day [Tukey post hoc test (α=0.05)]). Spiders were given a palatable cricket following training and each testing trial to control for hunger.


a
ab
ab
b
ab
Mean attack latency (s)
300
250
200
150
100
50
0
1
24
48
72
96
Testing time (h)

Obj. 2: Discussion

The duration of a spider's learned aversion to toxic prey was impacted by both memory and hunger level, but in complex ways. Support for the predictions of aversion persistence decreasing with time and/or hunger level was provided by the results of these experiments. As predicted, spiders' acceptance of toxic prey increased over time without re-exposure to *O. fasciatus*. Short-term memory of the learned aversion to the toxic prey was strongest soon after acquisition, but waned thereafter, as prey aversion varied between individuals after 24 hours following acquisition. Spiders tested one hour following initial aversion training had attack latencies significantly longer than all other groups tested, but no statistically significant differences were seen between other intervals despite an apparent trend for intermediate levels on days 1-2. Generally, spiders' mean number of bites upon toxic prey increased as time without re-exposure and starvation increased. However, the mean number of bites by spiders tested one hour after training (mean = 0) was significantly different from those tested on days 3 and 10, but again no statistically significant differences were seen between all other comparisons. This may indicate that once spiders attack toxic prey, they have similar requirements of exposure to toxic secretions in order to trigger memory of the learned aversion. This may also reflect a small sample size. Re-sampling of prey may be beneficial to these predators, as foraging patch relocations frequently occur (Persons & Uetz 1997).

The impact of hunger level upon learned aversion persistence was more difficult to discern because of the confounding effect of prey presentation order. When spiders were kept without palatable prey, but were re-exposed daily to milkweed bugs, acceptance of *O. fasciatus* decreased significantly over time. However, spiders subjected to alternating sequences of toxic milkweed bugs and cricket prey daily (to control hunger), continued to attack toxic prey throughout the testing period. This suggests that the relative availability of palatable prey may take a primary role in the spider's decision to attack or avoid potentially harmful prey. The effects of hunger upon predation were less influential than predicted. Similar to research concerning oviposition by the apple maggot fly, the tendency for predators to accept prey may be influenced by the pattern of successful predatory encounters (Prokopy et al. 1984). Uncertainty of prey palatability may have increased the likelihood of spider attack. During aversion training, each prey item the spider received was unpalatable. Repeated exposure to unpalatable prey was also maintained in the experiment where separate groups of spiders were given no prey until testing with a toxic milkweed bug. However, in the controlled hunger experiment, the alternating pattern of prey palatability may have confounded the spiders' responses to toxic prey. Taking the wolf spiders' natural behavior into account may help to explain these results. It is likely that if unpalatable prey were repeatedly encountered in a natural environment, spiders would change foraging patch residence. Theoretically, spiders with daily exposure only to toxic milkweed bugs were placed into the "same" patch during each trial (same testing arena and prey type [toxic *O. fasciatus*] at each chance for predation). In natural environments where all prey attacked within a patch are distasteful it may be beneficial for the spider to discontinue predatory behaviors in order to reduce exposure to toxins. In contrast, for spiders kept well fed throughout the testing period, every other prey presented was palatable. Furthermore, palatable crickets were given to and remained with spiders in their home container, but toxic prey was presented in a standard testing arena. Consequently, the predator may have perceived each predation opportunity as occurring in a different patch (different sensory stimuli encountered with change in prey type and testing arena). In a new foraging patch or in a patch where a predator may encounter palatable prey half of the time, it may be an advantage to continue to react in a predatory manner. While larger sample sizes would strengthen the results, these experiments suggest that *S. ocreata* effectively retains learned aversions up to one hour or more following training, but that ability declines over a period of days without recurring contact. However, upon repeated exposure, acceptance levels of toxic prey decreased over time. This may indicate that predatory behaviors may also be biased by some type of long-term memory of prey palatability within a foraging patch. The incorporation of past foraging experiences as an influence upon the decision to leave a foraging site has been suggested to occur in a web building spider (Nakata & Ushimaru 1999), so it may be possible for a ground dwelling forager to use prior experience as well.

Objective 3: To examine the sensory cues used for prey recognition.

Wolf spiders use vibratory, chemical, and visual cues to recognize potential prey (Witt & Rovner 1982; Uetz 2000; Uetz & Roberts 2002). Like many toxic insects, the milkweed bug *O. fasciatus* is aposematically colored (bright, contrasting warning coloration) and chemically defended (toxic cardenolide-containing secretions from repugnatorial glands). As do predators that form a "search image" which results in more accurate detection of cryptic, palatable prey (Kamil & Bond 2001), *S. ocreata* may associate noxiousness with the prey's physical, behavioral, or chemical characteristics and form a recognition image-- resulting in avoidance of a toxic prey item. I investigated the specific cue or cues most critical to *S. ocreata* for the recognition and subsequent avoidance of potentially toxic prey.

I tested two null hypotheses separating prey type and sensory cues [H_{01}: pattern of predatory behavior is independent of sensory mode of stimulus presented; H_{02}: pattern of predatory behavior is independent of predator experience with cardenolide-containing *O. fasciatus*] and the alternative hypotheses [H_{a1}: pattern of predatory behavior is not independent of sensory cues presented; H_{a2}: pattern of predatory behavior is not independent of predator experience with toxic milkweed bugs]. This was accomplished by experimentally manipulating potential cues available for recognition (color patterns, chemical cues, and tactile cues) of prey in cue isolation experiments using trained and untrained spiders.

Obj. 3 Methods:

Experiment 1.) Visual cues alone

Mechanical (vibratory and tactile) and chemical signals were removed through the use of video presentations of milkweed bugs and crickets. Video playback has been used in previous studies involving spiders. It allows each test subject to be shown an identical stimulus, thereby controlling for a source of behavioral variation that would occur if live stimulus organisms were used (Clark & Uetz 1990; Uetz 2000; Uetz & Roberts 2002). *S. ocreata* has previously been shown to respond to video images of spiders and prey as if the images were real and behave appropriately (McClintock & Uetz 1996; Persons & Uetz 1997).

Videotapes of live prey items' movements during locomotion were made by filming *O. fasciatus* and *A. domesticus* with a Canon XL1 3CCD digital video camcorder with a 16X zoom lens. The best representative individual recording of each species (i.e., a single exemplar) was chosen from review of numerous tapes. Single exemplars were used as representatives of different prey species to reduce variation arising from activity level. Each exemplar was then digitally edited using Adobe Premiere 5.1 and Adobe Photoshop 5.0 to produce a continuous loop with a controlled activity level. These images were shown to spiders on an RCA color LCD TFT microtelevision (2.7" screen). Standard video playback arenas (see McClintock & Uetz 1996) were used in these trials. The test arenas were of larger size (15.5cm diameter, 7.5cm high) compared to those used in other experiments, and one side of the arena was cut away to allow for closer positioning of the arena to the displayed video image. Trained (acquired learned aversion to toxic *O. fasciatus*) (N=14) and untrained (N=18) spiders were used and each spider was presented the cricket and milkweed bug video in random order in five-minute trials. Due to the small number of counts in certain cases, two-tailed binomial tests (Siegel & Castellan 1988) were used to examine differences in the presence or absence of orient, approach, and reach (extending legs as if to capture prey) behaviors based on the videos presented to the spiders within both the trained or untrained experience level. If naïve or trained (acquired learned aversion to toxic *O. fasciatus*) wolf spiders do not use visual cues alone to discriminate prey type, it was predicted that there would be no difference between the probabilities of spiders displaying the behavior in question toward either video when presented with the milkweed bug video (p) and the cricket video (q) [H_0: $p=q=0.5$].

Experiment 2.) Mechanical (tactile and chemical) cues

To test the importance of tactile and chemical cues in a spider's recognition image of *O. fasciatus,* color and pattern of milkweed bugs were altered using non-toxic black paint. The black paint masked the insect's aposematic coloration and pattern. Cardenolide-free *O. fasciatus* (sunflower-fed) were used in order to eliminate the presence of sequestered cardiac glycosides, although other cuticular hydrocarbons and glandular secretions were present. Painted milkweed bugs were presented to trained (N=11) and untrained (N=6) spiders in a covered, plastic test arena (9cm diameter, 1.5cm high). A Fisher's exact test was conducted to determine if there was a significant difference between attack responses (combined attack/feed and attack/reject behaviors) of trained (acquired learned aversion to toxic *O. fasciatus*) and naive spiders.

Experiment 3.) Chemical cues

The importance of chemical cues in aversion behavior was examined independently by harvesting glandular secretions from cardenolide-containing and cardenolide-free milkweed bugs. Milkweed bugs were squeezed with forceps until defensive secretions were produced, and then these chemicals were applied to the dorsal surface of typical non-toxic prey items (*A. domesticus*). Treated crickets were presented to trained (acquired learned aversion to toxic *O. fasciatus*) (N=32) and untrained (N=31) spiders in the standard 9cm diameter testing arena. Differences in predatory behavior between trained and untrained spiders for each of the secretion treatments were investigated. It was predicted that the spiders' response to the treated crickets (cardenolide-containing [N=36] or cardenolide-free [N=27]) would be independent of the spiders' experience with *O. fasciatus.*

Obj. 3 Results:

Experiment 1.) Visual Cues alone

In these trials video playback methods were used, therefore chemical and mechanical (vibratory, tactile) signals were removed and only a visual stimulus was presented to the wolf spiders. No significant differences were detected in the probability of spiders to display orient (two-tailed binomial tests: p=.624, untrained; p=1.0, trained), approach (p=.376, untrained; p=.624, trained), or reach (p=.07, untrained; p=.688, trained) behaviors based upon the video presented (milkweed bug or cricket) within spider experience level (Table 3.1). Trained and untrained spiders' responses were independent of the video presented.

Experiment 2.) Tactile and chemical cues

I tested the value of tactile and chemical cues in a spider's recognition image of milkweed bugs by altering the aposematic color and pattern of *O. fasciatus* with non-toxic black paint. Cardenolide-free milkweed bugs (fed on sunflower seeds) were used; therefore the presence of harmful cardiac glycosides could not be utilized in prey recognition. A Fisher's Exact test detected a significant difference between trained and naive spiders in attack responses (two-tailed p= 0.009). All untrained spiders (6/6) attacked, but most trained spiders (8/11) avoided the painted, cardenolide-free milkweed bugs (Table 3.2).

Experiment 3.) Chemical cues

The importance of chemical cues in the development of a *S. ocreata* recognition image of potentially harmful prey was examined by applying glandular secretions from cardenolide-containing and cardenolide-free milkweed bugs to the dorsal surface of non-toxic *A. domesticus*. I predicted that there would be no difference in attack (biting) responses between trained and untrained spiders when presented with crickets that had cardenolide-containing or cardenolide-free secretions applied. Using a Pearson's Chi-square, differences in predatory behavior between trained and untrained spiders for each of the secretion treatments was investigated. When crickets that were treated with cardenolide-free milkweed bug secretions were presented to trained and untrained spiders, no significant difference in avoidance and predatory behaviors were detected (X^2= 0.77, df=1, p=0.3811). However, when crickets that had cardenolide-containing secretions applied to them were presented to wolf spiders, a significant difference was

Table 3.1: Percentages of trained and untrained spiders displaying behaviors typical of an attack sequence toward a video presentation of *A. domesticus* or *O. fasciatus* (MWB= milkweed bug video).

Observed Behavior	Untrained *S. ocreata* (N = 18)			Trained *S. ocreata* (N = 14)		
	Video Stimulus		p	Video Stimulus		p
	Cricket	MWB		Cricket	MWB	
Orient	83%	94%	0.624	93%	93%	1.0
Approach	78%	94%	0.376	79%	93%	0.624
Reach	28%	61%	0.07	64%	79%	0.688

Table 3.2: Number of trained and naïve spiders displaying attack or avoidance behaviors toward a cardenolide-free *O. fasciatus*. The insect's aposematic color and pattern were eliminated by non-toxic black paint.

Spider Experience	Attack	Avoid	Fisher Exact Test
Untrained	6	0	p = 0.009
Trained	3	8	

detected in attacking behaviors (X^2= 8.44, df=1, p=0.0037). Most untrained spiders (14/16) attacked crickets treated with cardenolide-containing secretions. However, sixty percent of trained spiders avoided *O. fasciatus* defensive secretions, even on palatable prey (12/20) (Table 3.3).

Obj. 3: Discussion

The short-term aversion to toxic *O. fasciatus* was facilitated by a recognition image composed of particular sensory cues. In video playback experiments, only visual cues were presented to the trained and naïve wolf spiders. While only one exemplar for each insect was used, no significant differences were detected between the two spider groups when presented with either milkweed bug or cricket video. The majority of the observed behaviors occurred during presentations of both videos to both spider groups, which indicated that the spiders were able to detect the image and respond to it in a predatory manner. However, spiders did not behave differently toward a cricket image versus a milkweed bug image regardless of prior experience with toxic prey. This indicates that the milkweed bug was not recognized as an aversive stimulus on the basis of visual cues, even if the spiders detected a difference between the prey type of the two video images. Although wolf spiders use visual cues for prey detection, their ability to distinguish colors is restricted (D'Eath 1998; Fleishman et al. 1998; Uetz 2000). It is likely that no significant differences between video types occurred due to this characteristic. Additionally, spiders may not have been able to detect differences in prey type based on movement or body shape. This suggests that *S. ocreata* did not exclusively use visual cues in their recognition image of potentially harmful prey. While highly

Table 3.3: Number of trained and untrained wolf spiders displaying attack or avoidance behaviors toward *A. domesticus* treated with the secretions from toxic cardenolide-containing *O. fasciatus.*

Spider Experience Level	Attack	Avoid	Pearson's X^2	p
Untrained	14	2	8.44	0.0037
Trained	8	12		

visual, "seek and find", avian predators often respond to aposematic prey with neophobic or unlearned avoidance (Coppinger 1970; Schuler & Roper 1992), "sit and wait", wolf spider predators rely on movement, vibrations or chemical cues for detection of prey, with color being of lesser importance (Uetz & Roberts 2002). Consequently, the milkweed bugs' aposematic coloration was not effective in preventing initial spider predation or activating a previously learned aversion. Furthermore, when trained and untrained *S. ocreata* were presented with cardenolide-free milkweed bugs with aposematic colors and patterns eliminated by black paint, significant differences based on prior experience with toxic prey were detected. Although the aposematic coloration and pattern, and the presence of toxic cardenolides in the insects' secretions had been eliminated, the trained wolf spiders were still able to recognize and subsequently avoid the *O. fasciatus*. This suggests that while the aposematic patterns and the presence of toxic cardenolides were not critical to induce avoidance behaviors, some tactile or chemical and/or behavioral cues were used by *S. ocreata* to identify potentially harmful or non-cricket prey. It is possible that trained spiders were able to recognize some cuticular hydrocarbon, odor, or some behavioral characteristic that indicated the prey was a milkweed bug, irrespective of the presence of toxic cardenolides. However, when the milkweed bugs' defensive chemical secretions were applied to palatable cricket prey, only secretions containing cardenolides were effective in producing aversion by trained wolf spiders. Normally, tactile or olfactory cues plus the chemical cardenolide secretions function together to protect milkweed bugs. This research suggests wolf spiders that have experienced toxic prey may use either a tactile and/or chemical cue in their recognition and subsequent avoidance.

Previous research has indicated that spiders can use chemical cues to perceive and/or distinguish prey (Persons & Uetz 1996; Punzo & Kukoyi 1997), likely through the use of pit organs and specialized hairs. Spiders also possess specialized hairs and slit sensillae that aid reception of tactile cues (Barth 2002). Many sensory hairs and organs are located on spiders' legs which may account for the frequent occurrences of touching behavior preceding recognition and subsequent avoidance of toxic prey. Spiders exhibited discrimination based on input from their mechanical and chemical sensory receptors located upon their legs or pedipalps, sampling cues in a manner analogous to insects that recognize and reject prey based on contact with antennae (Traugott & Stamp 1996).

CONCLUSIONS

Results of this research indicate that *Schizocosa ocreata* are capable of learning to avoid toxic prey. As experience with toxic *Oncopeltus fasciatus* increased, the wolf spiders were less likely to attack them. While complete avoidance persisted for less than a day, over time fewer trials were necessary for reinforcement. In experimental studies, the interspersion of palatable prey presentation impacted the persistence of the learned aversion more than spider hunger level (i.e. spiders given palatable cricket prey between milkweed bug trials did not retain learned aversion). This may indicate that spiders' predatory behavior may be biased by incorporating recent foraging experiences. The ability of *S. ocreata* to acquire learned aversion, recognition and subsequent avoidance of harmful prey may be important, as these spiders are generalist predators. Animals that are considered to be specialists and/or those that do not easily move from a food resource have little need for aversion learning (Bernays 1993; Ratcliffe et al. 2003). On the other hand, generalist predators may encounter and sample many types of food; therefore recognition and learned avoidance may readily develop over the course of their lifetime and have significant impact upon predation.

Although wolf spiders use visual, chemical, and tactile sensory modalities to perceive their environment, only chemical and tactile cues (possibly including behaviors) were used to form the recognition image of the toxic milkweed bug prey. *S. ocreata* has previously demonstrated the ability to recognize chemical cues from palatable prey and increase their patch residence time accordingly (Persons & Uetz 1996; Punzo & Kukoyi 1997). Therefore, it is plausible that wolf spiders may leave foraging patches faster if unpalatable prey chemical cues are sensed. Furthermore, due to recognition of toxicity, the prey may have increased survivorship caused by decreasing attack severity following encounters with experienced predators (Vasconcellos-Neto & Lewinsohn 1984; Paradise & Stamp 1991). Because experienced wolf spiders may detect prey toxicity externally via tactile and/or chemical cues, both the predator and prey may benefit and co-occur without either organism being harmed. Similar to reactions by praying mantids (Paradise & Stamp 1991), experienced wolf spiders may allow non-toxic or less toxic conspecific prey to escape if they resemble a previously formed recognition image. Although it is not likely for these wolf spiders to encounter *O. fasciatus,* they may find other distasteful prey within their natural habitat. Since *S. ocreata* uses primarily tactile and/or chemical cues for recognition, cryptically colored toxic prey (which wolf spiders may encounter) could be learned to avoid as well.

Results from these experiments indicate that wolf spiders would be able to modify their predation patterns to increase foraging efficiency and possibly individual fitness and survival. Learned prey aversions have been demonstrated in jumping spiders and web building spiders (Bays 1962; Vasconcellos-Neto & Lewinsohn 1984; Edwards & Jackson 1994). As learning has rarely been studied in spiders, and never in the well-studied model *Schizocosa ocreata*, the results of this research contribute the groundwork for future studies in behavioral ecology. It may be interesting to explore the importance of rearing condition or the impact of various sensory modalities upon the acquisition of the learned aversion. Also, using prey items typically found within the wolf spiders natural habitat (aposematically colored or otherwise) could be worthwhile to investigate aversion learning and/or foraging behavior that may be occurring in the field. Finally, examining the capability of male and/or juvenile wolf spiders to learn may reveal more information on the cognitive abilities of this species.

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