

**The effect of prior exposure to predator cues on
chemically-mediated defensive behavior and survival
in the wolf spider *Rabidosa rabida*
(Araneae: Lycosidae)**

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Summary

Adults of the wolf spider *Pardosa milvina* are intraguild predators of spiderlings of the larger co-occurring wolf spider *Rabidosa rabida*. We examined the effect of prior exposure to various *Pardosa* cues (visual, vibratory, and chemical) on predator-naïve *Rabidosa* spiderling activity and survival. Each trial consisted of a pre-exposure movement test, 24-h exposure to one of five sensory treatments, a post-exposure movement test and finally a predation experiment with live *Pardosa* and associated chemical cues to assay the effectiveness of the spiderling's response. The five 24-h sensory treatments were ($N = 20$ /treatment): (1) *Pardosa* visual and vibratory cues, (2) *Pardosa* chemical cues only (silk and excreta), (3) visual, vibratory and chemical *Pardosa* cues, (4) chemical cues from a cricket (*Acheta domesticus*) and (5) no 24-h exposure to any *Pardosa* cues but chemical cues during the post-exposure movement test and during the predation experiment. In a sixth treatment we also measured spiderling movement and survival without any prior experience and no chemical cues from a predator. *Rabidosa* significantly reduced activity after 24-h exposure for all sensory treatments except the cricket and two no experience control groups. Treatments involving 24-h exposure to *Pardosa* chemical cues resulted in increased survival in the predation portion of the experiment relative to other sensory treatments. We conclude that (1) *Rabidosa* innately responds to *Pardosa* silk and excreta, (2) extended exposure to predator chemical cues enhances *Rabidosa* antipredator response and survival and (3) predator recognition is influenced more strongly by chemical rather than visual or vibratory predator cues.

Keywords: multi-modal, antipredator, predator recognition, learning, chemical cue, lycosid.

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Introduction

Animals that range widely through the environment need to gather as much information as possible regarding the presence of predators. Early detection and recognition of a predator allows for effective deployment of defensive responses such as freezing, hiding, cover seeking, and avoidance (reviewed in Kats & Dill, 1998). These responses, in turn, often increase survival of prey (Downes & Shine, 1998). Animals may use a variety of sensory modalities to perceive and recognize predators as a potential threat. Such recognition may be largely innate (Giles, 1984; Riechert & Hedrick, 1990; Veen et al., 2000; Hawkins et al., 2004), learned through association (Suboski, 1990; Chivers & Smith, 1995; Chivers et al., 1996; Wildy & Blaustein, 2001; Blumstein et al., 2002) or may be innate but subsequently modified by experience (Magurran, 1990; Wisenden & Millard, 2001). Different animals may have particular sensory biases with respect to learned associations (Garcia et al., 1974), as well as biases with regard to specific sensory channels that induce predator recognition and anti-predator responses (Stauffer & Semlitsch, 1993; Mathis & Vincent, 2000). There are also a number of non-associative effects that may give rise to changes in response as well (Terry & Hirsch, 1997). After longer periods of exposure, animals may become sensitized or primed to particular types of sensory cues and exhibit increasingly stronger responses rather than become habituated to these cues (Papaj & Prokopy, 1989; Turlings et al., 1992).

The mechanism of predator recognition as it relates to the effectiveness of presumed anti-predator responses is far from understood in many animals. However, for most animals it is likely that predator recognition involves a complex interplay of innate responses, prior experience and information acquired via interactions of multiple sensory modalities. Studies with vertebrates have shown that prey may have learning biases with respect to the sensory modes used to recognize predators (Brown et al., 2000). For example some species of fish learn anti-predator behaviors through association with combinations of diet-based chemical cues released by the predatory fish and the visual confirmation of the predator (Brown & Godin, 1999). However, visual confirmation of the predator alone may not induce as much predator avoidance as visual and chemical cues combined. Further, some characin fish are not able to identify the predator at all without chemical cues present (Brown & Godin, 1999).

Associative learning of chemical cues within the context of host localization, foraging, and prey preferences have been well demonstrated in a number of different arthropods (Van Lenteren & Bakker, 1975; Turlings et al., 1989; Papaj & Vet, 1990; Godfray, 1994; Morse, 2000; Persons & Rypstra, 2000), but the influence of prior experiences on arthropod antipredator behavior has received less attention (Craig, 1994; Chivers et al., 1996; Wisenden et al., 1997; Wcislo & Schatz, 2003). Further, most studies of learned antipredator behavior have not tested the effectiveness of such responses in the presence of a live predator, and of these studies, the majority have focused on fish (Mirza & Chivers, 2000; Chivers et al., 2002; Darwish et al., 2005). In this study we examined the effect of exposure to various sensory cues associated with a potential predator on naïve spiderling response and subsequent survival in the presence of a live predator.

Wolf spiders (Family Lycosidae) are often among the most abundant ground-dwelling predators throughout agroecosystems of the Eastern United States (Marshall & Rypstra, 1999a). Different species often engage in intraguild predation, feeding on smaller heterospecific lycosids, as well as smaller conspecifics (Hallander, 1970; Wise & Wagner, 1992). As such, predator-prey relationships are not static among these predators and therefore wolf spiders may benefit from a flexible predator-recognition system.

Wolf spiders employ a variety of sensory modes to obtain information about their environment. They can use their large simple eyes, which are particularly good movement detectors, during mate searching and prey localization (McClintock & Uetz, 1996; Rovner, 1996; Scheffer et al., 1996; Persons & Uetz, 1997; Hebets & Uetz, 1999). Lycosids also have various slit sensillae and lyriform organs used to detect substratum-borne vibratory cues from prey and conspecifics (Rovner, 1967; Persons & Uetz, 1996a; Scheffer et al., 1996; Hebets & Uetz, 1999). In many cases, these different sensory modes interact. For example, in the nocturnal capture of fireflies by one species of wolf spider, vibratory cues initiate prey stalking and visual cues are secondarily used for targeting and tracking prey (Lizotte & Rovner, 1988). However, other species of wolf spider localize prey primarily through visual information (Persons, 1999; Persons & Uetz, 1999). Lycosids are also known to use chemical information while searching for prey (Persons & Uetz, 1996b; Punzo & Kukoyi, 1997; Persons & Rypstra, 2000; Hoefler et al., 2002) and avoiding predators (Punzo, 1997; Persons et al., 2001; Barnes et al., 2002).

Tactile or chemical cues found in spider silk and excreta appear to be the principle source of information used to detect the presence of a potential predator for at least one species of wolf spider, *Pardosa milvina* (Persons et al., 2001, 2002). *Pardosa milvina* shows freezing behavior, reduced activity, and avoidance in the presence of silk and excreta from a larger predatory species of wolf spider, *Hogna helluo* (Persons et al., 2001; Barnes et al., 2002). Given the diversity of sensory modalities used during intraspecific communication, prey detection, and predator avoidance, it remains largely unknown how other types of sensory information may interact with chemical cues to shape predator recognition among lycosids. The relative importance of prior predator exposure versus innate responses on predator recognition also remains largely unknown among most species of spider; however studies have shown that wolf spiders are capable of modifying their behavior based on prior experience (Punzo, 1997, 2000; Persons & Rypstra, 2000; Hebets, 2003).

Rabidosa rabida and *Pardosa milvina* are syntopic lycosids that frequently engage in intraguild predation (personal observation). Adult *Rabidosa* may be more than ten times the mass of an adult *Pardosa*, but spiderlings and small juvenile *Rabidosa* are well within the preferred prey size of adult *Pardosa* and are readily consumed under field and laboratory conditions. Field surveys indicate that *Pardosa* are at least ten times more common, with mid-summer densities in no-till corn fields reaching nearly 6 individuals/m² (unpub. data; Marshall & Rypstra, 1999b). At such high densities, it is likely that *Pardosa* may be one of the most common predators of *Rabidosa* spiderlings.

We had two objectives for this study. First we compared the relative importance of different predator (*Pardosa milvina*) and non-predator (domestic crickets) sensory experiences (visual and vibratory cues together, chemical cues alone, visual, vibratory and chemical combined) on modifying the activity level of predator-naïve *Rabidosa rabida* spiderlings. Second, we determined whether any shifts in behavior due to prior predator sensory experiences translated into increased survival when in the presence of a live predator coupled with and without silk and excreta cues from that predator.

We predicted that if chemically-mediated antipredator responses are innate, predator naïve *Rabidosa* spiderlings will reduce activity in the presence of predator silk and excreta compared to cues from another novel source, the cricket, *Acheta domesticus*, or a blank control treatment. Also multi-modal

sensory information about a predator (visual, vibratory, and chemical combined) should result in even greater antipredator response. Studies with *Pardosa milvina* and its predator, *Hogna helluo*, found that *Pardosa* survival was significantly affected by the presence of *Hogna* silk and excreta when paired with live *Hogna* (Persons et al., 2001, 2002; Barnes et al., 2002). These results suggest that antipredator responses are induced primarily or wholly by *Hogna* chemical cues. If such responses exist among most lycosids, then prior experience with visual or vibratory information about *Pardosa* should have a lesser effect on *Rabidosa* spiderling activity levels than chemical information. Finally, variation in activity levels due to prior exposure to different sensory treatments should result in differential survival in the presence of a live predator if such responses serve an antipredator function among spiderling prey.

Materials and methods

General methods for spider collection and maintenance

Two species of wolf spider were used in this experiment and both species were housed and maintained in a similar manner. Adult female *Pardosa milvina* to be used as predators were collected by hand during the day in September 2000 within or adjacent to soybean and cornfields owned by Susquehanna University, Selinsgrove, Snyder County, PA, USA. Adult female lycosids were used in our study because they are considerably larger and heavier than males and are both more motivated to feed and are more likely to be dangerous predators than males (Lehmann et al., 2004). Spiders were chased into small transparent vials for transport back to the laboratory. Each spider was housed in a separate container under identical conditions. Individuals were maintained in small round plastic containers (5 cm h × 8 cm d) with 1-2 cm of moistened peat moss to serve as a substrate and source of water. Spiders were watered ad libitum and fed a weekly diet of adult *Drosophila melanogaster*. All spiders were maintained at room temperature (23-25°C) on a 13:11 h light/dark cycle. *Pardosa milvina* were fed *D. melanogaster* to satiation 48-h prior to use in any experiment to standardize their hunger level. Three adult female *Rabidosa rabida* with egg sacs were collected in early October 2000 and maintained in opaque round plastic containers (9 cm h × 12 cm d) with 2-3 cm of moistened vermiculite as a substratum. Three

0.5-cm-long cricket nymphs (*A. domesticus*) were presented once weekly to these spiders as food, and water was replenished as needed. After feeding, adult females were transferred to new containers that were devoid of odor cues and withheld food immediately after spiderlings emerged. One week after spiderlings emerged from the egg sacs, they were removed from the mother and housed individually in small round plastic containers (9 cm h \times 12 cm d) with 1 cm moistened vermiculite for substratum. A total of 200 *Rabidosa* spiderlings were maintained and fed twice weekly a diet of *D. melanogaster* and *Sinella curviseta* (Collembola). Spiderlings were not used in experiments prior to their third instar (one molt occurring during post-egg sac emergence). The *Rabidosa* spiderlings, like *Pardosa*, were fed *D. melanogaster* to satiation 48 h before their use in any experiments to standardize hunger level prior to testing. Spiderlings from each egg sac were randomly assigned across each treatment.

Stimulus preparation and testing procedures

We tested the effect of prior exposure to predator visual and vibratory cues combined, chemical cues alone, or combinations of visual, vibratory and chemical predator cues on the activity level and survival of naïve *Rabidosa* spiderlings. We compared spiderling responses to these different experiences with predator cues to either chemical cues produced from a domestic house cricket (*Acheta domesticus*) or no experience at all.

A total of six treatments were used (Figure 1). All trials were conducted in 9 cm h \times 12 cm d round plastic containers with a layer of moistened vermiculite on the bottom. Twenty spiderlings were used in each treatment. For each replicate, a test container was prepared by being rinsed with 95% ethanol solution and allowed to dry. A 1-cm layer of moistened vermiculite was then added to the bottom of the container as a substrate. An individual spiderling was then placed in the container along with four fruit flies and, after a 1-min acclimation period, the time spent in forward motion was recorded during a 10-min period. This pre-exposure test served as a baseline measure of spiderling activity (Figure 1). After this initial movement test (pre-exposure to the sensory treatment), the spider was again offered fruit flies in excess and placed into its designated experimental sensory treatment container for a 24 h period (Figure 1). Spiderling movement was recorded again (post-exposure to the sensory treatment) in a new container that was

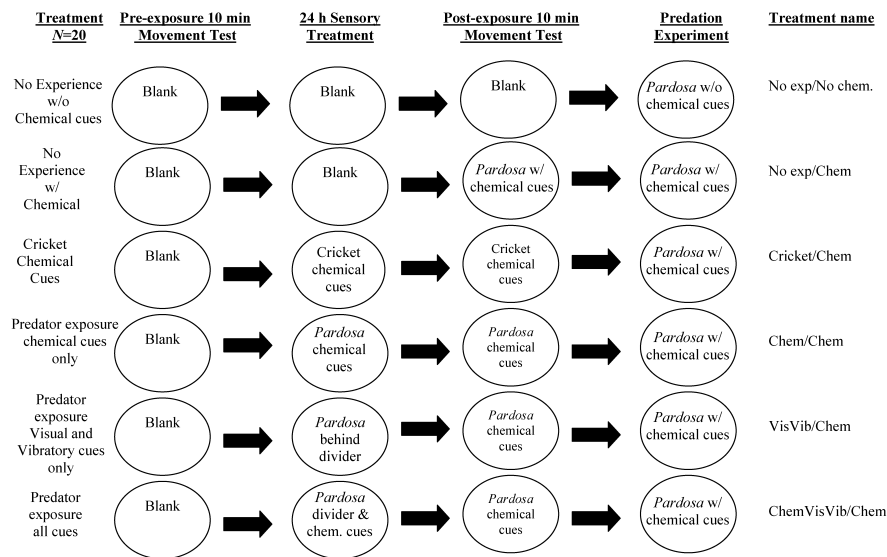


Figure 1. Diagram of the experimental design. Six treatments each with 20 spiderlings were established. Predator-naïve spiderling movement was measured initially for 10 min to establish baseline levels of activity (pre-exposure movement test). Spiderlings were then placed into containers with various predator cues for 24 h (sensory treatments). After this period of time, spiderling movement was measured again for 10 min (post-exposure movement test). After the post-exposure test, spiderlings were placed in a separate container with (chemical cues) or without silk and excreta (no chemical cues) of a larger predatory wolf spider *Pardosa milvina*. Predation latency was then measured across treatments (predation experiment). See text for details about each treatment group.

perfused with either *Pardosa* chemical cues for the treatments that corresponded to predator cues, cricket chemical cues for the positive chemical control, or no chemical cues for one of the two no experience treatments (Figure 1). Thus, spider activity was measured twice, once prior to exposure to one of the sensory treatments, and again 24 h post-exposure to one of the six treatments.

Following the pre- and post-exposure movement tests, a predation experiment was conducted to determine the effectiveness of reduced movement in the presence of a live *Pardosa*. Following the post-exposure movement test, the spiderlings were placed inside transparent circular walls composed of acetate nested concentrically within a container with silk and excreta produced by a single adult female *Pardosa milvina*. The predatory spider, which had been fed to satiation 24 h prior to use, was also added to the container, and following a 1-min acclimation period, the transparent wall was removed and

the time until predation was recorded. The experiment was terminated after a 3-h period.

Every spider from five of the six treatments was exposed to chemical cues from a live *Pardosa* after the post-movement test (Figure 1). In the sixth treatment group, *Pardosa* was not allowed to deposit cues during the predation portion of the experiment and *Rabidosa* spiderlings had no experience with *Pardosa* prior to adding the predator to the container (Figure 1). Thus, the six treatments were as follows: (1) exposure to a container devoid of predator or cricket cues for 24 h followed by no previous deposition of silk and excreta during the predation portion of the experiment (No experience/No chemical) (Figure 1). For this treatment, pre- and post-exposure spider movement was recorded in a container devoid of any type of predator cues; (2) as in No experience/No chemical, except that during the predation portion of the experiment, the predator, a single female *Pardosa milvina*, was allowed to deposit silk and excreta for 24 h in the container during direct interactions with the prey (No experience/Chemical); (3) excreta and silk produced from a single 1/4 inch long cricket nymph left in the container for 24-h that served as a positive control for novel chemical cues (Cricket/chemical); (4) silk and excreta produced from one adult female *Pardosa* during a 24-h period (Chemical/Chemical); (5) a predator visual and vibratory treatment of one adult female *Pardosa* separated from the spiderling by a circular transparent acetate divider arranged concentrically in the center of a container (Visual & Vibratory/chemical); and (6) combined chemical, visual and vibratory cues of an adult female *Pardosa* by the inclusion of an adult female *Pardosa* separated by a clear acetate divider concentrically arranged in the center of the container paired with silk and excreta from the same spider (ChemVisVib/chem).

A total of 260 *Pardosa milvina* were used during this study with no predator used more than once. Chemical cues from different predators were used for the sensory treatments, post-exposure, and survival experiment respectively to insure that the chemical cues were always fresh and to avoid possible confounding effects of silk and excreta deposited from the spiderlings during trials.

Statistical analysis

We used a one-way ANOVA to measure changes in activity between pre-exposure and post-exposure movement tests across sensory treatments. For

the survival portion of the experiment we used survival regression using a Kaplan Meier Product Limit Estimator to compare differences in survival distribution and test for a significant sensory experience treatment effect (Cox & Oakes, 1984; JMP, SAS Institute, 2002). Survival analysis is appropriate when the survival distributions are non-normal and when at least some of the data is censored (i.e., an actual time of death wasn't recorded for all individuals but it is known that survival times are greater than some specified value). We chose the Kaplan Meier Product Limit Estimator because it is nonparametric so requires no assumptions about the distribution of the data (Cox & Oakes, 1984). We then made pairwise comparisons of the survival distributions using the log-rank (Mantel-Cox) test (JMP, SAS Institute, 2002; see Persons et al., 2001; Lehmann et al., 2004 for more detailed information on the applicability of survival analysis to this type of data). We then used a sequential Bonferroni adjustment of the alpha level to compensate for the number of pairwise comparisons made (Rice, 1989).

Results

We compared the change in activity between pre-exposure and post-exposure movement tests across all sensory treatments (post-exposure minus pre-exposure). There was a significant effect of the type of 24-h experience on change in activity (ANOVA $F_{5,124} = 6.305$; $p = 0.0002$). Based on a Tukey post-hoc comparison of means test, treatments with any predator cues for 24 h resulted in spiderlings that moved significantly less than the Cricket/Chem, No exp/No chem., or No exp/Chem treatments (Figure 2), therefore *Rabidosa* spiderlings exhibit an innate reduction in activity when presented visual and vibratory or chemical cues from a predator for 24 h. The treatment with 24-h exposure to predator silk and excreta alone showed the greatest reduction in movement followed closely by the combination of chemical, visual, and vibratory cues together and finally visual and vibratory cues only respectively. Post-exposure to the No exp/Chem treatment resulted in an average increase in movement, but the net change in movement was not significantly different than the Cricket/Chem or No exp/No chem treatment (Figure 2). Most interesting, ten minutes of exposure to *Pardosa* silk and excreta (No exp/Chem and Cricket/Chem treatments), was insufficient to greatly reduce activity and these treatments were no different than when no chemical cues were available prior to being exposed to *Rabidosa* silk and excreta

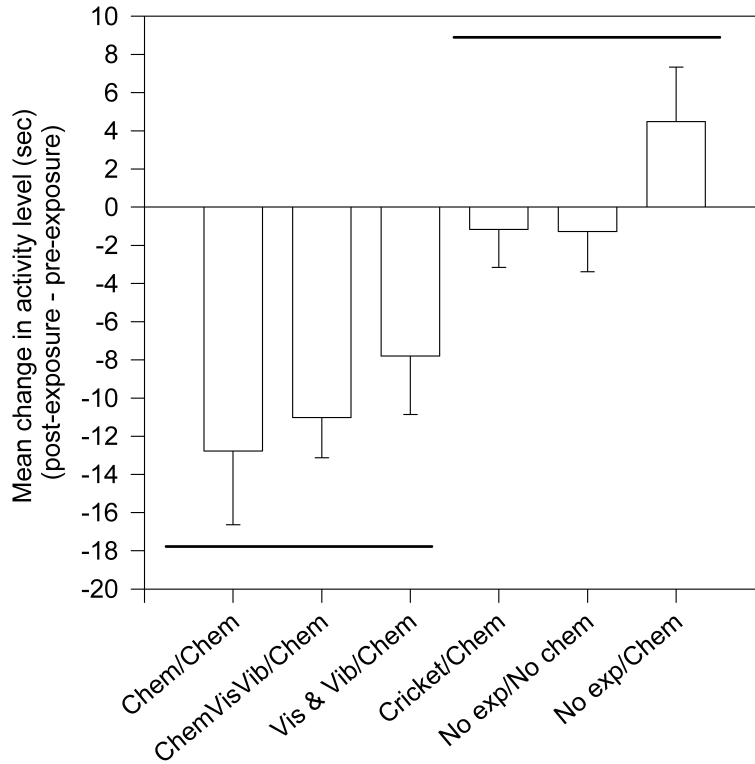


Figure 2. Index of change in time spent moving forward during two separate 10-min periods for *Rabidosa* spiderlings (mean \pm SE). Change in activity was calculated as post-exposure movement minus pre-exposure movement. Pre-exposure is baseline activity levels of spiderlings prior to being placed in a container with or without sensory cues from a predator for 24 h. Post-exposure is activity levels after the sensory treatment with and without chemical cues from the predator. Negative values indicate spiderlings that decreased average movement for that particular treatment. See Figure 1 and the text for further descriptions of the methods. Horizontal lines above each bar indicate treatment groups that are not significantly different from each other based on a Tukey post-hoc comparison of means test ($\alpha = 0.05$). Only treatments with spiderlings exposed to predator sensory cues for 24 h showed a significant decrease in activity.

(Cricket/Chem). Spiderlings required 24-h of predator cue exposure to sufficiently sensitize them to induce a significant reduction in activity (Figure 2).

In the predation experiment, we found a highly significant median difference in predation latency across predator experience treatments (Kaplan Meier Model, Mantel-Cox test: $\chi^2 = 20.79$, $p = 0.0003$). The lowest predation rate was the chemical/visual/vibratory treatment (ChemVisVib/Chem)

followed by the chemical only treatment (Chem/Chem) and visual and vibratory only treatments (Vis & Vib/Chem) respectively (Figure 3). The treatments with the lowest survival rates were the two no experience treatments (No exp/No chem and No exp/Chem) and the cricket treatment (Cricket/Chem). Spiderlings that had no previous predator experience and had no predator silk and excreta present when a live predator was present, had the shortest median survival time. Spiderlings without prior experience or with prior cricket chemical cue experience but with *Pardosa* silk and excreta present survived only slightly longer, but not significantly longer than when they had no prior experiences whatsoever (Figure 3). We conducted pair-wise comparisons of survival functions to determine which types of prior experience resulted in the highest survival levels. In general, spiderlings in treatments with 24 h of prior exposure to chemical cues survived significantly longer than spiderlings that did not have 24 h exposure to *Pardosa* silk and excreta. There was no significant increase in survival when visual, vibratory, and chemical cues were presented together previously than when chemical cues were presented alone and 24 h exposure to visual and vibratory cues from a predator resulted in no significant increase in survival than treatments without exposure to predator cues.

Discussion

The results of the pre- and post-movement tests indicate that naïve juvenile *Rabidosa* do adjust their behavior in the presence of silk and excreta from *Pardosa* when exposed for 24 h to either these cues or visual and vibratory cues from the predator (Figure 2). Although perceiving visual and vibratory information from a predator for 24 h significantly reduced overall activity of spiderlings, this did not translate into significantly increased survival in the presence of a live *Pardosa*. We also found no significant difference in survival between the No experience/no chemical treatment and the No experience/chemical treatment. This finding suggests that the presence of predator silk and excreta along with a live predator has little impact on survival unless spiderlings had been exposed previously to these cues. Our results run counter to previous experiments that have demonstrated that the presence of predatory wolf spider silk and excreta increases survival in the presence of a live predator (Persons et al., 2001, 2002; Barnes et al., 2002). However in

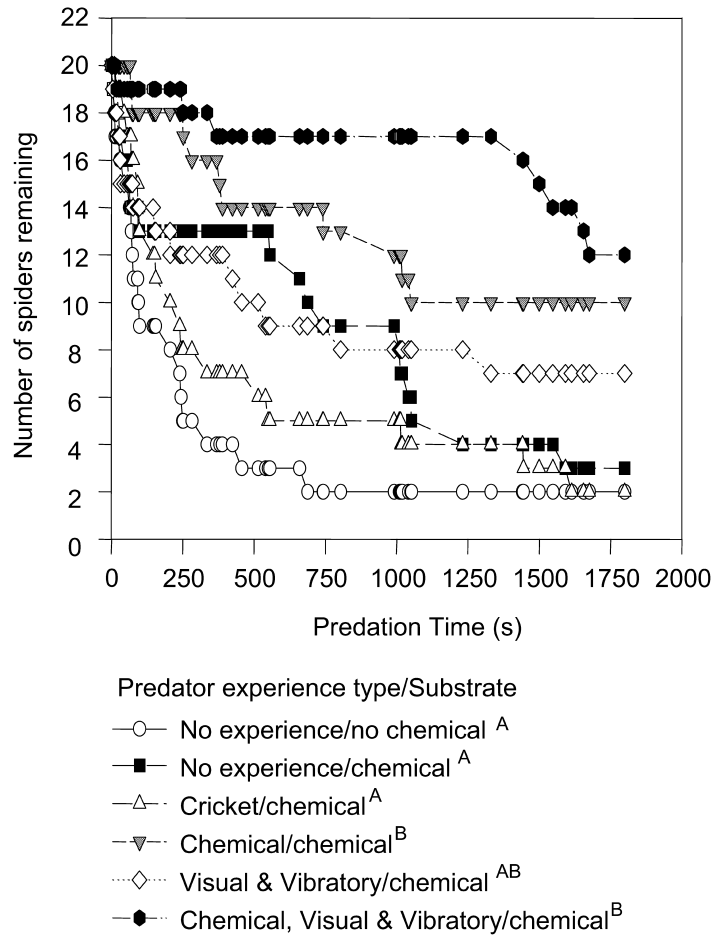


Figure 3. Survival of *Rabidosia rabida* spiderlings over time in containers with adult female *Pardosa milvina* and associated silk and excreta. Treatments are after 24-h exposure to one of five sensory treatments: (1) chemical, visual and vibratory cues from a *Pardosa* (Chemical, Visual & Vibratory/chemical), (2) chemical cues only from a *Pardosa* (Chemical/chemical), (3) visual and vibratory cues only from a *Pardosa* (Visual & Vibratory/chemical), (4) a positive control of chemical cues from a single cricket (Cricket/chemical); and (5) a negative control of a blank container (No experience/chemical) followed by chemical cues from a *Pardosa* during the 10-min post test. Additionally we included a sixth treatment of no chemical cues from the predator previously or during the predation experiment (No experience/no chemical). Different letters following each treatment indicate significant differences in median survival time across treatments based on pair-wise survival analysis (Kaplan Meier Product Limit Estimator Model) using a Mantel-Cox (Log-rank) test. Thresholds for significant differences are based on a step-wise Bonferroni adjustment of the alpha level to control for the number of pair-wise comparisons.

all of these other studies, adult or sub-adult field-caught subjects were used. These spiders may have had considerable previous experience with predators or their silk and excreta.

Our results indicate that extended exposure to *Pardosa* silk and excreta is critical for inducing effective anti-predator responses. We suggest that spiderlings became sensitized rather than habituated to *Pardosa* silk and excreta during this 24 h period. Once sensitized, *Rabidosa* spiderlings may have become primed to respond to other information about the predator more quickly resulting in increased awareness or vigilance toward the predator. Such priming effects have been observed in other species of spider, but have not been previously documented in the context of predator avoidance. For example, the jumping spider, *Habrocestum pulex*, shows enhanced attention to visual cues from prey if exposed to chemical cues concurrently (Clark & Jackson, 2000). It remains unclear if these responses are innate but modified strictly by exposure time, or if they are learned very quickly through association after exposure to a live predator. If *Rabidosa* spiderlings' responses are learned, then we can not rule out the possibility of rapid chemical-imprinting of a particular predator. Our experimental design did not allow for strict differentiation between these two possibilities. However, the spiderlings in the Chemical/chemical treatment did not have the opportunity to associate the silk and excreta cues with a live *Pardosa* prior to the predation test, yet these cues still induced significant changes in behavior and significantly greater survival. This result indicates that it is unlikely that observed changes in *Rabidosa* response were acquired through association, or if it occurred, the association occurred extremely quickly.

Under field conditions, it would be advantageous for *Rabidosa* spiderlings to emerge from the egg sac with an innate immediate response to the chemical cues left behind from one of their most abundant predators. Although such a response would certainly be adaptive, we did not test *Rabidosa rabida* spiderling responses while they were still on their mother's back. Persons & Lynam (2004) found that *Pardosa milvina* spiderlings climb off of their mothers significantly less often when the underlying substrate was previously occupied by a larger heterospecific wolf spider, *Hogna helluo*. In this study, the spiderlings sampled the substrate several times before finally disembarking permanently from their mother. Since this study involved interactions between spiderlings and their mother, it was unclear whether dispersal behavior was mediated by the mother, offspring, or an interaction between

them. *Rabidosa rabida* spiderlings also periodically climb off and climb back on their mothers at various points prior to permanently dispersing (Higashi & Rovner, 1975). This behavior appears mediated by silk draglines produced by the spiderlings. Higashi & Rovner (1975) also found that *Rabidosa rabida* spiderlings were prone to predation when placed in containers with heterospecific spiders or adult conspecific males. Since our sample of *Rabidosa* spiderlings was drawn from only three egg sacs, it is possible that maternal effects may have underestimated the range of responses of spiderlings compared to a sample of unrelated individuals.

Results from the predation experiment indicate that naïve juvenile *Rabidosa rabida* are capable of discriminating between predator sensory cues from that of crickets (Figure 2). By comparing the Chemical/Chemical to the Cricket/chemical treatment, we found that spiderlings are innately capable of distinguishing between predator and non-predator chemical cues. Antipredator behavior in the form of reduced activity is costly among lycosids, compromising foraging efficiency and reproduction (Persons et al., 2002; Taylor et al., 2005). Spiderlings that can discriminate between chemical cues from predators and less dangerous animals may be able to accurately assess predation risk and minimize the fitness-related costs associated with defensive behavior.

Our prediction that predator chemical cues will induce a greater antipredator response than visual cues and multi-modal sensory information will result in even greater antipredator response was not statistically supported by the post-exposure movement test (Figure 2) or by differences in survival (Figure 3). However, 24 h of predator cues resulted in both the greatest reduction in activity and the longest predation latency among all the treatments. It is important to consider that we measured only one component of spider antipredator behavior, namely reduced movement. Wolf spiders also show substrate avoidance, long periods of immobility, reduced distance traveled, reduced speed of movement (Person et al., 2001), and increased vertical movement (Persons et al., 2002) when encountering cues from predatory spiders. It is likely that these other unmeasured antipredator responses may have contributed to the large differences in survival time among treatments as well. Spiders may be more sensitive to chemical cues from a predator compared to visual or vibratory cues since this allows for the earliest possible deployment of antipredator behavior well before an encounter whereas responding to visual or vibratory cues may not.

Under natural conditions, *Rabidosa* spiderlings range widely over the environment, often occurring in disturbed habitats such as agricultural fields. Within these habitats, larger lycosid spiders such as *Pardosa* are likely to be a common predator. These laboratory observations suggest that naïve *Rabidosa* spiderlings sample their environment primarily through the use of chemical information on the substrate in determining potential predator risks. Based on post-hoc comparisons of exposure to different predator sensory cues, it appears that visual and vibratory cues, although equally important in mediating reductions in activity, are less effective sources of information when attempting to avoid a live predator. Further, the presence of another novel biogenic cue, cricket excreta, was no better than no information at all in modifying behavior. Collectively, these data suggest that *Rabidosa* spiderlings have an innate response to heterospecific lycosid silk and excreta that becomes stronger with exposure time. However the experience of combining these chemical cues with visual and seismic confirmation of the predator, did not significantly enhance recognition and survival beyond that of 24-h exposure to predator silk and excreta.

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