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The Influence of Predator Sex on Chemically Mediated Antipredator Response in the Wolf Spider *Pardosa milvina* (Araneae: Lycosidae)

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Abstract

The wolf spider, *Pardosa milvina*, reduces activity in the presence of chemical cues (silk and excreta) from a larger predatory wolf spider, *Hogna helluo*. *Hogna* is sexually dimorphic in body size and males and females differ in their propensity to attack prey. Consequently, each sex may present different levels of risk to *Pardosa*. We measured predation risk of *Pardosa* in the presence of male or female *Hogna*. We also assessed *Pardosa* antipredator responses and survival in the presence or absence of previously deposited chemical cues from male or female *Hogna*. In the absence of predator chemical cues, *Pardosa* survived significantly longer in the presence of male *Hogna* compared with female *Hogna*. We then assessed *Pardosa* survival in the presence of chemical cues from each *Hogna* sex by placing *Pardosa* in containers previously occupied by a female *Hogna*, a male *Hogna*, or no *Hogna* (control). We then introduced a female *Hogna* into each container and measured predation latency. *Pardosa* survived significantly longer in the presence of female and male cues compared with the control treatment. Median survival time of *Pardosa* was over four times longer on substrates with female *Hogna* cues compared with male cues, but this difference was not statistically significant. We tested *Pardosa* activity levels in the presence of chemical cues from male or female *Hogna*. Both *Hogna* sexes were maintained in separate containers after which we placed an adult female *Pardosa* in one of the containers or a blank control container. *Pardosa* significantly decreased activity in the presence of chemical cues from either sex relative to the control. Activity was lowest on substrates with female *Hogna* cues, but not significantly lower than on substrates with male *Hogna* cues. Results suggest that chemical information from male or female *Hogna* significantly reduces *Pardosa* activity which results in increased survival.

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Introduction

Animals show a variety of behavioral defenses against predators. These include hiding, reduced activity (Lima & Dill 1990; Lima 1998), and cover seeking (Endler 1991). Antipredator behavior is often most effective if the prey detects the predator first; therefore, many animals increase the probability of early predator detection by using indirect cues such as excreta or other metabolic waste products to identify a predation threat at a distance (reviewed in Kats & Dill 1998). However, there are also costs associated with antipredator behavior such as reduced feeding efficiency (Lima 1998; McPeek et al. 2001) and reduced reproductive success (Forsgren 1992; Berglund 1993; Hedrick & Dill 1993; Godin 1995). Consequently animals may benefit from accurate assessment of predation risk and show graded levels of defensive behavior proportional to the perceived risk (Kats & Dill 1998; Lima & Bednekoff 1999).

Predator chemical cues may provide information about the level of risk as well as the possible presence of the predator. For example, many species discriminate between predators that have or have not fed on conspecifics (Madison et al. 1999; Murray & Jenkins 1999; Venzon et al. 2000; reviewed in Chivers et al. 1996). Animals may also show elevated levels of antipredator behavior in the presence of fresh predator chemical cues compared with cues that are older (Venzon et al. 2000). In these cases, prey appear capable of extracting information about motivational state, relative predation risk, or the likelihood of a predator encounter based on chemical information alone, and modify their defensive behavior accordingly.

Wolf spiders (*Pardosa milvina*; Lycosidae) are ground-dwelling intraguild predators that often feed on smaller conspecific and heterospecific spiders (Edgar 1969; Hallander 1970; Samu et al. 1999; Marshall et al. 2000). Lycosids are also known to respond to chemical cues from conspecifics (Dondale & Hegdekar 1973; Tietjen 1979; Ayyagari & Tietjen 1986; Searcy et al. 1999; Rypstra et al. 2003), predators (Punzo 1997; Persons & Rypstra 2001; Barnes et al. 2002) and prey (Persons & Uetz 1996; Punzo & Kukoyi 1997; Persons & Rypstra 2000; Hoefler et al. 2002) and are therefore an interesting taxon in which to study chemically mediated predator–prey interactions. In addition to excreta, wolf spiders leave a silk dragline behind them as they move through the environment in search of prey. Silk draglines are often used to communicate among conspecifics (Tietjen & Rovner 1982), but such information is also readily available to heterospecific lycosids for assessment of predation risk.

Pardosa milvina is preyed upon by a larger syntopic species of wolf spider, *Hogna helluo* (Persons & Rypstra 2000). These two species are among the most common cursorial predators in agricultural systems of the mid-western United States (Marshall & Rypstra 1999). *Pardosa milvina* occur at densities of 5–30 individuals/m^2 but may reach population densities in excess of 100/m^2 (Marshall & Rypstra 1999; Marshall et al. 2002). *Hogna helluo* is less common with densities around 0.8–2.1 individuals/m^2 (Marshall & Rypstra 1999; Marshall et al. 2000). Given the population density of these two species, encounters with each other are

likely to be extremely common if not unavoidable. Adult *H. helluo* is much larger, up to 30 times the mass of an adult *P. milvina*, and is capable of consuming up to 10 *Pardosa* at a time (Persons et al. 2001). *Pardosa milvina* is extremely active, often covering large distances over short periods of time (Marshall et al. 2000). In contrast adult female and juvenile *Hogna* tend to be facultative burrowers that may move much less and accumulate considerable amounts of silk and excreta around their burrow entrances (Walker et al. 1999).

In the field, *Pardosa* numbers do not decrease in areas where *Hogna* are experimentally added to agricultural plots (Marshall et al. 2000) despite field observations of *Hogna* predation on *Pardosa*. However, *Pardosa* body condition is poorer in these same plots compared with areas without additional *Hogna* (Marshall et al. 2000). These observations suggest that *Pardosa* do not simply leave areas with high predator density, but modify their behavior to avoid predation, and in doing so, suffer decreased foraging success while remaining in areas with *Hogna*.

Adult female *Pardosa* show significant reductions in activity and prolonged periods of immobility in the presence of silk and excreta produced by adult female *Hogna*, yet do not show any change in activity in the presence of conspecific silk and excreta (Persons et al. 2001, 2002). These reductions in activity are likely to be adaptive as wolf spider visual systems are strongly biased toward movement rather than shape detection (Rovner 1996; Persons & Uetz 1997), and increased periods of movement greatly increase the lunging probability of a predatory wolf spider (Persons & Uetz 1997). More recent studies show that *Pardosa* defensive behavior in the presence of *Hogna* cues incurs significant fitness-related costs (Taylor et al. in press). In the presence of *Hogna* silk and excreta, but no actual predators, female *Pardosa*, show reduced feeding efficiency, poorer body condition, lighter egg sacs, and produce fewer eggs (Persons et al. 2002). *Pardosa milvina* is able to mitigate these fitness costs by acquiring additional chemical information about predation risk and then matching the degree of antipredator behavior with the level of perceived risk. For example, adult female *Pardosa* show greater reductions in activity in the presence of silk and excreta from adult female *Hogna* that have fed on *Pardosa* compared with those that have fed on other prey types (Persons et al. 2001). Adult female *Pardosa* also show significantly less activity in the presence of freshly deposited female *Hogna* silk and excreta compared with cues that are 1 week old (Barnes et al. 2002). Further, *Pardosa* can modify the level of activity based on the size of the *Hogna* that produces the chemical cues (Persons & Rypstra 2001). These graded antipredator responses to subtle differences in *Hogna* silk and excreta have been shown to directly affect the probability of *Hogna* predation on *Pardosa* (Persons et al. 2001, 2002; Barnes et al. 2002). These studies collectively demonstrate that the information content of predator chemical cues influences not just the presence or absence of antipredator responses, but the magnitude of such responses as well. Further, chemical information that conveys accurate information about risk (e.g. size of predator, time since it was in the area, diet of the predator etc.) may allow *Pardosa* to mitigate the fitness costs associated with defensive behavior.

The sex of a predator may be an important source of chemically acquired information if male and female predators present different levels of risk. Numerous studies provide direct and indirect evidence that male and female wolf spiders may not present the same level of risk to prey, and that the chemical cues deposited on the substrate by each sex may be qualitatively as well as quantitatively different. *Hogna helluo* is sexually dimorphic, with adult females being considerably larger and heavier than adult males (Edgar 1971; Dondale & Redner 1990; Walker 2001; Walker & Rypstra 2002). Female *Hogna* are more likely to kill and consume prey than are males, and are more likely to exhibit superfluous killing (Walker & Rypstra 2002). Also, previous studies have found that male and female wolf spiders respond differently to sensory information about prey (Persons & Uetz 1999; Persons 1999). Male and female lycosids may be distinguishable based on chemical cues alone. Females of some species of wolf spider are known to produce sex pheromones (Hegdekar & Dondale 1969; Richter et al. 1971; Dondale & Hegdekar 1973; Searcy et al. 1999; Rypstra et al. 2003), whereas male sex pheromones appear to be less common (Ayyagari & Tietjen 1986). Because sex-based differences in response to prey sensory cues exist among some lycosids (Persons & Uetz 1999; Persons 1999), wolf spiders may show different antipredator strategies depending upon the sex of the predatory wolf spider. If this is the case, lycosid prey may benefit by discriminating between chemical cues produced by males or females of a given species of spider.

We had three objectives for this study; (i) to determine whether adult male and female *Hogna* pose different levels of predation risk to *Pardosa* in the absence of silk and excreta cues; (ii) to determine if silk and excreta from adult male or female *Hogna* results in differences in predation of *Pardosa* in the presence of a live predator; (iii) to quantify possible differences in activity levels of *Pardosa* in the presence of chemical cues from adult male or female *Hogna*. We predicted that adult female *Hogna* should present a greater predation risk to *Pardosa* than adult male *Hogna* because of the female's greater energy needs for egg sac production and tendency toward greater weight gain after maturity. We also predicted that as adult female *Hogna* are larger than adult males, they are likely to produce more silk and excreta and therefore elicit a greater chemically mediated reduction in activity in *Pardosa* than chemical cues from males. If reduction in activity is an effective antipredator response, then *Pardosa* that have access to female *Hogna* and its associated silk and excreta should survive longer than *Pardosa* that have access to chemical cues from a male *Hogna* or no chemical cues at all.

Methods

Collection and Maintenance

Juvenile and subadult *P. milvina* and *H. helluo* were field caught during 12 September to 10 November 1999 and 25 May to 26 June 2001 in alfalfa and corn fields on Susquehanna University property adjacent to campus (Selinsgrove,

Snyder County, Pennsylvania). All spiders were allowed to mature in the laboratory prior to testing. Only adult female *Pardosa* (weight; $\bar{x} \pm SE$ 23.6 ± 1.3 mg; $n = 20$) were used as test subjects for all experiments. Spiders were individually maintained in white plastic containers with transparent lids. Each container was 9 cm diameter, 7 cm height with 2 cm of moistened peat moss cover serving as a source of water and humidity. All spiders were kept at room temperature (23–25°C) and maintained on a 13.00 : 11.00 hours (L : D) photoperiod. *Pardosa milvina* were fed a diet of 10 *Drosophila melanogaster* twice weekly. *Hogna helluo* were maintained on a diet of four subadult house crickets (*Acheta domesticus*) twice weekly except where otherwise noted. A total of four experiments were completed. In two of the four experiments, expts II and III, data used in the analysis were collected at two different times: October–December, 1999 and June–August, 2001. For these experiments, a blocking factor was included in the analyses to test and control for possible temporal effects on experimental results.

Experiment I: Measuring *Pardosa* Predation Risk in the Presence of Female or Male *Hogna* without Previously Deposited Chemical Cues

We tested for a difference in *Pardosa* predation risk in the presence of female (weight; $\bar{x} \pm SE$ 789.6 ± 15.2 mg; $n = 20$) or male *Hogna* (weight; $\bar{x} \pm SE$ 220.5 ± 7.0 mg; $n = 20$) when no previously deposited silk or excreta cues were available. Forty adult *H. helluo* ($n = 20$ per sex treatment) were fed as many prey as they could consume within a 24-h period and then withheld food for 10 days to standardize hunger levels prior to testing. We rinsed 40 plastic containers (9 cm diameter, 7 cm height) with 95% ethanol to remove any potential odor cues, after which they were allowed to dry. We then added a shallow lid with a few drops of water to each container to increase ambient humidity and provide a direct source of water for test spiders. A single adult female *Pardosa* was introduced into each container under a clear plastic vial followed by the immediate introduction of a single hungry male or female *Hogna* under a separate vial. Both spiders were allowed to acclimate for a 5-min period under their respective vials. The vials were then removed and the spiders were watched for 3 h or until *Hogna* preyed upon *Pardosa*. All predation times were recorded to the nearest second.

Using the Proportional Hazards Model to Determine Relative Predation Risk

There are many methods for analyzing time-to-event data with censored observations (e.g. Collett 1994), and several have been suggested for use in ecological applications (e.g. Dixon & Newman 1991; Fox 1993; Moya-Laraño & Wise 2000). We chose to use the proportional hazards model (Cox 1972). This is an alternative to parametric regression methods that make assumptions about the hazard function [a function that relates the instantaneous rate of failure to time (Collett 1994; Piegorsch & Bailer 1997)] and non-parametric methods that are useful in determining differences between groups (Collett 1994). The Cox

proportional hazards model makes the assumption that the effect of a covariate on the baseline hazard function is multiplicative and does not change over time. Thus, the parameters estimated using the proportional hazards model estimate the effect of a covariate on the baseline hazard function. In this experiment we are interested in the effects of predator sex on the hazard or instantaneous rate of failure of *Pardosa*. The proportional hazards model can be written as follows:

$$h_i(t) = e^{\beta * I_{sex}} h_o(t) \quad (1)$$

where $h_i(t)$ is the hazard function for the ith group (either male or female), $h_o(t)$ is the baseline hazard function, I_{sex} is an indicator variable that takes on a value of 1 if the predator is female and 0 if the predator is male. Thus, when I_{sex} equals zero (indicating males), $e^{\beta * I_{sex}} = 1$ and the baseline hazard is not modified. However, when $I_{sex} = 1$ the baseline hazard is multiplied by e^{β}. We have parameterized the model in a fashion that results in the baseline hazard function being the instantaneous rate of death of a *Pardosa* in the presence of a male predator and e^{β} represents the effect of having a female predator on the instantaneous rate of death relative to a male. Rearranging equation 1 results in the following:

$$\frac{h_f(t)}{h_m(t)} = e^{\beta} \quad (2)$$

Exponentiation of β gives an estimate of the ratio of the two hazard functions and can be interpreted as a time-independent relative risk (Collett 1994; Piegorsch & Bailer 1997). This is an extraordinarily useful property of the proportional hazards model; i.e. assume that $e^{\beta} = 2$, means that the risk of death for individuals placed into containers with female predators is twice that of individuals in containers with male predators. We will use this interpretation throughout the study. Confidence intervals (CI) for the hazard ratios can be found by exponentiation of the upper and lower estimates 'z'-based CI of β (Lee 1992; Collett 1994). If the CI for the hazard ratio includes 1 this suggests that there is no significant difference between the two hazard functions. Throughout the manuscript we will refer to the hazard ratios as relative risk. In addition, we compared the hazard functions between different groups (e.g. males and females) by constructing a likelihood-ratio test (Collett 1994).

Experiment I: results

None of the *Pardosa* survived the 3-h period with a live *Hogna*, irrespective of the sex of the predator. However, female *Hogna* and male *Hogna* significantly differed in the median time to capture and consume *Pardosa* (likelihood-ratio test, $\chi^2 = 4.49$; $p = 0.0339$; median (for female) 222 s, median (for male) 363.50 s), with males generally capturing prey more slowly (Fig. 1). The risk of *Pardosa* being consumed by female *Hogna* was 2.07 (lower 95% CI = 1.06, upper 95% CI = 4.13) times higher than their risk of being consumed in the presence of a male *Hogna*. The mean predation latency among male predators was also over

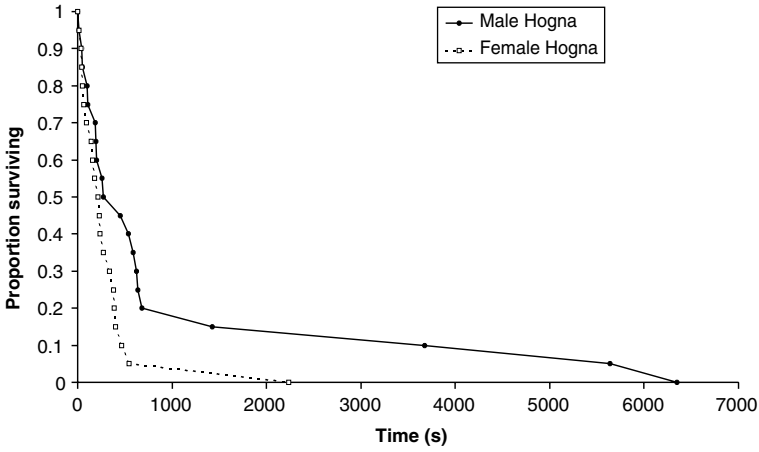

Fig. 1: Cumulative survival of *Pardosa milvina* over time in containers with an adult female or male *Hogna helluo* without chemical cues. See expt I for details

three times longer than that of female predators (female: $\bar{x} = 324.20$ s, SE = 106.47; male: $\bar{x} = 1102.60$ s, SE = 416).

Experiment II: *Pardosa* Predation Risk in the Presence of Previously Deposited Female and Male *Hogna* Chemical Cues

Methods

We tested the effects of previously deposited silk and excreta from cricket-fed male or female *Hogna* on survival of *Pardosa* in the presence of a live adult female *Hogna*. Thirty-five satiated adult female and 25 satiated adult male *Hogna* were placed in individual containers (9 cm diameter, 7 cm height) for 24 h. Another 35 containers remained empty and served as a control treatment. Spiders were then removed from their respective containers. Another set of 95 adult female *Hogna* from which food had been withheld for 10 days were then randomly assigned to each of the three treatment groups (previously deposited male chemical cues, previously deposited female chemical cues, or control). Similar to expt I, a single adult female *Pardosa* was introduced into each container under a clear plastic vial followed by the introduction of an adult female *Hogna* under a separate plastic vial. Both spiders were allowed to acclimate for a 5-min period under their respective vials after which time the vials were removed and predation time was recorded. The experiment was terminated after 3 h. Hazard functions were compared across treatments using the same method as in expt I except that we initially included block effects in this model because this experiment was conducted twice, once in the fall of 1999 (n = 15 males, 15 females, 15 control) and once in the summer of 2001 (n = 10 males, 20 females, 20 control). In this model there were two indicator variables. One indicator variable was for male

cues which was coded as 0 if male cues were not present and 1 if they were. The second indicator variable was for female cues which were also coded as 0 if female cues were not present and 1 if the cues were present.

Experiment II: results

We found no effect of block (e.g. experiments conducted in the fall of 1999 vs. the summer of 2001, likelihood-ratio test; $\chi^2 = 1.316$; d.f. = 1; p = 0.2512). As there was no block effect, we left this effect out of the models. There was a significant cue effect on *Pardosa* survival (likelihood-ratio test; $\chi^2 = 9.90$; d.f. = 2; p = 0.0071) (Fig. 2). The survival time of *Pardosa* in the female and male *Hogna* chemical cue treatment was much longer compared with controls. As there was no block effect in the model we can obtain estimates of the hazard ratio or relative risk for *Pardosa* tested in the presence of male cues compared with controls, and for *Pardosa* tested in the presence of female cues by exponentiating β parameters associated with each indicator variable (see 'Methods' in expt I). In addition, the estimate of relative risk in the presence of male predator cues relative to female predator cues, and the 95% CI, can be obtained using the existing model and the parameter variance–covariance matrix (see Collett 1994 p. 90). *Pardosa* in control treatments were significantly more likely to be consumed by *Hogna* than

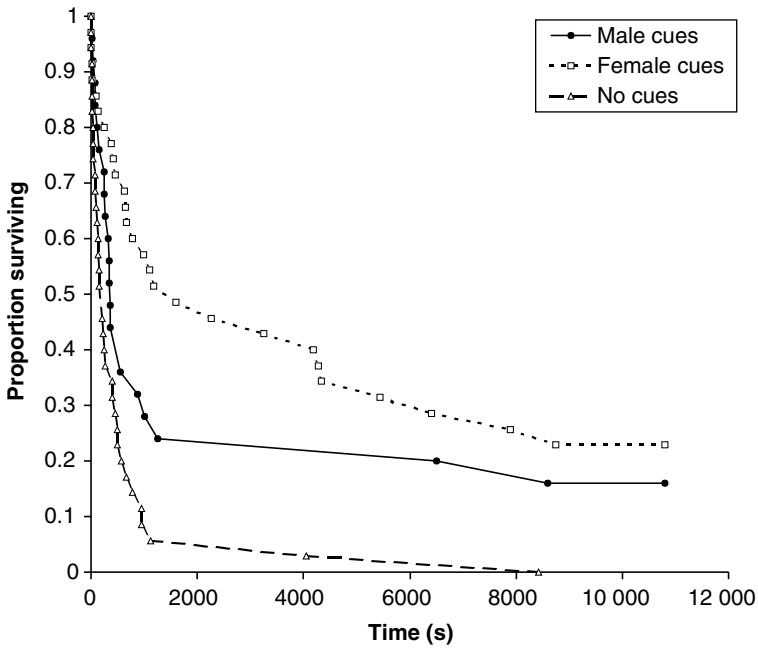

Fig. 2: Cumulative survival of adult female *Pardosa milvina* in the presence of adult female *Hogna helluo* with chemical cues of either a single adult female *Hogna*, a single adult male *Hogna* or no chemical cues (control). See expt II for details

individuals in the presence of male (likelihood-ratio test: $\chi^2 = 7.21$; d.f. = 1; $p = 0.0072$; risk relative to control = 0.48; lower 95% CI = 0.27; upper 95% CI = 0.82) or female (likelihood-ratio test: $\chi^2 = 18.67$; d.f. = 1; $p < 0.0001$; risk relative to control = 0.31; lower 95% CI = 0.18; upper 95% CI = 0.53) cues. There was no significant difference between survival of *Pardosa* in the presence of male vs. female *Hogna* cues (likelihood-ratio test; $\chi^2 = 2.014$; d.f. = 1; $p = 0.1558$; male risk relative to female = 1.51; lower 95% CI = 0.85; upper 95% CI = 2.70).

Very few *Pardosa* remained at the end of the 3-h period. All of the spiders in the control treatment were consumed within the first 25 min, 16% survived the 3-h period in the male *Hogna* cue treatment (four spiders), and 22% survived in the female *Hogna* cue treatment (eight spiders; Fig. 2). No *Pardosa* remained alive in the control treatments at the end of the experiment. The median survival times for each treatment were 204 s for the control, 364 s for male cues, and 1603 s for female cues. The mean survival times for each treatment were (in $\bar{x} \pm$ SE): control, 628.8 ± 258 s; male cues, 2623 ± 832.2 s; female cues, 4073 ± 733.7 s. There was an association between treatment and the number of individuals surviving, controlling for block (Cochran–Mantel–Haenszel test, $\chi^2 = 4.3657$, d.f. = 1; $p = 0.0367$). This was mainly driven by the low survival in the control treatment as there was no significant difference in the proportion of *Pardosa* that survived the 3-h period in the *Hogna* female or *Hogna* male treatments (Cochran–Mantel–Haenszel test, $\chi^2 = 0.3943$, d.f. = 1; $p = 0.5300$).

Experiment III: Shifts in *Pardosa* Activity Level in the Presence of Chemical Cues from Female, Male, or no *Hogna* Cues

Methods

We compared possible differences in *Pardosa* activity toward female and male *Hogna* chemical cues relative to substrates lacking predator cues. Satiated adult female or male *Hogna* (n = 30 per treatment) were individually placed in closed clean plastic containers for 24 h (9 cm diameter, 7 cm height) with a small plastic cap filled with water. The container was otherwise empty. *Hogna* were then removed and a single adult female *Pardosa* was introduced into each container under a clear plastic vial and allowed to acclimate for a 5-min period. The vial was then lifted and the spider could move freely within the container for a 30-min period.

Each test *Pardosa* was presented either an empty container devoid of male or female predator cues (control), cues from an adult female *Hogna*, or cues from an adult male *Hogna*. Each predator cue treatment container housed a different individual, and no container was used more than once. Similarly, no *Pardosa* was used more than once for any cue treatment. All spiders were tested between 07.00 and 19.00 hours. For each spider, we recorded the following behaviors separately for each chemical cue treatment: (1) time spent moving forward (walking) (W),

(2) time spent in non-forward movement (any movement of the appendages or turning of the body in place without walking) (NF), (3) time spent being immobile (no visible indications of movement) (I), (4) distance traveled (cm) (D), and (5) speed of movement, derived by dividing distance traveled by time spent moving forward (cm/s).

Spider movements were measured continuously by monitoring the spiders remotely using an automated digital data collection system (Videomex-I®; Columbus Instruments, Columbus, OH, USA) integrated into a black and white charge-coupled device (CCD) video camera. All locomotor behavior listed above was recorded automatically (see Persons et al. 2001 for a more detailed description of the video-tracking system). As none of the behavior classes showed a significant deviation from a normal distribution (Wilk–Shapiro normality statistic, Statistix®; Statistix Analytical Software 1996), parametric statistics were used for analyses. Differences in activity between predator sex treatments were compared using a randomized complete block ANOVA for each behavioral category. Time of testing (fall of 1999 or summer, 2001) was used as the blocking factor. The sample sizes used for each block were 20 individuals per treatment in the fall of 1999 and 10 individuals per treatment for the summer of 2001.

Experiment III: results

We did not find significant block effects among any behavioral categories ($F_{1,86} < 3.43$, $p > 0.07$). *Pardosa* showed a significant reduction in activity in the presence of chemical cues from male or female adult *Hogna* compared with control containers devoid of predator cues (Fig. 3). *Pardosa* traveled significantly shorter distances ($F_{2,86} = 22.96$; $p < 0.0001$) and spent significantly less time walking when on substrates previously occupied by male or female *Hogna* ($F_{2,86} = 22.67$; $p < 0.0001$). *Pardosa* also showed a significant reduction in non-forward movement ($F_{2,86} = 5.51$; $p = 0.0056$) and increased time being spent immobile ($F_{2,86} = 10.36$; $p < 0.0001$). In addition, there was a significant difference in mean speed across treatments ($F_{2,86} = 8.94$; $p < 0.003$). Based on Tukey–Kramer post hoc comparison of mean tests, we did not find significant differences in *Pardosa* activity in the presence of male or female cues for any behavior tested (Fig. 3).

Experiment IV: Female *Hogna* Responses to Female and Male *Hogna* Cues

Differences in survival of *Pardosa* across each chemical cue treatment could be attributed to differences in predator as well as prey behavior (Persons et al. 2001). Therefore, we measured the locomotor response of female *Hogna* toward chemical cues from other female and male *Hogna*, or to no chemical cues to determine if these cues significantly affected the predator's behavior. We prepared chemical stimuli and measured *Hogna* behavior with the automated video tracking system used in expt III (Videomex-I®).

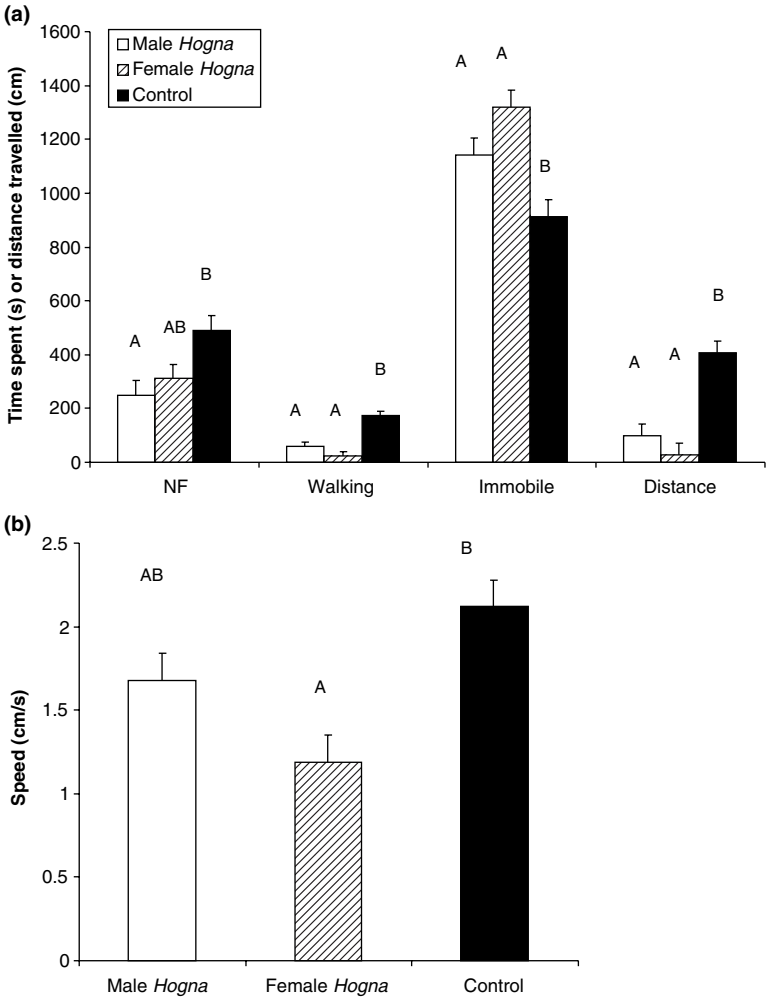

Fig. 3: (a) Activity levels (mean + SE), and (b) speed (mean + SE; cm/s) of adult female *Pardosa milvina* in the presence of male or female *Hogna* chemical cues compared with a blank control. Different letters above walking and distance are indicative of significant differences between treatments based on a Tukey–Kramer post hoc comparison of means test

Fifteen male and 15 female *Hogna* were all fed with 2-wk-old crickets, which were 2–3 cm in length. The adult female *Hogna* (n = 15) were left individually in plastic containers (20 cm diameters, 8 cm height) on filter paper for 24 h, taken out, and a different adult female *Hogna* (n = 15) was used for the 30-min recording of spider movement. The procedure was repeated for the chemical cue treatment with adult male *Hogna*. A third control treatment was used to record *Hogna* activity level on a substrate devoid of *Hogna* cues. We recorded the same behaviors for *Hogna* as for *Pardosa* in expt III. All other protocols were also

identical to that described in expt III except for the species tested. Behaviors included time spent walking, time spent in non-forward movement, time spent immobile, distance traveled, and speed of movement.

Experiment IV: results

There was no significant difference in any behavioral category between male or female chemical cue treatments, nor was there a significant difference between chemical cue sex treatments and the control (Table 1).

Discussion

Female and male *H. helluo* present different levels of predation risk to *Pardosa*. In general, female *Hogna* attacked more quickly and were found to be more persistent in stalking *Pardosa*. This finding is consistent with other studies that have demonstrated sex differences in foraging behavior and activity level among cursorial spiders (Givens 1978; Cady 1984; Persons 1999; Persons & Uetz 1999; Walker & Rypstra 2001, 2002). Givens (1978) found that females of the dimorphic jumping spider, *Phidippus audax*, tended to kill more prey more frequently, and also tended to consume prey more completely, and in a shorter amount of time, than males. Thus, female cursorial spiders are generally more willing to attack prey and are likely more aggressive toward prey than males and represent a greater degree of risk to prey.

Our results suggest that silk and excreta from either male or female *Hogna* induce similar behavioral responses from *Pardosa* that translate into improved survival. We found no differences in *Pardosa* survival in the presence of male and female *Hogna* cues and there was no statistically significant difference in *Pardosa* activity levels across male and female *Hogna* cue treatments. The presence of cues from either sex results in a large decrease in activity, and *Pardosa* survive equally well in the presence of a female *Hogna* if provided silk and excreta from either a female or a male *Hogna*. Given the significant differences in risk between male and

Table 1: Comparison of mean activity levels (±SE) for adult female *Hogna helluo* responding to chemical cues of another adult female or male *Hogna* compared with a blank control substrate (n = 15 per treatment). F-ratios are based on one-way ANOVAs for each behavioral category

Category	Male *Hogna*	Female *Hogna*	Control	$F_{2,43}$-value	p-value
Distance (cm)	907.72 ± 195.95	858.32 ± 208.99	999.12 ± 247.95	0.10	0.8985
Time walking (s)	294.40 ± 54.20	266.56 ± 56.88	348.46 ± 72.66	0.45	0.6354
Non-forward movement (s)	543.80 ± 53.00	611.31 ± 46.20	522.73 ± 24.87	1.16	0.3210
Resting time (s)	967.60 ± 91.12	895.18 ± 81.42	928.80 ± 72.33	0.16	0.8478
Speed (cm/s)	2.79 ± 0.15	2.98 ± 0.21	2.60 ± 0.18	1.06	0.3538

female predators, this suggests that female *Pardosa* may be overestimating their risk of predation in the presence of cues from male *Hogna*. While this does not support our original hypothesis, there is evidence that overestimation of risk may have minimal fitness consequences (e.g. Koops & Abrahams 1998).

As female *Pardosa* do not differentiate between cues left by male and female *Hogna*, questions arise about differences in the silk and excreta produced by males and females, and about the information contained in these cues. Male and female silk may differ in the kinds of pheromones they contain. For at least some species of wolf spider, female silk contains contact sex pheromones (Tietjen & Rovner 1982; Rypstra et al. 2003) and male silk contains male inhibitory pheromones (Ayyagary & Tietjen 1986). Presumably *H. helluo* also produces substratum-borne sex pheromones, although this remains speculative. Moreover, female *Hogna* are approx. 3.5 times as heavy as males, and consume more prey than do males (Walker & Rypstra 2002), suggesting that they may produce more excreta and possibly silk. Despite these potential differences between the silk and excreta produced by male and female *Hogna*, *Pardosa* responds in a similar fashion to cues from either *Hogna* sex. This implies either that *Pardosa* do not detect or use the differences in silk or excreta composition to determine appropriate antipredator behavior or that silk and excreta do not qualitatively or quantitatively differ between the sexes in *Hogna*.

Previous studies have shown that adult female *Pardosa* can discriminate between silk and excreta produced by *Hogna* that differ in size (Persons & Rypstra 2001). *Pardosa* tends to respond with reduced activity to *Hogna* chemical cues from a spider equal to or larger than itself (Persons & Rypstra 2001). However, determination of body size of *Hogna* based on silk and excreta cues alone is not definitive. Persons & Rypstra (2001) found that the antipredator response of *Pardosa* to silk and excreta from large- or medium-sized *Hogna* was statistically indistinguishable from the response of *Pardosa* to eight *Hogna*, each of which was one-fourth the mass of the *Pardosa*. Thus quantity of chemical cues is a useful, yet imperfect means of assessing risk and, under some circumstances, results in overestimating risk (e.g. responding to many small harmless *Hogna* as though they were a single large predator). We found a significant reduction in activity in the presence of cues from either sex relative to control substrates, but no significant difference based on the sex of the predator. These data collectively suggest that female *Pardosa* exhibit a threshold response to the quantity of predator chemical cues from *Hogna* (i.e. if the quantity of silk and excreta from a heterospecific spider exceeds that produced by a single adult *Pardosa*, then exhibit reduced activity). Thus, above a certain quantity of *Hogna* cues, *Pardosa* exhibit strong responses and the quantity of cues produced by both males and females is above this threshold. We also note that males and females may not differ in the quantity of silk and excreta that they produce or that such differences may lie below the perceptual limits of *Pardosa*.

Exhibiting antipredator behavior above a certain threshold quantity of predator cues might result in increased costs of antipredator behavior because of overestimation of predation risk. However, overestimation of predation risk may

have minimal fitness consequences if there is imperfect information about predation risk. Theoretical studies suggest that prey should either over- or underestimate their risk of predation depending on the assumptions made in the model (Bouskila & Blumstein 1992; Sih 1992; Abrams 1994, 1995; Bouskila et al. 1995; Koops & Abrahams 1998). However, in general, selection should favor the overestimation of risk as underestimation can be fatal (Bouskila & Blumstein 1992).

Empirical studies suggest that the degree of certainty prey have about the presence of a predator influences their antipredator behavior. Under conditions of uncertainty about predators, prey overestimate their risk and increase their refuge use (Sih 1992) or vigilance (van der Veen 2002). While chemical cues left by predators can be used as information by prey to avoid predators or exhibit appropriate antipredator behavior, this information is by nature incomplete. Although the presence of silk and excreta from *Hogna* does not clearly indicate the presence of *Hogna*, it indicates the likelihood that one is present. Thus chemical cues are at best, uncertain indicators of predation risk.

Although we found no statistically significant difference in *Pardosa* response to *Hogna* cues based on the sex of the predator, we did find that the direction of the expected responses of *Pardosa* matched our predictions. Activity level was lower for four of the five behavioral categories measured when *Pardosa* were on cues from a female *Hogna* compared with a male *Hogna*. Also the median survival time of *Pardosa* on substrates previously occupied by female *Hogna* was over four times higher than that of *Pardosa* placed on substrates with male *Hogna* cues. Further, the mean predation latency was over one and a half times longer on substrates with female predator cues compared with male-cued substrates. Finally, a higher percentage of *Pardosa* survived on substrates previously occupied by female *Hogna* compared with male *Hogna*. Because of these observed differences, we suggest that future studies of prey responses to predators that are sexually dimorphic may be fruitful.

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