Learned avoidance of the Large Milkweed Bug (Hemiptera: Lygaeidae: *Oncopeltus fasciatus*) by jumping spiders (Araneae: Salticidae: Dendryphantina: *Phidippus*)

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Abstract: In the laboratory, *Phidippus* jumping spiders often attacked, but seldom fed upon nymphs and adult milkweed bugs (*Oncopeltus fasciatus*) when these were reared on milkweed (*Asclepias*) seeds. Spiders readily attacked and fed upon *Oncopeltus* reared on sunflower (*Helianthus*) seeds. *Phidippus* were shown to reject flies treated with either hemolymph, or with fluid from the lateral thoracic compartment, of *Oncopeltus*. They also rejected flies treated with β-Ecdysone, but accepted flies treated with lethal doses of the cardenolides g-Strophanthin (Ouabain) and Digitoxin. Single encounters with *Oncopeltus* significantly reduced the probability of attack in a subsequent encounter for *less than* two hours. Repeated encounters with *Oncopeltus* led to greater avoidance than did a single encounter. In the absence of repeated experience with these bugs, however, *Phidippus* recovered their tendency to attack over a period of several days. More satiated spiders were more discriminating in their choice of prey. Negative experience with *Oncopeltus* did not necessarily impact their predation on other insects, including flies (Diptera). Impact of measurement techniques on results in prey avoidance and acceptance studies are discussed. A preliminary model for selective avoidance and attraction to potential prey, the defenses of *Oncopeltus fasciatus*, and salticid contact chemoreception in general, are also reviewed.

Key words: cardenolide, chemical defense, chemical ecology, chemoreception, Digitoxin, Ecdysone, learning, Ouabain, predation, salticid

Introduction

Like most spiders, salticids of the genus *Phidippus* will attack and feed upon a wide variety of terrestrial arthropods (Edwards 1980; Edwards and Jackson 1994). Movement by potential prey elicits a turn by the salticid to face that prey, bringing the high resolution of the its large anterior medial eyes (AME) into play (Land 1971; Duelli 1978; Hill 2006a, 2010a). This *facing* behavior is readily elicited, but subsequent pursuit (rapid approach, stalking, and jumping attack) is contingent upon visible features of the potential prey. Once a pursuit sequence is initiated, it is usually completed, unless the prey escapes (Gardner 1964).

A jumping spider may encounter and turn to face many potential prey in the course of a single hour. The response of the spider may be indifference, sustained facing (presumed study or examination), pursuit, or escape. Clearly a broad range of relevant experience is available to the spider as it encounters many different animals. Salticids of the genus *Phidippus* are known to be deterred by immediate contact with many of the defensive chemicals employed by insects, including Z-dihydromatricaria acid from *Chauliognathus* soldier beetles (Meinwald *et al.* 1968; Eisner et al. 1981), and steroids (lucibufagins) from *Photuris* and *Photinus* fireflies (Eisner *et al.* 1997). *Phidippus* will also drop the lucibufagin-bearing diurnal firefly *Lucidota atra* upon contact, without harming the insect (Gronquist *et al.* 2006)

Phidippus are also known to avoid or to reject (after capture) the Large Milkweed Bug, *Oncopeltus fasciatus* (Jackson 1977; Givens 1978). During the course of the present work, and also as reported recently by Skow & Jakob (2006), *Phidippus* could be fed on *Oncopeltus* that have been reared exclusively on sunflower (*Helianthus*) seeds, with no apparent harm to the spiders. This suggests that unknown chemicals sequestered by *Oncopeltus* from its normal diet of milkweed (*Asclepias*) seeds produce the observed deterrent effect. A number of different insects that feed upon milkweed (*Asclepias*) are known to sequester plant steroids that contribute to their defense against vertebrate predators. The relationship between the monarch butterfly (*Danaus plexippus*) and *Asclepias* has received much attention in the past (Brower *et al.* 1968; Rothschild *et al.* 1975). Sequestration of steroids from *Asclepias* by *Oncopeltus* has also been well-studied (Duffey & Scudder 1972, 1974; Scudder & Duffey 1972; Isman 1977; Isman et al. 1977; Vaughan 1979). Since *Phidippus* has a strong contact reaction to lucibufagins associated with lampyrid beetles (*Photinus*, *Photuris*, and *Lucidota*), it could be expected that they would also react to cardenolides.

I have also observed large populations of the chrysomelid beetle *Trirhabda canadensis* in close association with both *P. clarus* and *P. princeps* in old field habitats, in both Minnesota and New York. The spiders frequently encountered these insects, but I never observed pursuit or feeding on them. Although inherited or innate recognition of aposematic features of these animals was a possible factor, the fact that *Phidippus* encounter a great variety of insect species in populations of varying density according to locality suggests that learned (modifiable or adaptable) avoidance would be advantageous. The objective of the present study was to further isolate the factors associated with rejection of *Oncopeltus* by *Phidippus*, and also to evaluate the effect of experience with unpalatable *Oncopeltus* on the tendency of *Phidippus* to attack them.

Since the time that the studies presented in this paper were completed in 1979, the extent to which experience can modify the subsequent predatory behavior of *Phidippus* has been studied by Edwards & Jackson (1994), Carducci & Jakob (2000), and Skow & Jakob (2006). Edwards & Jackson reported that almost all *Phidippus regius* spiderlings that attacked ants in their trials avoided them on subsequent encounters for up to four days. Carducci and Jakob found differences in behavior between laboratory-reared and field-caught *P. audax*, and noted that several different explanations, including selective pressure in the field, or variable experience, could explain these results. The studies of Skow and Jacob were similar to those presented here, and will be addressed in the discussion section.

Materials and methods

Studies of the degree of acceptance of prey by *Phidippus* were primarily conducted as staged encounters in which the reaction of each spider was observed after prey was added to a clean plastic Petri dish (90 x 15-20mm) containing that spider, in an arena designed to provide good lighting for these visual predators (Figure 1). Given our current understanding of the role of UV reception in these salticids (DeVoe 1975; Blest *et al.* 1981; Lim & Li 2006), future studies related to visual avoidance of prey by salticids should definitely consider impact of the relevant UV spectrum on the behavior of these animals. The present study used primarily incandescent lighting in the laboratory.

Control and experimental encounters were conducted in a uniform manner here, but the important recent finding that behavioral thresholds related to predation may change when these spiders are moved or change location (*context*, after Skow & Jakob 2006) suggests that much more attention to the visual and physical surroundings of these spiders is warranted during behavioral trials. Except for periods of continuous experience with insects as described here, each spider was placed into a clean Petri dish with

no accumulation of silk or debris prior to introduction of prey into the arena. The new context may have reduced or otherwise altered the attack threshold of these spiders, but at least both control and experimental groups received the same treatment.
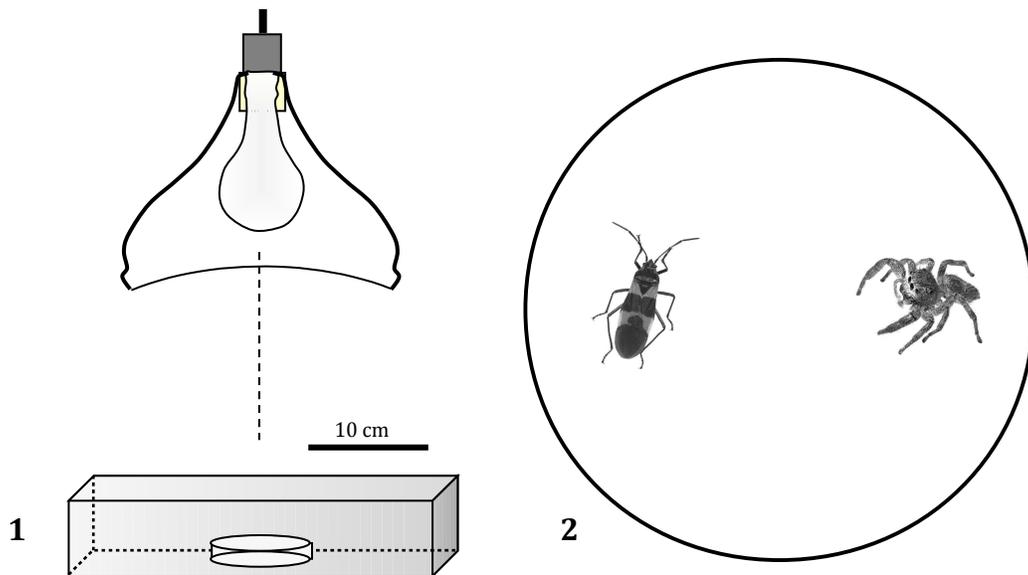

Figure 1.   Arena for testing encounters between *Phidippus* and insect prey.   Each encounter began when the spider turned to face prey that was placed into a clean 90 x 15-20 mm high plastic Petri dish containing the spider.   **1,** Side view of the arena, showing placement of the Petri dish within a 30 x 30 x 6 cm white cardboard box centered 30 cm under a 40 w incandescent reflector lamp.   **2,** Observer's top-down view of adult *Oncopeltus fasciatus* and *Phidippus princeps* within a Petri dish.

Phidippus audax (Hentz 1845) and *P. princeps* (Peckham & Peckham 1883) were field-captured in the vicinity of Ithaca, New York (Figures 2-3).
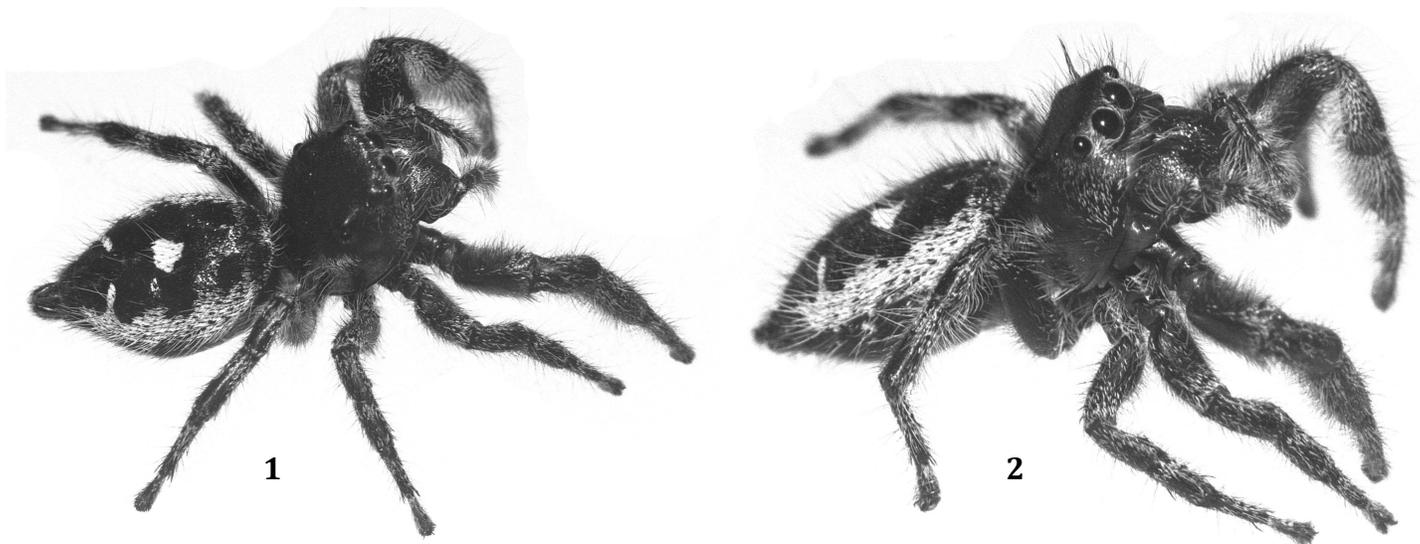

Figure 2. Two adult female *Phidippus audax* captured in an old field in Ithaca, Tompkins County, New York (1978). Many local varieties of *P. audax* do not have the broad lateral band of opisthosomal scales shown here.   *P. audax* appears to be a generalist with respect to habitat and it is widely distributed across much of North America, with many recent sightings in the far west. It can frequently be found living on herbaceous plants in old fields, but I have also found it near water, woodland margins, on trees, on fence posts, and even nesting on the ground under rocks.
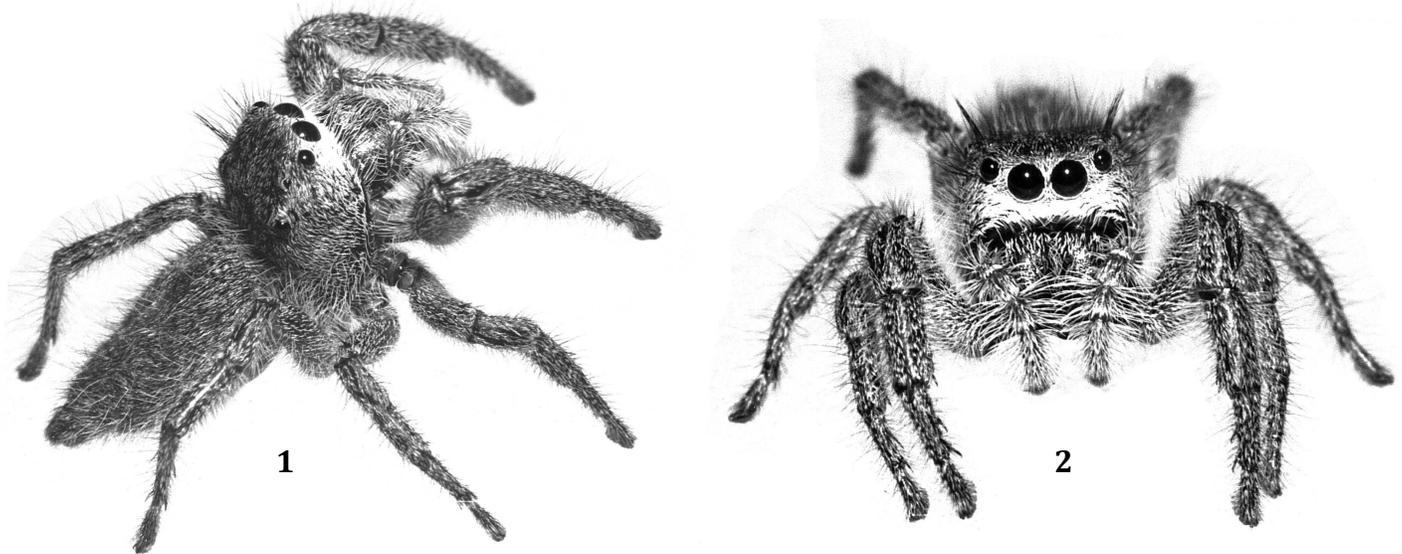
Figure 3. Two views of an adult female *Phidippus princeps* captured in an old field in Ithaca, Tompkins County, New York (1978). In this area female *P. princeps* were tan in color, often with abundant white or cream-colored facial scales as shown here. At least as juveniles, they build their nests and hunt on herbaceous plants in old field habitats. They are common in eastern North America, from Minnesota southeast to northwestern South Carolina and northern Georgia. Further to the southeast, they are replaced by the closely related *P. pulcherrimus* Keyserling 1885, also an inhabitant of old fields (Edwards 2004). Note the distinctive 'hair' tufts on the carapace, a characteristic of most *Phidippus* jumping spiders.

Hatchling *P. texanus* Banks 1906 were reared from a brood sac found in Lea County, New Mexico by David B. Richman. All males from this brood were fairly uniform in appearance after the description (Edwards 2004) for either *P. texanus* or *P. ardens* Peckham & Peckham 1901. About half of the reared females had the typical *P. ardens* coloration, and half had the typical *P. texanus* coloration (Figure 4). Per Edwards (2004, page 92), these two species, along with *P. purpuratus* Keyserling 1885, "might constitute one widespread variable species." No attempt was made to separate the two color forms in trials, which primarily involved immature animals for this species.
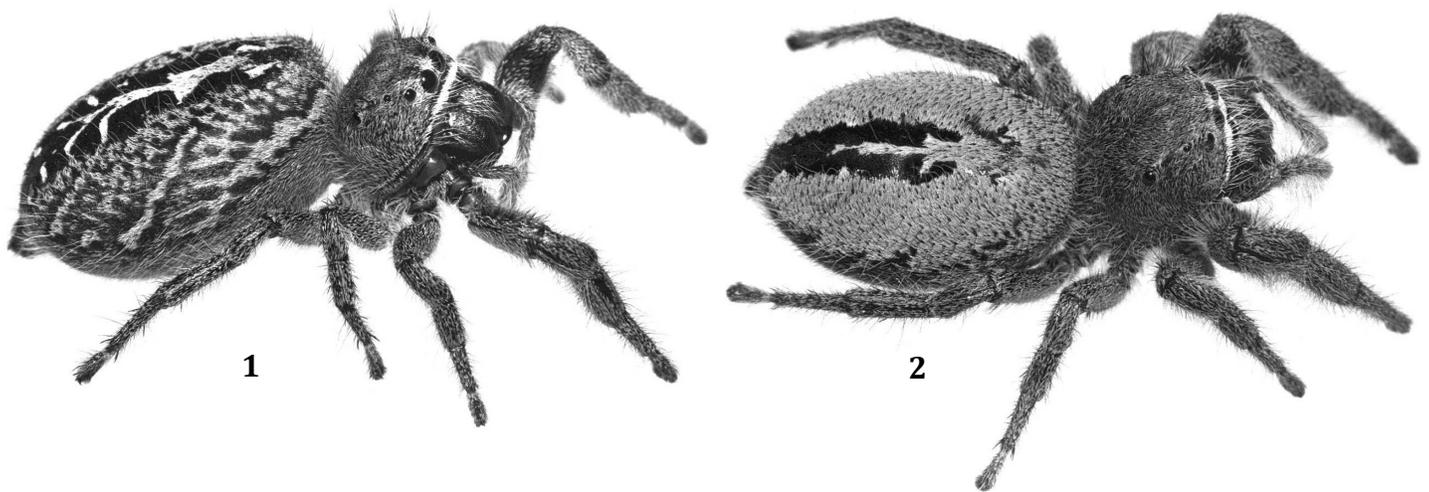

Figure 4. Two female *Phidippus texanus* (sisters) reared from the same brood sac found in Lea County, New Mexico, in August of 1978 (on mesquite 21 miles W of Jal on SR 128). Half of the females in this brood had the typical *texanus* form with cream to white scales on a black background (1), and the other half had a similar dorsal pattern with the coloration of the related *P. ardens*, with rust-red scales covering much of the dorsal opisthosoma. Edwards (2004) placed *P. ardens* and *P. texanus* in the *borealis* clade of the *purpuratus* group within *Phidippus*, but kept the species separate in part because of their parapatric ranges. However, he did report both species from Lea County, New Mexico where this brood sac was found, and both live on mesquite. These are very large *Phidippus*, with females averaging 13-15 mm in body length.

Spiders were reared in 90 x 15-20 mm plastic Petri dishes at laboratory temperature (about 23 C), under laboratory lighting conditions which included ambient fluorescent and window light supplemented by a 40 W incandescent lamp at a distance of about 0.4-0.5 m from the spiders. Spiders were reared under an approximate 14:10 light:dark cycle with testing near the middle of the light period. They were provided with water droplets and fed with either house flies (*Musca domestica*) or vestigial-wing fruit flies (*Drosophila melanogaster*).

All *Oncopeltus fasciatus* were reared from cultures provided by Dr. William S. Bowers of the Geneva Research Station in New York. Most *Oncopeltus* used in avoidance testing were reared solely on a diet of locally-collected (vicinity of Ithaca, New York) Common Milkweed seeds (Asclepiadaceae: *Asclepias syriaca*). Other *Oncopeltus* were reared solely on a diet of commercial sunflower (Asteraceae: *Helianthus annuus*) seed, through at least four generations prior to use in these experiments. *Helianthus*-reared animals tended to have lower fecundity and growth rates. All *Oncopeltus* colonies were also provided with water in a Petri dish filled with wet cotton. Another lygaeid bug, *Ortholomus scolopax* (used to assess the generality of learned avoidance) was collected locally in association with Common Cinquefoil (*Potentilla simplex*) seeds.

Spiders were deprived of physical contact with any insects for variable periods of time, ranging from 1-10 days prior to the onset of an experiment. Where applicable, numbered spiders were randomly assigned to control and experimental groups, and testing alternated between groups to vary the exact time of testing and to thereby control for the potential effect of circadian rhythms on spider behavior. When separate encounters were separated by a discrete interval, testing of individuals was staggered and completed according to a pre-planned schedule for efficiency.

For live studies, the individual test was usually a staged encounter between a spider and a living insect. Each encounter began, by definition, when the spider turned to face an insect which had been placed, with minimal disturbance, into its container (Figure 1). The response of the spider was either to jump upon (attack) the insect, after an approach, or to turn away from the insect (no attack). If a spider initially approached the insect, but did not execute a jump or otherwise contact the prey before turning away, then the result was scored as no attack. In these live studies the fraction of the spiders of each group (control and experimental) that attacked was compared. The chi-squared test was used to assess the significance of all group to group comparisons. As with insects (Schoonhaven 1977), considerable variation of individual preference and responsivity may exist, but this individuality was not considered in analysis of the pooled data.

Testing of spider responses to specific chemicals or fluids followed methods described previously (Eisner *et al.* 1981). Vestigial-wing *Drosophila melanogaster* were killed by freezing and then treated with respective solutions prior to presentation to spiders at the end of a hair, threaded through the legs of the respective fly. For these trials only attacks (which took place virtually all of the time) were scored according to a number of different behavioral categories related to acceptance. These unambiguous behaviors are described with the related results.

The solvents used to treat both control and experimental flies were the same. Fluid was obtained from the lateral thoracic compartment of adult *Oncopeltus fasciatus* following the methods of Duffey and Scudder (1974) by gently squeezing the anterior of each bug between thumb and forefinger until small droplets appeared at the posterodorsal margin of the metathoracic pleurites. This fluid was collected from several bugs with a 20 µl glass capillary tube until about 1-2 µl of fluid was obtained. This fluid was then blown out onto a surface as a droplet use to dip the fly. Hemolymph of *Oncopeltus* was obtained by severing the legs of one side and squeezing the bug gently. Care was taken to avoid mixing this fluid with

other exuded fluids, including the dorsolateral thoracic space fluid. This fluid was also collected in a 20 µl glass capillary tube, until about 2-4 µl of fluid was obtained and blown out as a droplet for dipping the fly. All fluids were collected and flies were treated immediately before testing with spiders. Flies were dipped in water as controls for these treatments.

To further study the ability of these spiders to detect steroids, each fly was dipped in a methanol solution of either Ouabain, Digitoxin, β-Ecdysone, or simply the methanol solvent (control) and briefly dried until a crystalline residue of the respective solute coated the integument (Figure 5). These flies were also prepared immediately before use.
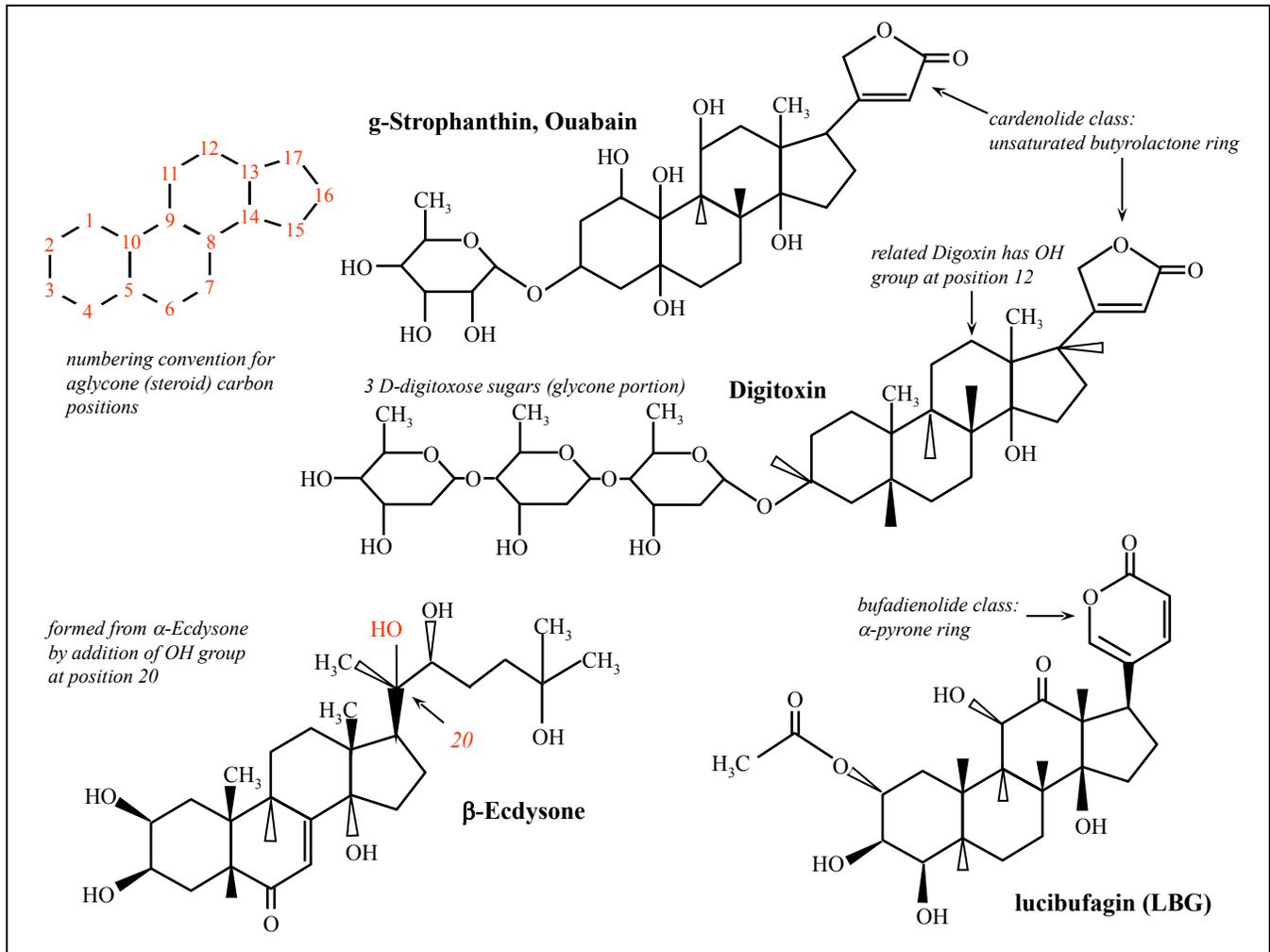

Figure 5. Comparative structure of steroid compounds mentioned in the text. Numbering for steroid (aglycone) positions is shown at upper left. The glycone portion (for example, the three D-digitoxose sugars in Digitoxin) is considered of less importance in differentiating these compounds. As shown, cardenolide class cardiac glycosides compounds are characterized by the presence of an unsaturated butyrolactone ring. Bufadienolide class cardiac glycosides bear an α-pyrone ring. Digitoxin is obtained from the Foxglove (*Digitalis purpurea*). Ouabain, or g-Strophanthin, is obtained from the ripe seeds of at least two African trees, *Strophanthus gratus* and *Acolkanthera ouabaio*, and was originally used for poison darts. β-Ecdysone is an important molting hormone for many insects, but was obtained from a commercial source as an extract from the fern *Polypodium vulgare* for this study. Only one of many different lucibufagins is shown here (after Gronquist *et al.* 2006).

Ouabain (g-Strophanthin, Sigma Scientific) was   prepared as a 0.1 M solution in methanol (72.86 g $C_{29}H_{44}O_{12} \circ 8H_2O$ per liter). By weighing flies before and after dipping and drying, it was estimated that treatment with this solution added about 50 µg of solid, crystalline Ouabain to the exterior of each fly. A

0.05 M solution of Digitoxin in methanol was obtained locally (2.3 mg $C_{41}H_{64}O_{13}$ in 60 µl methanol solution and suspension, molecular weight 764.96). Commercially available β-Ecdysone extracted from a fern (*Polypodium vulgare*, rhizomes of which may contain a 1% concentration), also obtained locally, was used in a 0.1 M solution in methanol (2 mg per 43 µl of solution, molecular weight ~466).

Results

Defense by Oncopeltus against an attack by Phidippus. The results of a series of 100 encounters between adult female *P. audax* and O. fasciatus (reared solely on a diet of *Asclepias* seeds) are shown in Table 1. Most of the time the bugs survived the attack. Even in the few cases where a bug was killed, the spider dropped it. In many cases, apparently after fluid had been released by *Oncopeltus*, spiders engaged in protracted mouth-wiping against the substratum (Figure 6). In many other cases, no fluid was visible and release was immediate upon contact. Note that the start of each encounter was defined by the execution of a facing turn by the spider in the direction of the prey, and only one encounter was tested per trial in the arena. In all of these trials, and in similar trials based on encounters, prey insects were not left with the spiders until the spiders attacked, but were removed at the end of the first encounter between spider and prey, and it was this encounter that was scored.

Table 1. Description of the rejection of adult *Oncopeltus fasciatus* (reared on *Asclepias*) as prey by adult female *Phidippus audax*. Trials (encounters) were conducted over a three day period, with no spider subjected to more than one trial in a day.

|	BEHAVIOR	N	N/attack
1	spider faced adult *Oncopeltus* (encounter)	100	—
2	no attack	21	—
3	spider attacked and contacted bug	79	1.00
4	spider released bug immediately or soon after contact with no apparent feeding	79	1.00
5	spider released bug immediately upon contact	75	0.95
6	spider wiped mouth against substratum after release of the bug	35	0.44
7	liquid observed on the surface of the bug after attack	52	0.66
8	liquid observed on the prothorax of the bug after attack	43	0.54
9	aldehyde detected by scent after attack	7	0.09
10	attacked bug appeared normal one day later	76	0.96
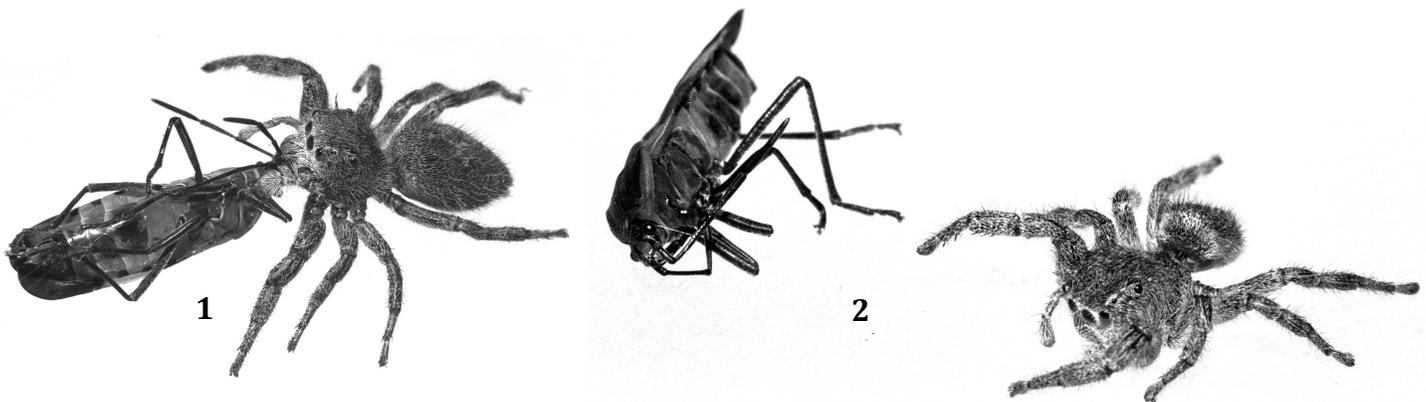
Figure 6. Violent reaction of an adult female *Phidippus princeps* to fluids associated with an adult *Oncopeltus fasciatus*. **1,** This spider first bit the bug on its head but held its legs and pedipalps far away from the prey. **2,** Moments later, the spider dropped the fatally-bitten bug, and began to wipe its mouthparts against the surface, leaving a trail of fluid behind (fluid cannot be seen in these photographs).

Impact of Asclepias diet on Oncopeltus defense. Adult female *P. audax* were separated into two groups, one of which was offered *Oncopeltus* reared on *Asclepias* seeds, and the other which was offered *Oncopeltus* reared on *Helianthus* seeds (Table 2). The *Asclepias* diet clearly gave *Oncopeltus* protection from these Phidippus (Figures 7-8).

Table 2. Comparison of attacks by adult female *Phidippus audax* on *Oncopeltus* reared on either *Asclepias* or *Helianthus* seeds. Encounters leading to attacks were staged over a 3 day period with a total of 21 different spiders, and no spider was used more than once in a given day. (P) indicates the probability of a null hypothesis (*Asclepias* result the same as *Helianthus* result) Feeding on *Asclepias* was clearly key to the survival of *Oncopeltus*.

	BEHAVIOR	*Oncopeltus* fed seeds of		P
		Asclepias	*Helianthus*	
1	spider attacked and contacted prey (N)	20	20	—
2	spider dropped prey immediately or soon after contact	20	4	<<0.0001
3	spider dropped prey immediately upon contact	20	3	<<0.0001
4	spider wiped mouth after dropping prey	14	2	<<0.001
5	spider repositioned for a second bite	0	17	<<0.0001
6	spider fed extensively on prey	0	16	<<0.0001
7	prey survived for at least 1 day after attack	20	4	<<0.0001

Figure 7. Predation on an *Oncopeltus fasciatus* reared solely on sunflower seeds (*Helianthus annuus*), by an adult female *Phidippus audax*. Adult bugs were usually grasped by the head, or just behind the head as shown here.
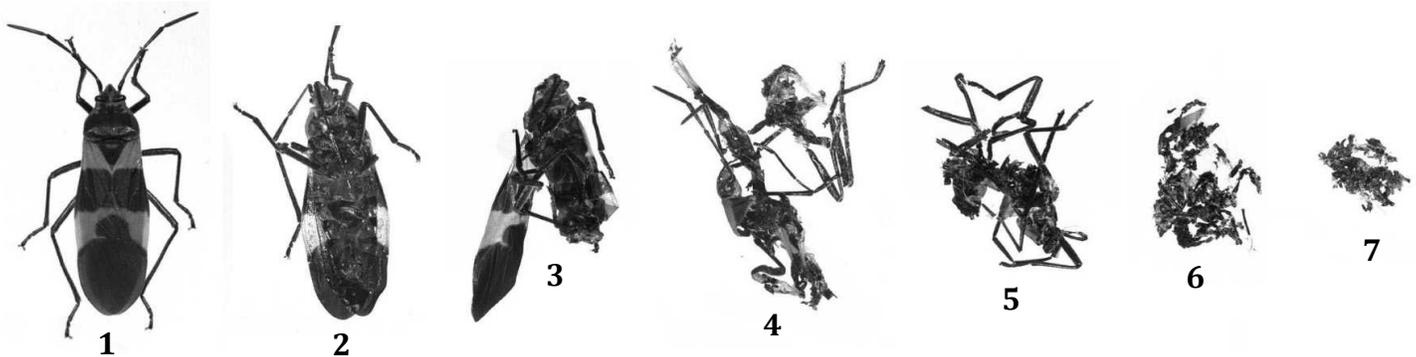

Figure 8. Remains of *Oncopeltus* fed on *Asclepias* (1, surviving) or *Helianthus* (2-7, dead) and subsequently attacked by six different adult female *Phidippus audax*. *Oncopeltus* fed on *Helianthus* were macerated to a variable extent during feeding as shown in this series (2-7).

Response of Phidippus to treated flies. In a series of trials, adult female *P. audax* were offered vestigial-wing *Drosophila melanogaster* treated with either fluid taken from *Oncopeltus* (Figure 9:1-2), or with a known steroid (Figure 9:3, Tables 3-4). Control flies were treated only with solvents. Results indicated that *Phidippus* were very significantly deterred by both lateral thoracic fluid and the hemolymph of *Oncopeltus*. They were also deterred by β-Ecdysone, but did not demonstrate any ability to detect the cardenolides Ouabain (g-Strophanthin) and Digitoxin. Unfortunately, spiders readily fed on a toxic dose of these cardenolides, and many of the spiders were immobilized and later died.
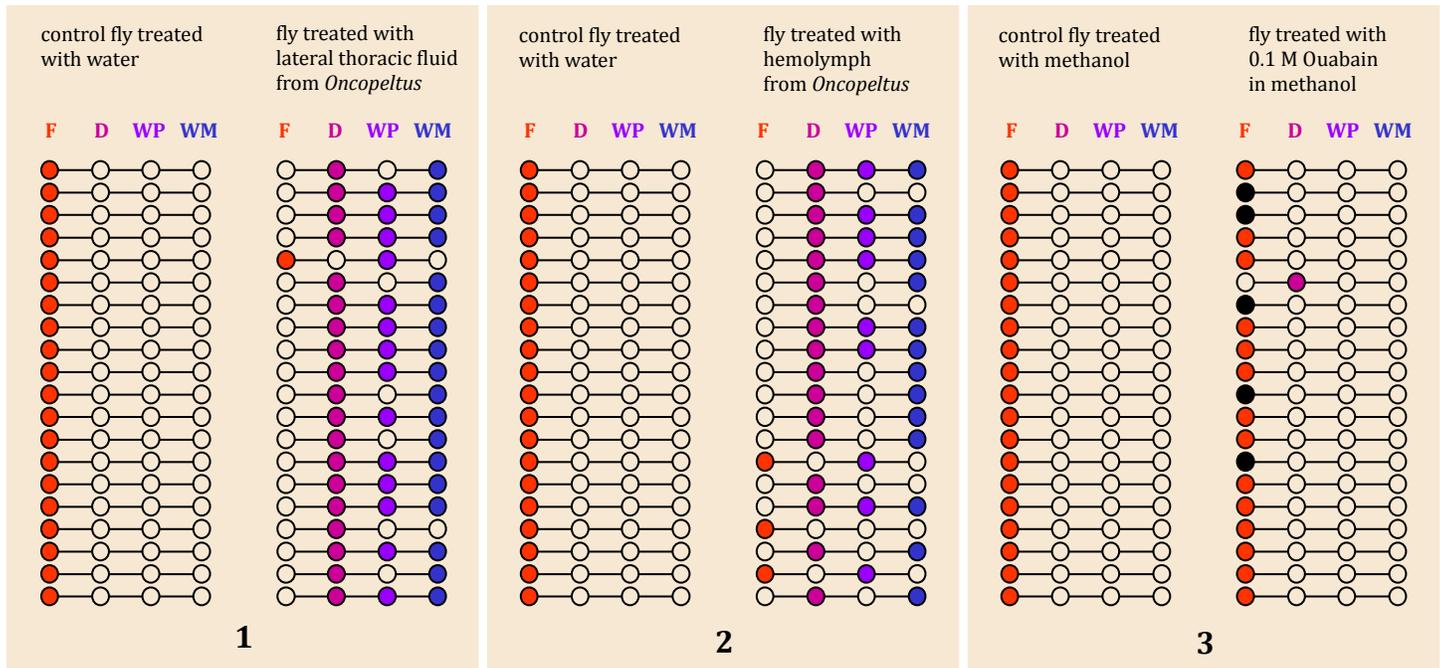

Figure 9. Bioassay based on reaction of adult female *Phidippus audax* to treated flies. In each case the spider was offered a recently killed and treated fly (vestigial-wing *Drosophila melanogaster*). Reactions of the spiders were scored with respect to whether they fed (F), dropped the fly without feeding (D), wiped the prey against the substratum (WP), or wiped their mouth against the substratum (WM), typically a more violent reaction. Note that all control flies were eaten normally, in all three series (A-C). In each series, there were 20 control and 20 experimental animals, assigned randomly. **1,** Experimental flies were treated with fluid from the lateral thoracic fluid of adult *Oncopeltus*, reared on *Asclepias*. All scored behaviors were highly significant when compared to controls (P<<0.001). **2,** Experimental flies were treated with hemolymph obtained from the legs of adult *Oncopeltus*, also reared on *Asclepias*. All scored behaviors were highly significant (P<0.001). **3,** Experimental flies were dipped in a 0.1 M solution of the cardenolide Ouabain in methanol, and dried. Flies so treated were coated with white, crystalline Ouabain, estimated by weighing at about 50 μg per fly. There was no significant difference between the reaction of control and experimental groups, even though the concentration of Ouabain on the surface of treated flies was close to 100%. Unfortunately, this also constituted a lethal dose, and five of the experimental subjects were completely immobilized and died within 12 hours of this experiment (black shaded feeding circles).

Table 3. Response of adult female *Phidippus audax* to fruit flies (*Drosophila melanogaster*) treated with Digitoxin. There was no significant difference between the observed response to Digitoxin and the control, treated with methanol, even though Digitoxin was toxic to these spiders.

	BEHAVIOUR	A. control (methanol)	B. Digitoxin	P(A=B)
1	spider attacked the treated fly (N)	18	18	—
2	spider wiped mouth or prey after attack	1	1	>0.05
3	spider dropped prey immediately or soon after capture of prey	1	0	>0.05
4	spider completedly macerated the prey	16	14	>0.05
5	spider was immobilized within 24 hours of attack	0	7	—
6	spider died without recovering mobility	0	4	—

Table 4. Response of adult female *Phidippus audax* to fruit flies (*Drosophila melanogaster*) treated with β-Ecdysone. These spiders reacted immediately to a very high concentration of β-Ecdysone on treated flies, and no spiders were immobilized or died as a result of these trials.

	BEHAVIOUR	A. control (methanol)	B. β-Ecdysone	P(A=B)
1	spider attacked the treated fly (N)	15	15	—
2	spider wiped mouth or prey after attack	1	7	—
3	spider dropped prey immediately or soon after capture of prey	0	15	<0.0001
4	spider completedly macerated the prey	15	0	<0.0001
5	spider was immobilized within 24 hours of attack	0	0	—

Avoidance effect of a single encounter with Oncopeltus. With few exceptions, naïve second instar *Phidippus texanus* (newly emergent, had never encountered any insect before) either turned away from sighted *Oncopeltus* (reared on *Asclepias*), or dropped the bugs immediately or shortly after capture. Apparent chemical punishment of the predator had a significant impact on the tendency to attack in a subsequent encounter one hour (± 5 min) after the initial attack (Table 5). The severity of subsequent attacks was reduced much more significantly, as the probability that the prey would be released immediately upon contact (and would therefore survive the attack) increased.

Table 5. Effect of a single encounter involving contact with a first instar *Oncopeltus* on the tendency of naïve (newly emergent from brood sac, had never encountered an insect), second instar *Phidippus texanus* to attack and to kill these bugs 1 hour (± 5 min) later. As in all subsequent avoidance experiments, these *Oncopeltus* were reared solely upon Common Milkweed (*Asclepias syriaca*) seeds. Each attack included both a pursuit and a jump at the prey.

	GROUP	N	fraction of spiders that:	
			attack the prey	kill the prey
A	naïve spiders that encounter bugs for the first time	99	0.83	0.52
			↕ P< 0.005	↕ P< 0.001
B	spiders with a single previous (1 hour earlier) encounter with a bug, involving physical contact	71	0.61	0.14
None of these spiders fed on a bug after it was captured				

The next set of trials involved older spiders (near sixth instar *Phidippus princeps*). Immediately (within 2 minutes) after a first encounter with *Oncopeltus*, almost all spiders refused to attack the bugs again (Table 6). The fact that the great majority of these spiders would still attack a vestigial-wing *Drosophila* of comparable size in this subsequent encounter shows that this avoidance of *Oncopeltus* was not associated with general suppression of all attack behavior.

Table 6. Effect of a single first encounter with a fourth instar *Oncopeltus* (reared on *Asclepias*) on the tendency of immature (near sixth instar) and naïve (no previous *Oncopeltus* experience) *Phidippus princeps* to attack either this bug (A), or a vestigial-wing *Drosophila* of comparable size (B) in a subsequent encounter within 2 minutes of the first encounter. The entire population of reared *P. princeps* spiderlings (N= 56) was divided randomly into groups A and B.

	GROUP	N	fraction of spiders that attack the prey
A+B	naïve spiders that encounter bugs for the first time	56	0.62
			↕ P< 0.001
A	second encounter with bug immediately after first encounter with bug	28	0.04
			↕ P< 0.001
B	second encounter with fly immediately after first encounter with bug	28	0.93

To determine whether there was a time based recovery of the tendency to attack, a different group of 60 immature (near sixth instar) *P. princeps* were divided randomly into 2 groups of 30 each. Both groups (A and B) were initially tested with a first encounter followed by an immediate (within 2 minutes) second encounter. The first group (A) was tested for a subsequent response 15 minutes after the first encounter with *Oncopeltus*, and the second group (B) was tested 120 minutes later. The results (Figure 10) show that recovery of the tendency to attack was virtually complete 120 minutes after the initial attack.
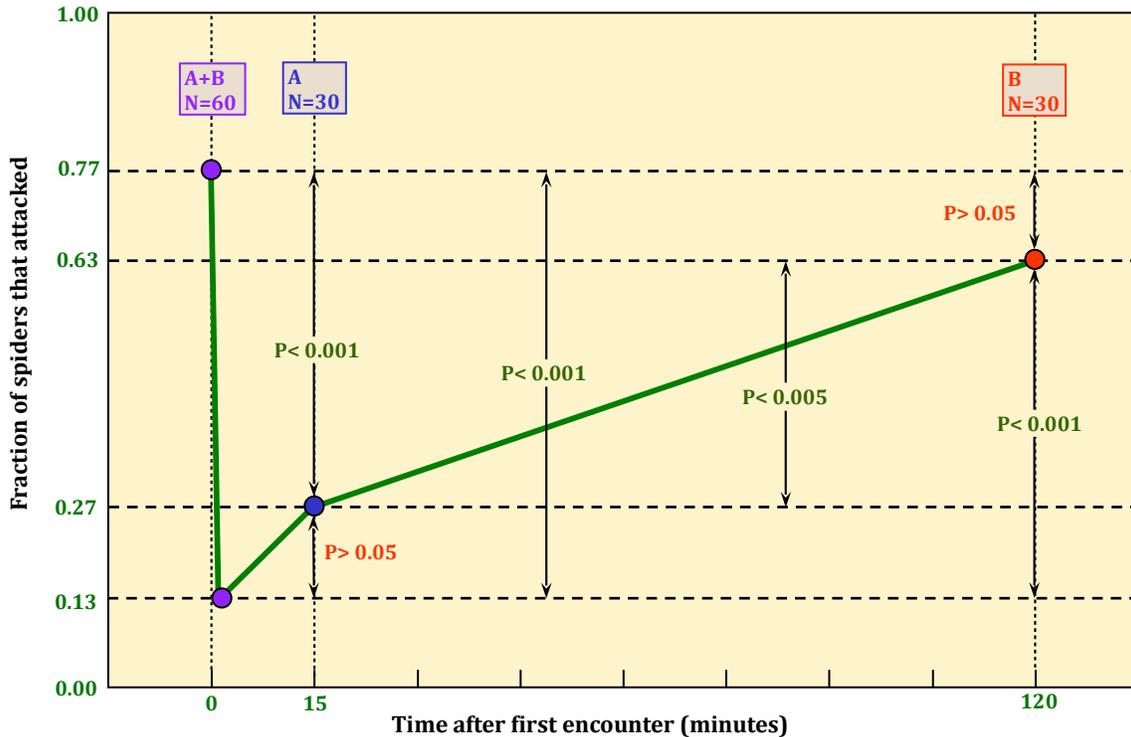

Figure 10. Recovery of tendency of immature (near sixth instar) *Phidippus princeps* to attack fourth instar *Oncopeltus* reared on *Asclepias*. Spiders of both groups (A+B) were tested with a first encounter at t=0, and a second encounter immediately (within 2 minutes) after this. Subsequently, group A spiders were tested 15 minutes after the first encounters, and group B spiders were tested 120 minutes after the first encounters. Recovery of the tendency to attack was significantly greater and almost complete after 120 minutes. All test spiders were fed a single fly (*Musca domestica*) 3 days before these trials.

Impact of repeated encounters with Oncopeltus. To investigate the cumulative impact of encounters with *Oncopeltus* on the attack behavior of *Phidippus*, a group of adult *P. audax* was divided into two groups of 40 spiders each (Figure 11). One group (B) was given a single encounter two hours in advance, otherwise both groups received the same treatment. The early encounter still had a significant impact four hours after it took place, supporting the idea that avoidance is a cumulative effect.
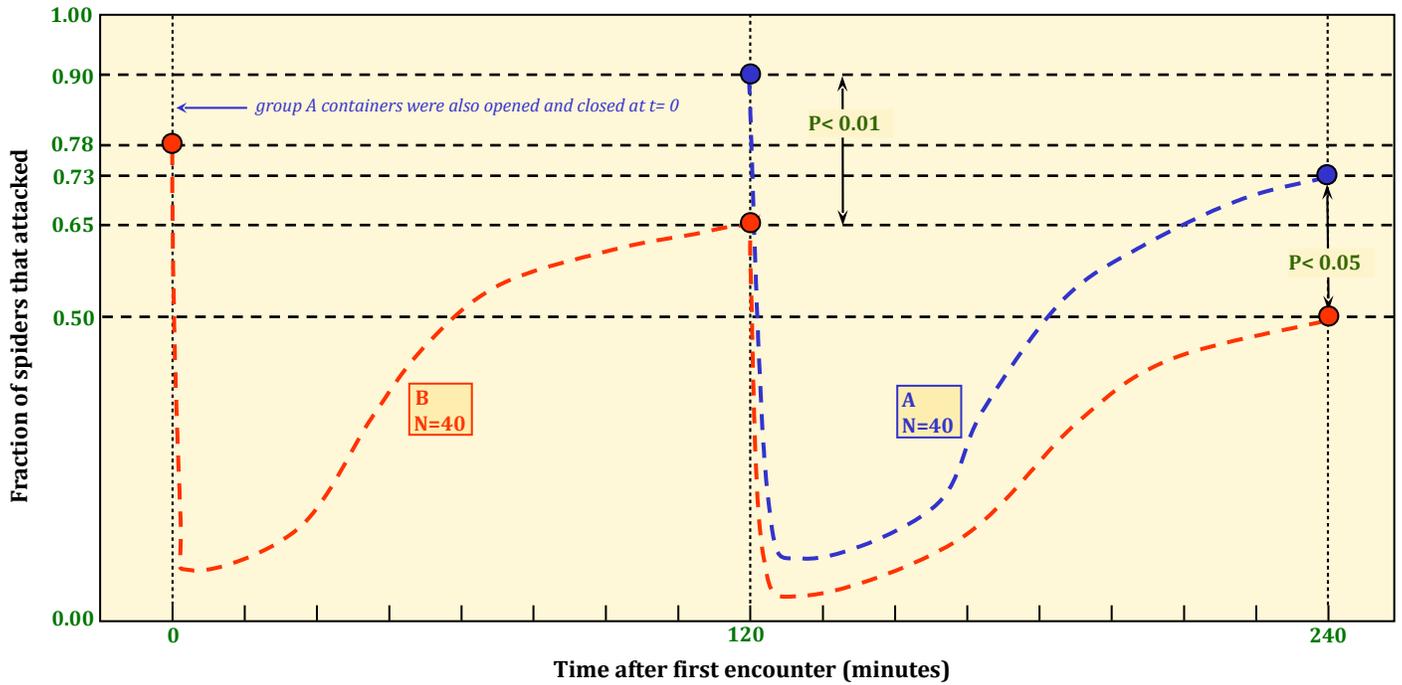
Figure 11. Impact of two isolated encounters on the tendency of adult *Phidippus audax* to attack adult *Oncopeltus fasciatus* reared on *Asclepias*. Group A spiders were tested with an encounter at t= 120 minutes and again at t= 240 minutes. Group B spiders were tested at t= 0 minutes, t= 120 minutes, and t= 240 minutes. Bugs were removed immediately after each encounter. The attack rate for Group B was significantly lower than that for Group A 4 hours after the initial encounter (far right). Circles represent actual trial data points, and connecting dashed lines show the estimated recovery trend based on other experimental results (see Figure 10). Spiders were deprived of all food for one week before these trials.

Repeated hourly trials with immature *P. princeps* (Figure 12) showed suppression of the tendency to attack after a series of repeated encounters, but also recovery of the tendency to attack by the next day.
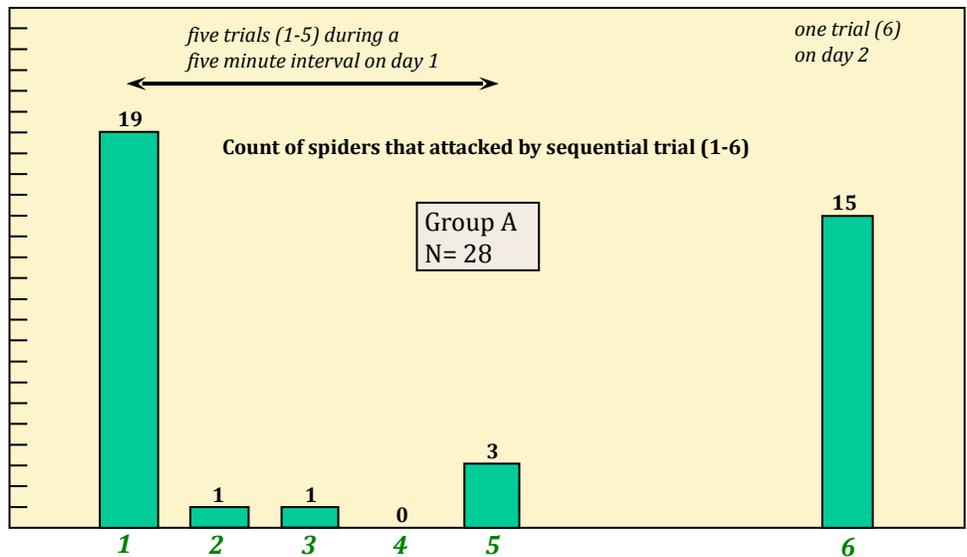

Figure 12. Each Group A spider associated with trials shown in Figure 10 (near sixth instar *P. princeps*, fourth instar *Oncopeltus* reared on *Asclepias*) was given 5 successive trials with *Oncopeltus* over a 5 minute period, and then a sixth trial on the next day. Note the recovery of the tendency to attack on the second day.

A longer period of exposure to *Oncopeltus* (10 hours) had a significant deterrent effect for several days. In the next series of trials (Table 7) each bug attack test was followed by a fly attack test. The tendency of *P. audax* to attack flies was not significantly changed by this experience with *Oncopeltus*, indicating again that this was prey-specific avoidance and not generalized feeding suppression.

Table 7. Impact of 10 hours cumulative experience with 4 fourth instar *Oncopeltus* reared on *Asclepias* on the tendency of immature (near 6 mm body length) *Phidippus audax* to attack. As part of each test, the spider was first tested with a bug, and then with a fly (vestigial-wing *Drosophila melanogaster*).

GROUP		N	fraction at t= 24 hours that		fraction at t= 48 hours that	
			attack bug	attack fly	attack bug	attack fly
A	no encounters for 10 hours, but dish was opened as a control at t= 0	30	0.87	0.93	0.73	0.86
			↕ P <0.001	↕ P >0.05	↕ P <0.01	↕ P >0.05
B	10 hours of exposure to 4 bugs in container beginning at t= 0	30	0.47	0.90	0.40	0.80

However, a much longer period of exposure (32 days) had no significant impact on the tendency of older *P. audax* to attack *Oncopeltus* 10 days later (Table 8).

Table 8. Apparent recovery of tendency of older *Phidippus audax* (seventh instar to adult) to attack fifth instar *Oncopeltus fasciatus* reared on *Asclepias* reared within 10 days. The test group (B) lived with 1-2 of these bugs in the same container for 32 days, then the bugs were removed 10 days before testing. At the onset, 60 spiders were assigned to each group, but some of these were in molting sacs at the scheduled test time and could not be tested. Feeding and watering schedules were the same for both groups. Spiders of both groups had no alternative prey for 17 days prior to testing.

	GROUP	N	fraction of spiders that attack the prey
A	control (no laboratory contact with *Oncopeltus* prior to testing)	56	0.41
			↕ P >0.05
B	32 days with 1-2 living *Oncopeltus*, ending 10 days before testing	53	0.45

Two hours of continuous experience with adult *Oncopeltus* had a significant impact on the tendency of adult P. audax to attack two hours after the last contact (Figure 13). At the same time, there was also a surprising recovery of the tendency to attack in only two hours, considering the many encounters that these spiders had with *Oncopeltus* during this two hour period.
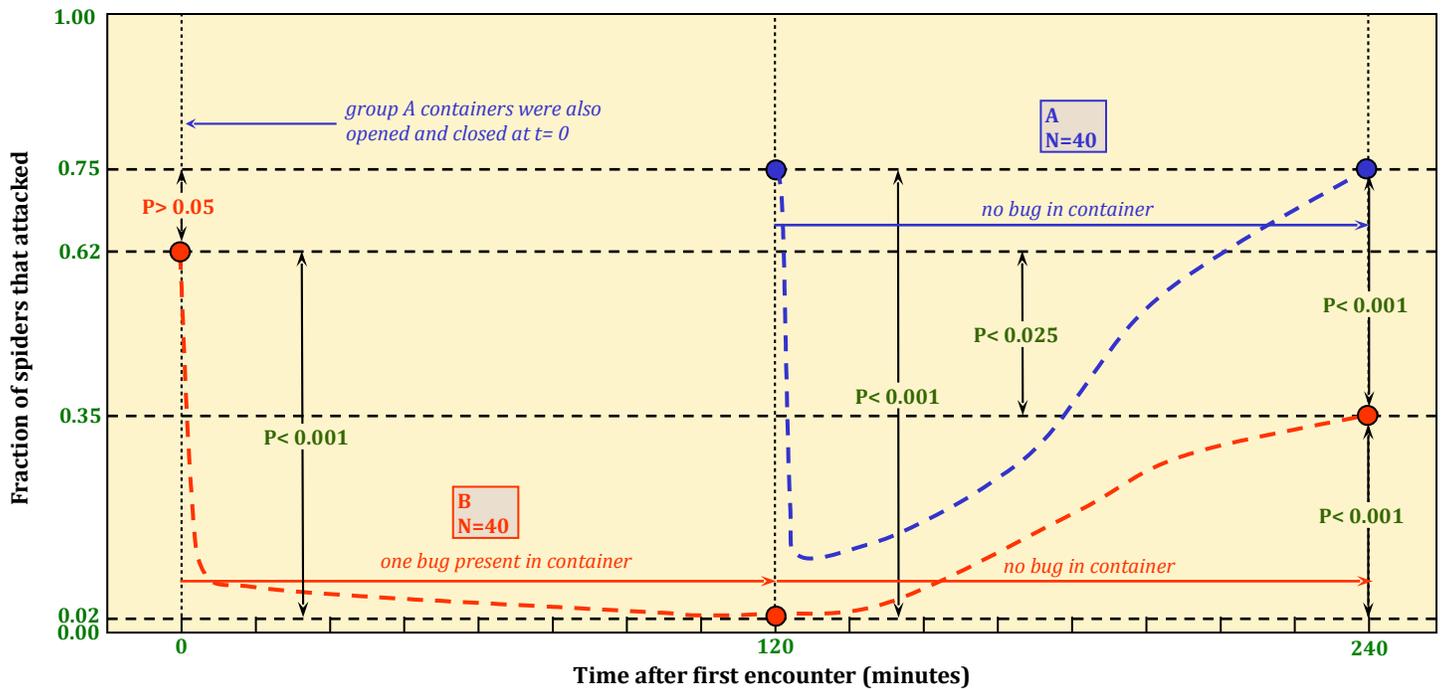
Figure 13. Impact of cumulative experience on the tendency of adult *Phidippus audax* to attack adult *Oncopeltus fasciatus* reared on *Asclepias*. Group A spiders were tested with an encounter at t= 120 minutes and again at t= 240 minutes. Group B spiders were tested at t= 0 minutes, t= 120 minutes, and t= 240 minutes. In addition, a bug was present in the container of each group B spider between t= 0 and t= 120 minutes. The attack response of spiders with only a single previous encounter (group A) recovered completely in 2 hours (far right), whereas the recovery of spiders exposed to bugs continuously over two hours (group B) was significantly slower. Circles represent actual trial data points, and connecting dashed lines show presumed trend based on other experimental results. In both groups, there was not a subset of spiders that consistently attacked or did not attack in the sequential trials. For example, many that did not attack in the first trial, did attack in the second trial. Thus this fraction that attack measurement appears to be a good estimate of the probability of attack by a given spider in a single encounter.

In each trial of the next series, an adult *Phidippus audax* was placed in the same container with an adult *Oncopeltus fasciatus*. A total of 40 different spiders were observed over a period of either 15 minutes, or until the third attack by the spider, whatever came first (Figure 14). From these data, it was possible to construct an averaged or cumulative recovery curve for attacks on *Oncopeltus* over a 15 minute interval (Figure 15). This approach differed significantly from previous experiments in that this was not a test of behavior during single encounters, but observation of *continuous encounters*. It might take 2 hours for the probability of attack during a single encounter to recover completely (Figure 11), but most of these adult spiders would attack a second or even a third time within 15 minutes in the continuous presence of an *Oncopeltus* adult.
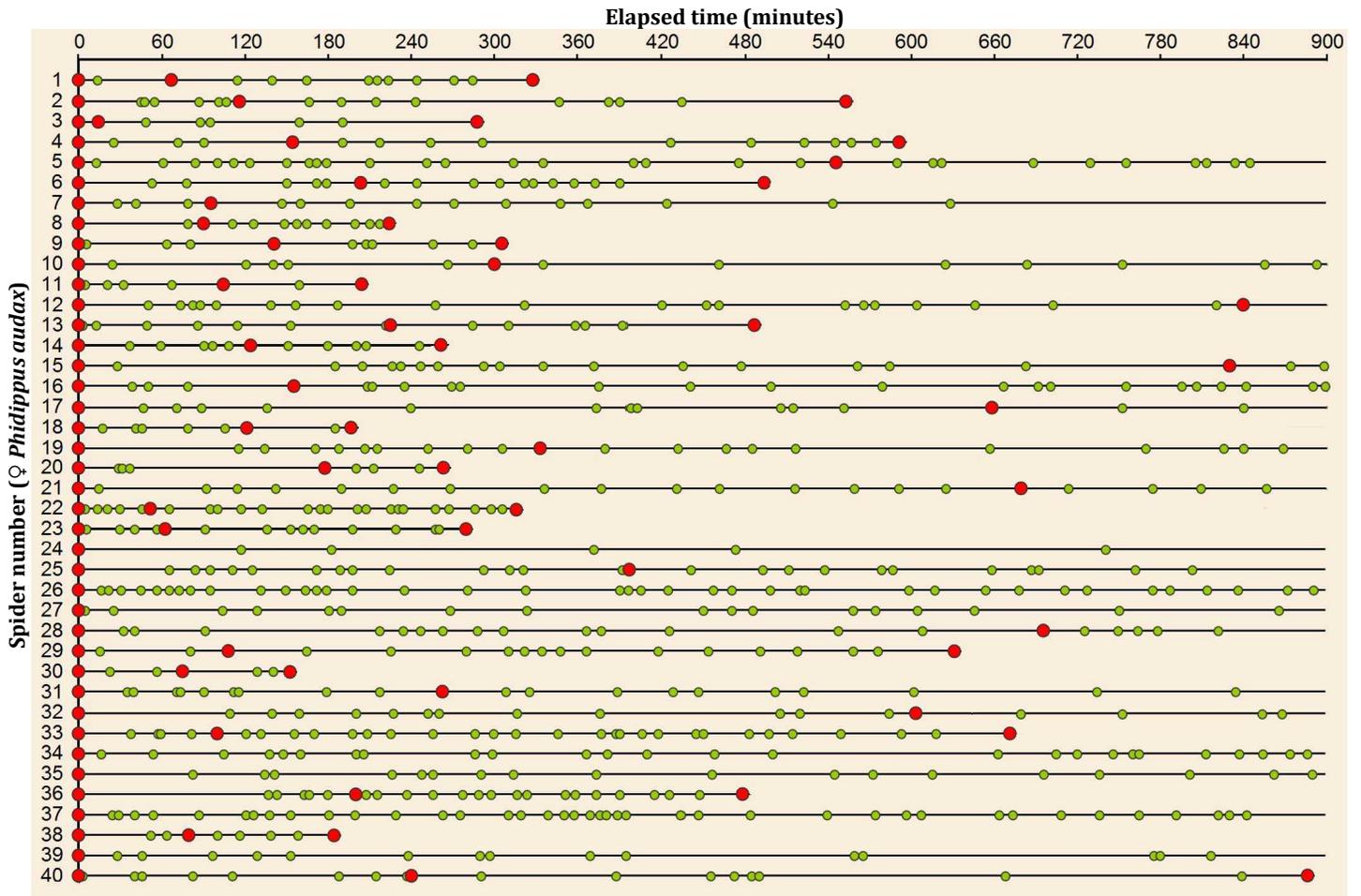
Figure 14. Recovery of tendency to attack by adult female *Phidippus audax*. Each spider was placed in a clean Petri dish with one adult Oncopeltus reared on Asclepias reared. After an initial attack (t=0), the behavior of 40 spiders (numbered at left) was charted through either the third sequential attack, or until 15 minutes had elapsed, whatever came first. Turns to face the bugs are shown as green circles, and attacks (jump and contact) are shown as red circles.
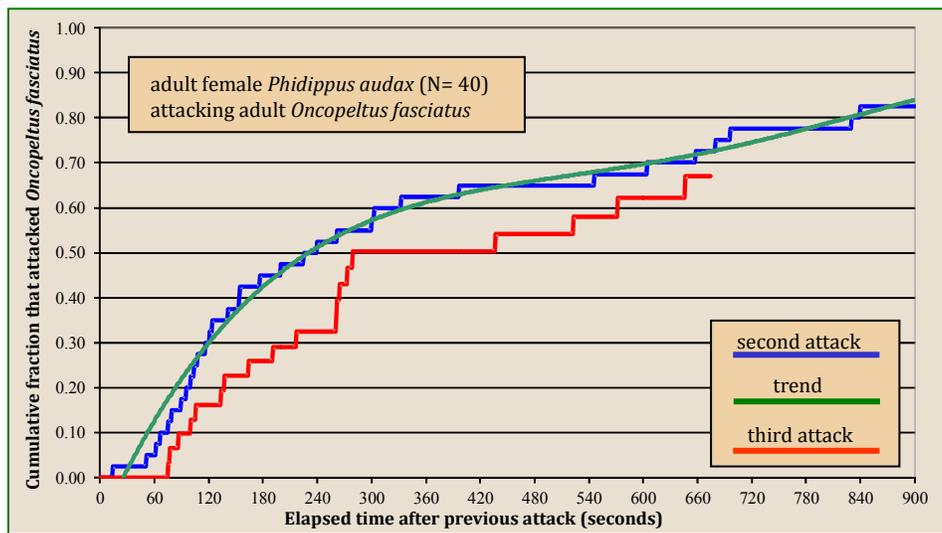

Figure 15. Cumulative fraction of spiders that attacked a second and third time as a function of elapsed time, based on data shown in Figure 14. In most cases the interval between second and third attacks was greater than the interval between first and second attacks (P<0.001).

Impact of alternative prey or feeding before trials. Feeding on a single fly significantly reduced the tendency to attack *Oncopeltus* the next day (Table 9), but did not reduce the tendency to attack other flies. This suggests both the positive impact of satiation on avoidance, as well the prey-specificity of that avoidance.

Table 9. Impact of satiation on the tendency of adult female *Phidippus audax* to attack adult *Oncopeltus* reared on *Asclepias*. None of the spiders were fed for 10 days prior to this experiment. House flies (*Musca domestica*) reared in the laboratory were used as alternative prey.

GROUP		N	fraction of spiders that	
			attack bug at t= 18 ±2 hours	attack fly at t= 19 ±2 hours
A	spiders not fed at t= 0	40	0.82	0.98
			↕ P <0.001	↕ P >0.05
B	spiders fed a single fly at t=0	40	0.40	1.00
None of these spiders fed on a bug after it was attacked				

Figure 16 depicts trials in which one group (B) of immature *Phidippus princeps* was given a single feeding (house fly, *Musca domestica*) on the day before testing. In this series of trials, both groups showed significant recovery of the tendency to attack on the second day, but avoidance was also significantly greater by all spiders that had eaten on the previous day.
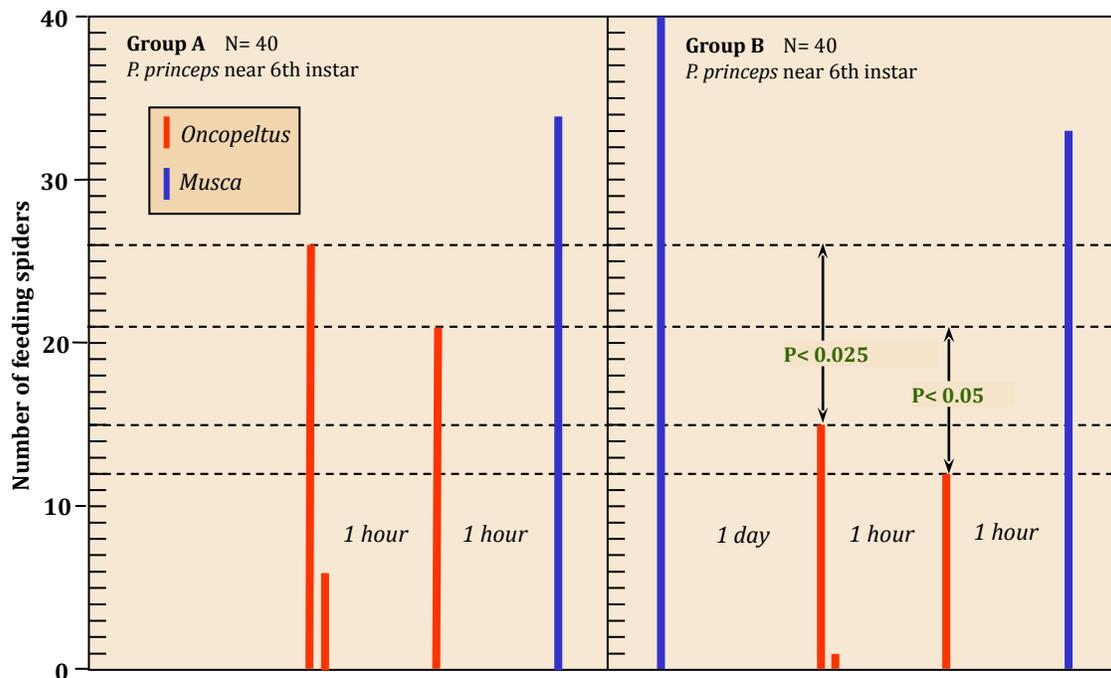

Figure 16. Count of attacking immature *Phidippus princeps* (near 6th instar) by sequential encounter. Spiders were deprived of all prey for at least 5 days prior to these trials. Sequential trials involved presentation, as shown above, of either *Oncopeltus* (7 mm nymphs, *Asclepias* reared) or much larger house flies, *Musca domestica*. Group B spiders were offered and fed upon a single *Musca* on the first day. Otherwise all testing for both groups took place on the second day and involved two successive encounters with *Oncopeltus* within one minute, followed by an encounter with an *Oncopeltus* one hour later, and then another presentation of a *Musca* one hour after that. All attacked flies were captured and fed upon, and all *Oncopeltus* were dropped. The tendency to attack *Oncopeltus* was significantly reduced for spiders that had eaten a fly on the previous day.

In a series of trials that were less controlled, but perhaps more indicative of a real-world situation, immature *P. audax* (N= 30) were given a series of hourly contacts over several days (Figure 17). Toward the end of the second day and into subsequent days, encounters with *Oncopeltus* alternated with feedings with flies. This was associated with a significant decline in the tendency to attack *Oncopeltus* relative to either of the fly species, a decline which persisted for at least 4 days when the spiders were not fed.
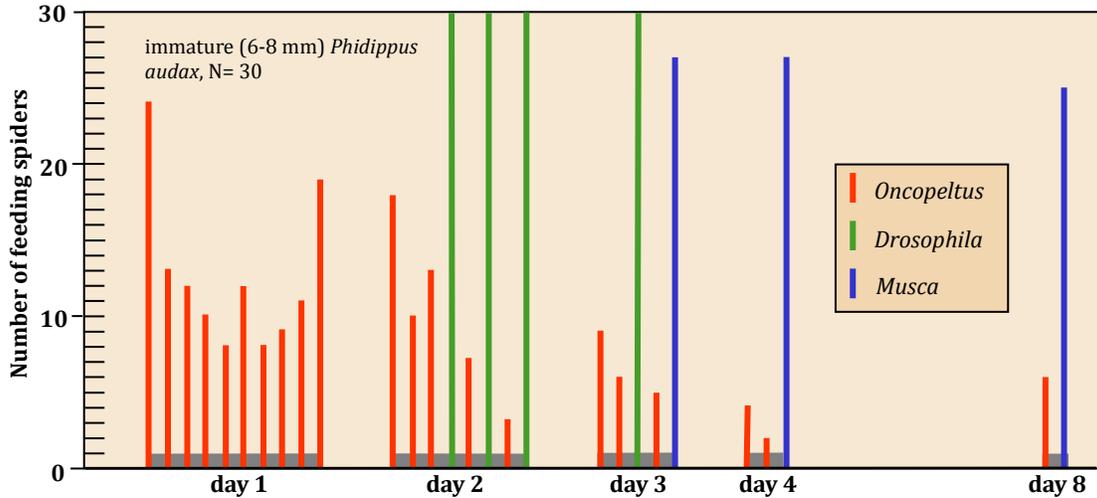

Figure 17. Count of attacking immature (6-8 mm) *Phidippus audax* by sequential encounter. Spiders were deprived of all prey for at least 7 days prior to these trials. Sequential trials involved presentation, as shown above, of either *Oncopeltus* (7 mm nymphs reared on *Asclepias*), vestigial-wing *Drosophila virilis*, or the much larger *Musca domestica*. Trials were separated by 1 hour during the first day. *Oncopeltus* trials were separated by 1 hour, but fly feedings followed these by 30 minutes, and *Oncopeltus* trials followed fly feedings by 90 minutes. There was no feeding or exposure to prey animals between day 4 and day 8.   All attacked flies were captured and fed upon, and all *Oncopeltus* were dropped.

Impact on tendency to attack other insects. As shown in Table 9 and Figures 16-17, negative experience with *Oncopeltus* did not reduce the tendency of *Phidippus* to attack flies (either *Musca* or *Drosophila*). A negative encounter with *Oncopeltus* also impacted the tendency of *Phidippus* to attack *Oncopeltus* more than it impacted the tendency to attack adult *Ortholomus scolopax*, a different lygaeid bug (Table 10). In addition, rejection of *Ortholomus* on the first encounter was associated with a higher rate of rejection of *Ortholomus* than of *Oncopeltus* on a second encounter. This is just a preliminary, isolated result. It does suggest that a great deal can be learned about the prey-specificity of avoidance through successive encounter experiments with different insects.

Table 10. Impact of deterred attacks on one lygaeid on the tendency of *Phidippus princeps* (6-8 mm, near 6th instar) to attack a lygaeid of a different genus. Both bugs (adult *Ortholomus scolopax* and fourth instar *Oncopeltus fasciatus* reared on *Asclepias*) were close to 5 mm in length. The second prey was presented 1-5 minutes after the first prey was rejected. Experiments were conducted on four different days, and no spider was tested more than once in a day.

	GROUP	N	fraction that attack prey		
A	spiders offered *Ortholomus* after attacking and rejecting *Oncopeltus*	64		0.48	
			↑	↕ P< 0.005	
B	spiders offered *Oncopeltus* after attacking and rejecting *Oncopeltus*	32	P <0.01	0.16	
			↓	↕ P >0.05	↑
C	spiders offered *Ortholomus* after attacking and rejecting *Ortholomus*	16		0.13	P <0.05
				↕ P>0.05	↓
D	spiders offered *Oncopeltus* after attacking and rejecting *Ortholomus*	58		0.36	

Discussion

Measurement of avoidance. There are many different ways to measure avoidance by salticids, each of which can produce a different result. As shown here, single encounter tests are useful, but they do not reflect a natural situation where encounters between many different kinds of insects take place, or where multiple encounters between a spider and one species of chemically-defended insect may take place in a short span of time. The fact that the tendency to attack recovers over a 1-2 hour interval after one encounter does not imply that protected insects are safe for that period of time, as with multiple encounters the probability of attack in a given interval increases greatly. Probability to attack per encounter is perhaps the best way to make a direct and controlled measurement of a change in tendency on the part of a spider.

Defense by Oncopeltus fasciatus. Under natural conditions, *Oncopelus fasciatus* often live in large aggregations (Sauer & Feir 1973; Figure 18). Ralph (1976) found that survival of nymphs in the field, but not in the laboratory, was greater if they were part of a large aggregation. As Ralph suggested, aggregation may improve access to milkweed seed pods, but it is also reasonable to assume that individuals in groups benefit from the reduced chance of injury related to the training of predators in the vicinity.

Figure 18. *Oncopeltus fasciatus* aggregating on *Asclepias* leaves and seed pods in southern Greenville County, South Carolina. These insects pierce seed pods to feed on seeds. **1,** Pair of immatures resting on top of an *Asclepias* leaf. **2,** Two adults feeding on seed pod. **3,** Aggregation of mating adult pairs. **4,** Lateral view of adult showing long stylus. **5,** Dorsal view of adult.

Oncopeltus may employ a series of defenses in nature, including escape. Other defenses are: aposematic coloration (visual advertisement), broadcast chemical advertisement, deterrent or identifying contact chemicals on the surface, deterrent taste of body fluids, and toxic effect of body fluids (very costly to the individual). Different defenses may be relevant to different predators. For example, mantids (*Tenodera*) were shown to feed upon *Oncopeltus* reared on *Asclepias*, but later regurgitated and subsequently avoided these insects (Berenbaum & Miliczky 1984; Gelperin 1968).

Phidippus (Figure 19) can clearly learn to avoid these bugs based on their physical appearance, at least for the short-term. However, the aposematic coloration by itself did not trigger a predictable response, at least under laboratory conditions. The fact that *Phidippus* readily fed on *Oncopeltus* that had been reared only on Sunflower (*Helianthus annuus*) seeds (also reported by Skow & Jakob 2006) supports the view that these bugs acquire critical components of their defense from *Asclepias*. *Phidippus* react strongly to both lateral metathoracic fluid and hemolymph of *Oncopeltus*. The presence of deterrent chemicals on the surface of *Asclepias*-fed Oncopeltus can also be inferred from the tendency of spiders to release these bugs immediately upon contact, in the absence of any visible fluid loss or injury.

Figure 19. Feeding *Phidippus* from southern Greenville County, South Carolina. **1,** Penultimate female *P. audax* with two leafhoppers. This spider held one leafhopper as it jumped and captured the second. **2,** Adult female *P. audax* fedding on a large brachyceran fly. **3,** Adult female *P. princeps* feeding on spider. **4,** Adult female *P. princeps* feeding on a captured bug after wiping it against the surface. Each scale bar = 1.0 mm.

The chemical deterrents that are used by *Oncopeltus* against *Phidippus* are not known. Aldehydes can be discharged from the metathoracic scent glands of adult *Oncopeltus* (Games & Staddon 1973a) and the dorsal abdominal glands of nymphs (Games & Staddon 1973b). However, a reduced role of these glands is indicated by their relatively poor development (Schaefer 1972), and the low aldehyde content of their secretion (Everton & Staddon 1979). Cardiac glycosides may be sequestered in the dorsolateral spaces of the adult (Duffey & Scudder 1974). These cardenolides are thought to have an emetic effect on a vertebrate predator (Brower *et al.* 1968), but their effect on spiders has not been demonstrated. As shown here, some well-known cardenolides (Ouabain or g-Strophanthin, and Digitoxin) are toxic to *Phidippus*, but these spiders did not recognize them and they had no inhibitory effect. However, in related studies *Phidippus* were found to immediately detect and reject defensive steroids (lucibufagins, Figure 5) associated with fireflies of the genera *Lucidota*, *Photinus* and *Photuris* (Eisner *et al.* 1997; Gronquist *et al.* 2006). More recently an alkyl methoxypyrazine (2-isobutyl-3-methoxypyrazine) considered to be a warning odorant was isolated from the fluids sequestered by these bugs from a milkweed (*Asclepias*) diet, in addition to the cardiac glycosides (Aldrich *et al.* 1997).

Learned avoidance Many arthropods are known to avoid stimuli associated with a punishing experience. For example, fruit flies (*Drosophila*) can be trained to avoid such odorants as benzaldehyde and 3-octanol after a single experience with an associated electric shock (Quinn *et al.* 1974; Dudai 1977). Gelperin (1968) trained mantids (*Paratenodira*, *Mantis*) to selectively avoid flies on a red (as opposite to white) background, through the administration of electric shocks to his subjects. These mantids, like *Phidippus*, also refused to attack *Oncopeltus* after several encounters, but likewise continued to attack flies. Bays (1962) found that the araneid spider *Araneus* could associate quinine-treated flies with a certain frequency of web vibration. According to frequency these spiders would either bite acceptable prey, or wrap and cut the unacceptable prey out of their webs.

Earlier descriptive accounts of salticid behavior support the view that these spiders have a selective, short-term memory with respect to the acceptability of potential prey. Dahl (1885) found that *Evarcha arcuata* would attack, and afterwards avoid, certain beetles (*Coccinella* and *Phyllobius*). Drees (1952) found that some *Salticus scenicus* could be trained to selectively avoid a cross or a triangle. However, this demonstration lacked a quantitative basis. After it was sprayed by the ant *Crematogaster clara*, *Anasaitis canosa*, normally a predator on ants, selectively avoided ants of this species (Edwards *et al.* 1974).

The present study suggests that learned avoidance may decay rather quickly, within a few hours. Others (Drees 1952; Precht & Freytag 1958; Dalwigk 1973) have described a similar recovery of the tendency to attack in salticids after habituation to a stimulus. Plett (1975) described this as stimulus-specific inhibition. We may think of volatile memory as a deficiency, but in fact this volatility may be very useful for salticid spiders. Short-term avoidance is useful if a chemically-defended insect is abundant in a given area, but it comes at the high cost of screening out many acceptable prey insects, on the basis of limited experience (as noted by Drees 1952).

Suppression of attacks may extend to more than just the aposematic species, but at least as shown with flies here (and with crickets, Skow & Jakob 2006), this suppression is not general. If a spider moves to a different site, it is also reasonable to assume that the prey that it encounters according to its scheme of classification have a greater probability of being different from those in the previous location (*context*). Skow & Jakob (2006) similarly described the learned avoidance of *Oncopeltus fasciatus*, reared on *Asclepias* seeds, by *Phidippus princeps*, and found that a change of context could erase this avoidance.

Impact of positive experience with prey. This has not been investigated here, but it is quite possible that positive experience with certain prey types leads to repeated and specific search behavior by salticids. If this can be demonstrated, then it is quite likely that this change of behavior is also associated with volatile memory and context-specificity.

Effect of feeding. Feeding, or satiation, can have a significant impact on the prey-selectivity of these spiders. For this reason, it is very important to maintain related controls (*e.g.*, same food and water regime for control and experimental animals) in this kind of experiment. Earlier studies (Drees 1952; Gardner 1964, 1966) also demonstrated this impact.

Visual taxonomy of the arthropod fauna by salticids. Whether we compare the response of *Phidippus* to *Oncopeltus* to their response to flies, as shown here, or to their response to crickets (Skow & Jakob 2006), it is clear that these salticids treat different insects in different ways. At one extreme, I have seen salticids take free-fall (no dragline) jumps in response to the sight of a quickly flying large metallic wasp. Clearly, salticids recognize conspecifics, or near-conspecifics, even if they frequently prey on them as well. The ability of *Phidippus* to vary their attack with respect to different types of prey is well-known (Edwards 1980; Edwards & Jackson 1993, 1994). It is as if there were a *fly type*, a *bug type*, a *large grasshopper type*, a *caterpillar type*, an *aphid type*, a *leafhopper* type, a *spider type*, an *ant type*, and so on.   As noted earlier, there is much opportunity for sequential encounter experiments involving different insects or even spiders as prey. There are also many published studies of prey selectivity by salticids (*e.g.*, Jackson 1997; Jackson & van Olphen 1991, 1992). There continues to be much opportunity to work out the details of the taxonomy of prey actually used by these spiders. Many spider studies (*e.g.*, *Argiope*, Robinson & Robinson 1976, and *Phidippus*, Edwards & Jackson 1994) suggest that this is an inherited taxonomy.

Chemical taxonomy of the arthropod fauna. Investigation of the ability of salticids to react to and to resolve a spectrum of different chemicals upon contact is in its infancy. As noted previously, several insect-related chemicals that are environmentally relevant to *Phidippus* are readily detected by them, and other toxic chemicals (*e.g.*, Ouabain) that have no known environmental relevance are not detected at all.

Gamesmanship by salticid spiders. We can combine hypotheses related to predatory avoidance and attraction into a more general model of the threshold for pursuit by jumping spiders that encompasses both facets of interaction with prey (Table 11). For animals that live at most for only several seasons, reliance on the inherited T as their long-term memory (*species memory*), and on E for only short-term memory many represent a very satisfactory solution. A more elaborate mathematical model linking behavior to risk and reward, and ultimately to survival and natural selection, would be instructive.

Table 11. Simple arithmetic model to communicate the concept of an attack threshold. This includes several of the most important factors related to the probability of attack, and addresses the general subjects mentioned in this paper. Hunger may also cause changes in foraging strategy, or drive a spider to relocate (Givens 1978). As noted by Skow & Jakob (2006), E values may be modified or reset when the spider moves to a different location (context). Identity of the prey taxon is definitely associated with T and E and, since it has something to do with the selection of the technique used to approach prey (Edwards 1980; Edwards & Jackson 1993, 1994), it may also impact the cost of pursuit (C).

A	ATTACK	fixed threshold for attack or pursuit
C	COST	work or effort associated with pursuit, including impact of relative position of the prey
E	EXPERIENCE	impact of recent interaction with prey on attraction (negative value after aversive experience)
H	HUNGER	appetite or hunger of the spider, including readiness to feed based on molting condition, breeding status, time of day, or other behavioral context
T	TAXON	attraction value for prey taxon, most likely inherited, and encompassing factors related to relative prey size, risk of injury to the predator, nutrient value, ease of capture, relative effort to subdue, optimal approach, and also linked to identifying characteristics related to form and movement
If (H + T + E) > (A +C), then the spider will attack		

Contact detection of deterrent chemicals by Phidippus. *Phidippus* exhibit a strong aversive reaction to contact between the ends of legs I and the surfaces of insects bearing either certain steroids (lucibufagins per Eisner *et al.* 1997, or β-Ecdysone per this study) or Z-dihydromatricaria acid (Eisner *et al.* 1981). A recent study (Hoefler *et al.* 2002) found no signs that *P. audax* used chemical cues left on filter paper to detect prey, but did find that these were used by a lycosid spider, *Pardosa milvina*. However, an earlier study (Clark *et al.* 2000) found that *Habrocestum pulex* reacted to chemical cues left by its ant prey on a soil substratum, or in the air, but did not react to chemical cues left on filter paper.

The legs of salticid spiders carry whorled setae (*spondylae*) in association with the adhesive tenent setae of the pretarsus (Figure 20; Hill 1977, 2010b). These are also found on the ends of the pedipalps, often in considerable abundance. Based on structure and distribution, they have long been thought to be contact chemoreceptors (Foelix 1970, Foelix & Chu-Wang 1973), and this has been confirmed recently in wolf spiders (Lycosidae) through electrophysiological recording of receptors (Drewes & Bernard 2005). This study confirmed the earlier morphological evidence, and identified several mechanoreceptor neurites, as well as numerous chemoreceptor neurites in association with an open pore at the end of each spondyla. The pore is at the apex of the terminal cone shown in Figure 20.
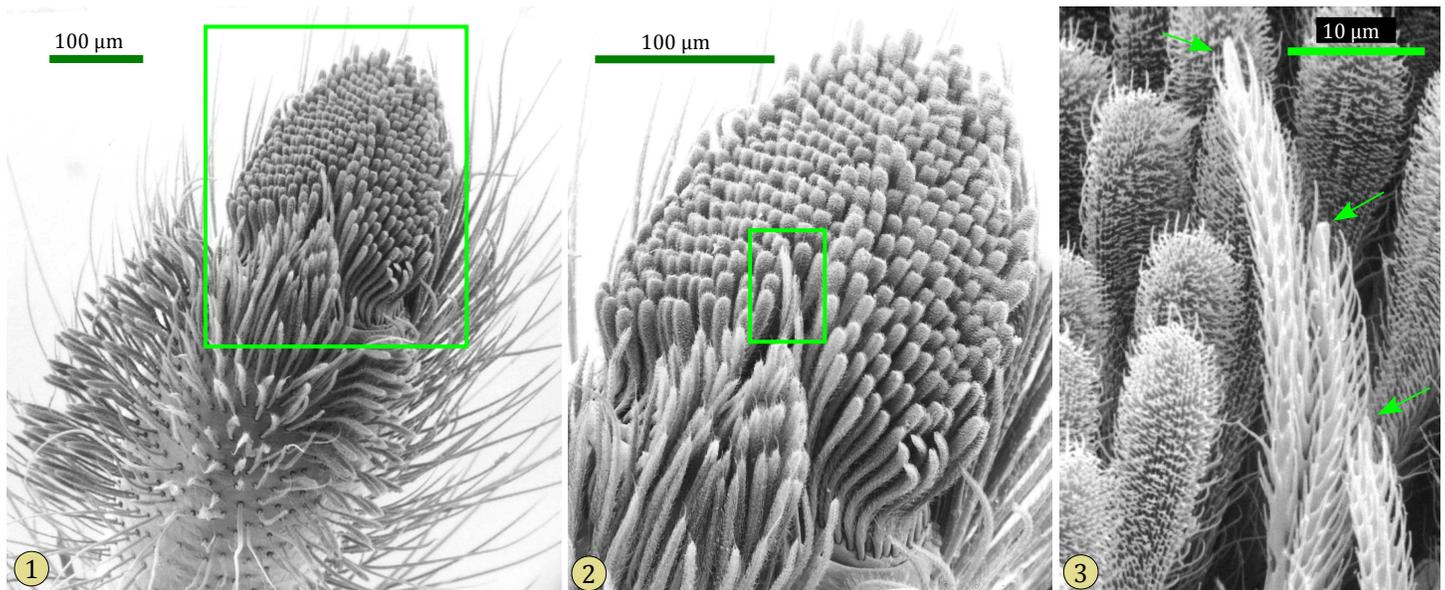

Figure 20. SEM of chemosensory setae (spondylae) associated with the pretarsus or foot of an adult male *Phidippus audax* from Iowa City, Iowa. **1-3,** Ventral views of the distal end of right leg I at three levels of magnification. **2,** A group of spondylae (inset) originates between the anterior and posterior plates of flattened tenent setae. **3,** Detail showing the conical tip (arrows) at the end of three spondylae. These are surrounded by flattened tenent setae bearing, ventrally, regular rows of bifid filaments that adhere to a smooth surface. Each spondyla bears an open sensory pore at the apex of the cone.

Insects that advertise with contact chemicals that can be detected have less risk of injury in an encounter with *Phidippus* than do those that rely on internal fluids alone. Early detection must benefit the spider as well.

Acknowledgments

References

Aldrich, J. R., W. S. Leal, R. Nishida, A. P. Khrimian, C. J. Lee and Y. Sakuratani. 1997. Semiochemistry of aposematic seed bugs. Entomologica Experimentalis et Applicata 84: 129-135.

Banks, N. 1906. Descriptions of new American spiders. Proceedings of the Entomological Society of Washington 7: 94-100.

Bays, S. M. 1962. Training possibilities of *Araneus diadematus*. Experientia 18: 423-424.

Blest, A. D., R. C. Hardie, P. McIntyre and D. S. Williams. 1981. The spectral sensitivities of identified receptors and the function of retinal tiering in the principal eyes of a jumping spider. Journal of Comparative Physiology 145: 227-239.

Berenbaum, M. R., and E. Miliczky. 1984. Mantids and milkweed bugs: efficacy of aposematic coloration against invertebrate predators. The American Midland Naturalist 111 (1): 64-68.

Brower, L. P., W. N. Ryerson, L. L. Coppinger and S. C. Glazier. 1968. Ecological chemistry and the probability spectrum. Science 161: 1349-1351.

Carducci, J. P., and E. M. Jakob. 2000. Rearing environment affects behaviour of jumping spiders. Animal Behaviour 59: 39-46.

Clark, R. J., R. R. Jackson and B. Cutler. 2000. Chemical cues from ants influence predatory behavior in *Habrocestum pulex*, an ant-eating jumping spider (Araneae, Salticidae). The Journal of Arachnology 28: 309-318.

Dahl, F. 1885. Versuch einer Darstellung der psychischen Vorgange in den Spinnen. Vierteljahrsschrift für wissenschaftliche Philosophie und Soziologie 9: 84-103.

Dalwick, H. B. von. 1973. Über die Temperaturabhangigkeit von Instinkthandlungen bei Libellenlarven (*Aeschna cyanea* Mull.) und Springspinnen (*Salticus scenicus* Cl.). Zoologisches Anzeiger 169: 292-298.

DeVoe, R. D. 1975. Ultraviolet and green receptors in principal eyes of jumping spiders. Journal of General Physiology 66: 193-207.

Drees, O. 1952. Untersuchungen uber die angeborenen Verhaltenswiesen bei Springspinnen (Salticidae). Zeitschrift für Tierpsychologie 9: 169-207.

Drewes, C. D. and R. A. Bernard. 2005. Electrophysiological responses of chemosensitive sensilla in the wolf spider. Journal of Experimental Zoology 198 (3): 423-428.

Dudai, Y. 1977. Properties of learning and memory in *Drosophila melanogaster*. Journal of Comparative Physiology 114: 69-89.

Duelli, P. 1978. Movement detection in the posterolateral eyes of jumping spiders (*Evarcha arcuata*, Salticidae). Journal of Comparative Physiology 124: 15-26.

Duffey, S. S., and G. G. E. Scudder. 1972. Cardiac glycosides in North American Asclepiadaceae, a basis for unpalatability in brightly colored Hemiptera and Coleoptera. Journal of Insect Physiology 18: 63-78.

Duffey, S. S., and G. G. E. Scudder. 1974. Cardiac glycosides in *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae) I. The uptake and distribution of natural cardenolides in the body. Canadian Journal of Zoology 52: 283-290.

Edwards, G. B. 1980. Taxonomy, ethology, and ecology of *Phidippus* (Araneae: Salticidae) in eastern North America. Ph.D. Dissertation, University of Florida: 1-353.

Edwards, G. B. 2004. Revision of the jumping spiders of the genus *Phidippus* (Araneae: Salticidae). Occasional Papers of the Florida State Collection of Arthropods 11: i-viii, 1-156.

Edwards, G. B., J. F. Carroll and W. H. Whitcomb. 1974. *Stoidis aurata* (Araneae: Salticidae), a spider predator of ants. Florida Entomologist 67: 337-346.

Edwards, G. B. and R. R. Jackson. 1993. Use of prey-specific predatory behaviour by North American jumping spiders (Araneae, Salticidae) of the genus *Phidippus*. Journal of Zoology, London 229: 709-716.

Edwards, G. B. and R. R. Jackson. 1994. The role of experience in the development of predatory behaviour in *Phidippus regius*, a jumping spider (Araneae, Salticidae) from Florida. New Zealand Journal of Zoology 21: 269-277.

Eisner, T., M. A. Goetz, D. E. Hill, S. R. Smedley and J. Meinwald. 1997. Firefly "femmes fatales" acquire defensive steroids (lucibufagins) from their firefly prey. Proceedings of the National Academy of Sciences, USA 94: 9723-9728.

Eisner, T., D. Hill, M. Goetz, S. Jain, D. Alsop, S. Camazine and J. Meinwald. 1981. Antifeedant action of Z-dihydromatricaria acid from soldier beetles (*Chauliognathus* spp.). Journal of Chemical Ecology 7 (6): 1149-1158.

Everton, I. J., and B. W. Staddon. 1979. The accessory gland and metathoracic scent gland function in *Oncopeltus fasciatus*. Journal of Insect Physiology 25: 133-141.

Foelix, R. F. 1970. Chemosensitive hairs in spiders. Journal of Morphology 132: 313-334.

Foelix, R. F. and I-W. Chu-Wang. 1973. The morphology of spider sensilla. II. Chemoreceptors. Tissue and Cell 5: 461-478.

Games, D. E. and B. W. Staddon. 1973a. Chemical expression of sexual dimorphism in the tubular scent glands of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). Experientia 29: 532-533.

Games, D. E. and B. W. Staddon. 1973b. Composition of scents from the larva of the milkweed bug *Oncopeltus fasciatus* (Hem. Het. Lygaeidae). Journal of Insect Physiology 19: 1527-1532.

Gardner, B. T. 1964. Hunger and sequential responses in the hunting behavior of salticid spiders. Journal of Comparative Physiology and Psychology 58: 167-173.

Gardner, B. T. 1966. Hunger and characteristics of the prey in the hunting behavior of salticid spiders. Journal of Comparative Physiology and Psychology 62: 475-478.

Gelperin, A. 1968. Feeding behavior of the preying mantis: a learned modification. Nature 219: 399-400.

Givens, R. P. 1978. Dimorphic foraging strategies of a salticid spider (*Phidippus audax*). Ecology 59: 309-321.

Gronquist, M., F. C. Schroeder, H. Ghiradella, D. Hill, E. M. McCoy, J. Meinwald and T. Eisner. 2006. Shunning the night to elude the hunter: diurnal fireflies and the "femmes fatales." Chemoecology 16: 39-43.

Hentz, N. M. 1845. Descriptions and figures of the araneides of the United States. Boston Journal of Natural History 5: 189-202.

Hill, D. E. 1977. The pretarsus of salticid spiders. Zoological Journal of the Linnean Society, London 60: 319-338.

Hill, D. E. 2006a. Use of location (relative direction and distance) information by jumping spiders (Araneae, Salticidae, *Phidippus*) during movement toward prey and other sighted objectives. Version 2. Peckhamia Epublication. 1-72.

Hill, D. E. 2006b. Learned avoidance of the large milkweed bug (Hemiptera, Lygaeidae, *Oncopeltus fasciatus*) by jumping spiders (Araneae, Salticidae, *Phidippus*). Version 1 (August 15, 2006). Peckhamia Epublication. 1-21.

Hill, D. E. 2010a. Use of location (relative direction and distance) information by jumping spiders (Araneae, Salticidae, *Phidippus*) during movement toward prey and other sighted objectives. Peckhamia 83.1: 1-103.

Hill, D. E. 2010b. Jumping spider feet (Araneae: Salticidae). Peckhamia 85.1: 1-48.

Hoefler, C. D., M. Taylor and E. M. Jakob. 2002. Chemosensory response to prey in *Phidippus audax* (Araneae, Salticidae) and *Pardosa milvina* (Araneae, Lycosidae). The Journal of Arachnology 30: 155-158.

Isman, M. B. 1977. Dietary influence of cardenolides on larval growth and development of the milkweed bug *Oncopeltus fasciatus*. Journal of Insect Physiology 23: 1183-1187.

Isman, M. B., S. S. Duffey and G. G. E. Scudder. 1977. Variation in the cardenolide content of the lygaeid bugs *Oncopeltus fasciatus* and *Lygaeus kalmii kalmii*, and of their milkweed hosts (*Asclepias* spp.) in central California. Journal of Chemical Ecology 3: 613-624.

Jackson, R. R. 1977. Prey of the jumping spider *Phidippus johnsoni* (Araneae: Salticidae). Journal of Arachnology 5: 145-149.

Jackson, R. R. and A. van Olphen. 1991. Prey-capture techniques and prey preferences of *Corythalia canosa* and *Pystira orbiculata*, ant-eating jumping spiders (Araneae, Salticidae). Journal of Zoology, London 223: 577-591.

Jackson, R. R. and A. van Olphen. 1992. Prey-capture techniques and prey preferences of *Chrysilla*, *Natta* and *Siler*, ant-eating jumping spiders (Araneae, Salticidae) from Kenya and Sri Lanka. Journal of Zoology, London 227: 163-170.

Keyserling, E. 1885. Neue Spinnen aus America. VI. Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien 34: 489-534.

Land, M. F. 1971. Orientation by jumping spiders in the absence of visual feedback. Journal of Experimental Biology 54: 119-139.

Lim, M. L. M. and D. Li. 2006. Behavioural evidence of UV sensitivity in jumping spiders (Araneae: Salticidae). Journal of Comparative Physiology A 192: 871-878.

Meinwald, J., Y. C. Meinwald, A. M. Chalmers and T. Eisner. 1968. Dihydromatricaria acid: Acetylenic acid secreted by soldier beetle. Science 160: 890-892.

Peckham, G. W. and E. G. Peckham. 1883. Descriptions of new or little known spiders of the family Attidae from various parts of the United States of North America. Milwaukee. 1-35.

Peckham, G. W. and E. G. Peckham. 1901. Spiders of the *Phidippus* group of the family Attidae. Transactions of the Wisconsin Academy of Sciences, Arts and Letters 13: 282-358.

Plett, A. 1975. Über die Ermüdung von Teilhandlungen des Beutefangs bei Springspinnen (*Salticus scenicus* Cl.), Libellenlarven (*Aeschna cyanea* Mull.) und Ameisenlowen (*Euroleon nostras* Fourcr.). Zoologisches Anzeiger 195: 8-20.

Precht, H., and G. Freytag. 1958. Über Ermüdung und Hemmung angeborenen Verhaltensweisen bei Springspinnen (Salticidae). Zugleich ein Beitrag zum Triebproblem. Behaviour 13: 143-211.

Quinn, W. G., W. A. Harris, and S. Benzer. 1974. Conditioned behavior in *Drosophila melanogaster*. Proceedings of the National Academy of Science, Washington 71: 708-712.

Ralph, C. P. 1976. Natural food requirements of the large milkweed bug *Oncopeltus fasciatus* (Hemiptera : Lygaeidae) in their relation to gregariousness and host plant morphology. Oecologia 26: 157-176.

Robinson, M. H. and B. Robinson. 1976. Discrimination between prey types: an innate component of the predatory behavior of araneid spiders. Zeitschrift für Tierpsychologie 41: 266-276.

Rothschild, M., J. van Euw, T. Reichstein, D. A. S. Smith and J. Pierre. 1975. Cardenolide storage in *Danaus chrysippus* (L.) with additional notes on *D. plexippus* (L.). Proceedings of the Royal Society of London 190B: 1-31.

Sauer, D. and D. Feir. 1973. Studies on natural populations of *Oncopeltus fasciatus* (Dallas), the large milkweed bug. American Midland Naturalist 90: 13-37.

Schaefer, C. W. 1972. Degree of metathoracic scent gland development in the trichopterus Heteroptera (Hem.). Annals of the Entomological Society of America 65: 13-37.

Schoonhoven, L. M. 1977. On the individuality of insect feeding behavior. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen C 80: 341-350.

Scudder, G. G. E. and S. S. Duffey. 1972. Cardiac glycosides in the Lygaeinae (Hemiptera: Lygaeidae). Canadian Journal of Zoology 50: 35-42.

Skow, C. D. and E. M. Jakob. 2006. Jumping spiders attend to context during learned avoidance of aposematic prey. Behavioral Ecology 17: 34-40.

Vaughan, F. A. 1979. Effect of gross cardiac glycoside content of seeds of common milkweed, *Asclepias syriaca*, on cardiac glycoside uptake by the milkweed bug *Oncopeltus fasciatus*. Journal of Chemical Ecology 5: 89-100.