2025–2026. Journal of Arachnology 53:174–182

Predation risk affects male activity and mating interactions with well-fed females in the wolf spider *Pardosa milvina* (Araneae: Lycosidae)

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Abstract. Courtship and mating behaviors are conspicuous and can attract eavesdroppers that pose a threat to both males and females. The body condition of females has significant effects on their reproductive output and so males should value well-fed females and be willing to take on increased risk in exchange for the opportunity to mate. We tested the hypothesis that predation risk affects male activity and mating behavior differently based on the female's body condition. We manipulated the body condition of female *Pardosa milvina* (Hentz, 1944) (Araneae: Lycosidae) and monitored the activity of males when placed on female substrate–borne cues with or without similar cues from their common predator, *Tigrosa helluo* (Walckenaer, 1837) (Araneae: Lycosidae). Males appeared to engage in a search for females on cues from well-fed females as they spent more time walking but covered less distance. Predator cues reduced this activity when coupled with cues from well-fed, but not food-limited females. In a second experiment, males were paired with females that differed in body condition and included treatments with and without predator risk. Females in good condition attacked males less and were more likely to mate. Males courted hungry females longer, but with the same intensity as well-fed females. Predation risk eliminated aggression toward males by well-fed females. In summary, males were able to identify the body condition of prospective mates from chemotactile cues alone, but when females were present, predation risk had little impact on the aspects of courtship and mating that we documented.

Keywords: Anti-predator behavior, female condition, mating success, male courtship

https://doi.org/10.1636/JoA-S-24-020

Courtship and mating activities are necessary but costly endeavors for many animals. These undertakings are not only energetically taxing but they also reduce the time available for other fitness-enhancing enterprises such as foraging (Scharf 2016; Karigo & Deutsch 2022). Thus, the resources accumulated prior to reproduction are critical, especially for females since they have direct impacts on the number of offspring produced, their size, and their prospects for long-term survival (Honek 1993; Bonduriansky 2001; Hunt et al. 2005; Scharf 2016). As a result, both partners have a vested interest in the body condition of the female which influences her ability to produce eggs and provide other necessary forms of parental investment. Hence, mature females with adequate reserves should be ready to mate just as selection should favor those males that can recognize their body condition as an indicator of their quality (Scharf et al. 2013; Iglesias-Carrasco 2018; Ando et al. 2020).

Courtship displays are advertisements as they have been selected for their efficacy in alerting potential partners to the signaler's location and quality. The conspicuousness of these activities means that they attract eavesdroppers such as predators and parasites in addition to prospective mates (Magnhagen 1991; Zuk & Kolluru 1998). Due to these risks, we expect that animals should have the ability to use environmental cues to assess the presence and proximity of any potential threats and adjust their behavior accordingly (Magnhagen 1991; Lind & Cresswell 2005; Dougherty 2021). Ultimately, mature animals must evaluate their own priorities and assess their capacity to reproduce successfully in the context of the current environmental situation. Through this evaluation, they can more effectively navigate the trade-off between engaging in the conspicuous activities necessary to ensure long term reproductive success versus defensive behaviors to avoid the risk of injury or death in the short run.

Spiders have proven to be an important model taxon for testing communication modalities and information exchange during male and female interactions (Schneider & Andrade 2011; Uhl & Elias 2011; Herberstein et al. 2014). Because hungry females are often voracious predators, selection favors males that recognize potential risk or receptivity on the part of the female and calibrate their responses early in the exchange (Schneider & Andrade 2011; Herberstein et al. 2014). Notably, silk and other chemical information convey key information during the interactions between sexes as they approach one another prior to mating (reviews in Gaskett 2007; Scott et al. 2018; Fischer 2019). For example, females of the orb-weaver, *Argiope brunnichi* (Scopoli, 1772) (Araneae: Araneidae) adjust their production of sex pheromones depending on their hunger level (Weiss & Schneider 2022) and male *Pisaura mirabilis* (Clerck, 1757) (Araneae: Pisauridae) are able to assess the reproductive value of a potential partner based on trails consisting of silk lines and body odor (Beyer et al. 2023). Intriguingly, widow spiders (*Steatoda grossa* (C.L. Koch, 1838) (Araneae: Theridiidae) actually attempt to manipulate males through their pheromones: when females approach starvation, they reduce the production and deposition of the chemical components of pheromones into the web but increase the rate at which any existing precursors are converted to the mate-attracting pheromones and ultimately are still able to attract males (Fischer et al. 2024). This reliance on chemicals is important because, in a recent synthesis, White et al. (2022) concluded that olfactory signals were more likely to attract the attention of eavesdropping predators than visual or vibratory displays.

When wolf spider species coexist, they can detect heterospecifics through silk and other excreta which stimulate the same chemosensory channels that are important to conspecific interactions during mate search and courtship. For example, *Rabidosa punctulata* (Hentz, 1844) (Araneae: Lycosidae) males court conspecific females in the presence of female silk cues alone but when cues from *Rabidosa rabida* (Walckenaer, 1837) (Araneae: Lycosidae), its co-occurring predator, are present, males forgo courtship and shift to a direct mount mating strategy (Wilgers

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et al. 2014). Similarly, male brush-legged wolf spiders, *Schizocosa ocreata* (Hentz, 1844), normally commence courtship when they encounter female silk and associated chemical excreta but, when those female cues are accompanied by similar cues from *Tigrosa helluo* (Walckenaer, 1837) (Araneae: Lycosidae), a larger intraguild spider predator, courtship ceases (Nickley et al. 2016). This reduction in courtship is not evident when *Pardosa milvina* (Hentz, 1944) (Araneae: Lycosidae) males and females are paired on cues from *T. helluo* (Taylor et al. 2005; Rypstra et al. 2016) but, when substrate-borne chemotactile cues from *either* a virgin female *P. milvina* or an adult *T. helluo,* are present, the survival of courting males is enhanced (Rypstra et al. 2017). When male *P. milvina* encounter silk and other excreta deposited on a substrate by females and by potential predators, there is a high probability that they will detect both and their behavioral reactions to different combinations of cues provide insight into how they manage risk during reproductive activity.

The goal of the studies reported here was to determine if predation risk affected male response to females that differed in their potential value as reproductive partners. We hypothesized that the body condition of female *P. milvina* would have different effects on the reactions of males to *T. helluo* cues and affect courtship and mating. In an initial laboratory experiment, we documented the activity of *P. milvina* males on substrate-borne chemotactile cues from food-limited and well-fed females with and without additional cues from the predator, *T. helluo.* In a second experiment, we introduced males to females that differed in condition and manipulated predation risk through chemotactile cues so that we might uncover any effects of these factors on courtship and mating interactions.

Study system.—*Tigrosa helluo* and *P. milvina* are the dominant spider species in the ground layer of agricultural fields of eastern North America (Young & Edwards 1990; Marshall & Rypstra 1999). Adult female *T. helluo* weigh 300–400 mg with the carapace widths measuring 5–7mm and they reliably prey on the smaller *P. milvina,* (weight: 20–40mg, carapace width: 2–2.5mm) (Persons et al. 2001). The relationship between these species makes them amenable to the study of the separate and combined effects of female condition and predation risk on male activity and mating behavior. Courtship by male *P. milvina* is initiated in response to substrate-borne silk and other excreta produced by females (Hoefler et al. 2008) and similar cues deposited by T. *helluo* elicit risk appropriate antipredator behavior to the size (Persons & Rypstra 2001), sex (Lehmann et al. 2004), hunger level (Bell et al. 2006), and diet (Persons et al. 2001) of the individual that produced the cues. Thus, we have a convenient and ecologically relevant way to manipulate perceived risk without interference from or death caused by a live predator. Males respond to females with spurts of intense activity that we refer to as courtship bouts. The male activity during these bouts includes two quantifiable metrics that have been associated with mating success (Brautigam & Persons 2003; Rypstra et al. 2003; Hoefler et al. 2009a). A body shake consists of a vigorous quiver while the animal is raised up on their tarsi, and a leg raise involves synchronously lifting the first pair of legs (Montgomery 1903; Kaston 1936; Godfrey et al., 2022). This courtship activity is an honest indicator of male quality (Hoefler et al. 2009a) but it is costly in that it renders males more susceptible to predation by the coexisting wolf spider predator, *T. helluo* (Hoefler et al. 2008). Finally, there is evidence that males recognize and value the recent feeding history of potential mates (Hoefler et al. 2009b).

METHODS

Spider collection and care.—All spiders originated from populations occupying the corn and soybean fields at Miami University's Ecology Research Center (39.5325°N, 84.7233°W Oxford, Ohio, USA). Because males can detect the female's mating status and prefer virgin females (Rypstra et al. 2003), all *P. milvina* females were collected as juveniles and completed their final molt in the laboratory, so we could be sure they had not had contact with males as adults. Depending on the experiment, we included some males that were collected from the field as adults and others were collected as immatures and matured in the laboratory. We used a combination of laboratory reared and field caught adult female *T. helluo* to provide predator cues. We elected to use females because there is strong evidence from past studies that *P. milvina* detect and react to their cues in a consistent manner that reduces risk (Persons & Rypstra 2001; Persons et al. 2001; Lehmann et al 2004; Bell et al. 2006). Seventy-two *T. helluo* females were randomly assigned to predator treatments in two separate experiments, with the caveat that each treatment had a combination of field caught and laboratory raised individuals. Each individual participated in only one trial of one experiment. When their role in these experiments was completed, spiders were either returned to the laboratory stock for possible use in other experiments or released near the collection site.

When not involved in experimentation, spiders were held individually in cylindrical containers with translucent walls and secure lids that allowed light to penetrate but prevented clear visual assessment of the surroundings. Each container had a 1–2 cm layer of damp peat moss on the bottom to provide substrate and moisture. Containers for *P. milvina* were 5.5 cm in diameter with 5.5 cm walls and containers for the larger *T. helluo* females were 10 cm in diameter with 8 cm walls. Ten to twenty spiders of the same sex and species were placed in boxes that were housed in an environmental chamber set at 25°C and 70% relative humidity with a 13:11 h light:dark cycle. When not involved in experimentation, spiders were fed a combination of crickets (*Acheta domesticus*) and fruit flies (*Drosophila melanogaster*) weekly. When we fed crickets, whether to experimental spiders or those in the general population, we selected individuals that were approximately 1/2 the body size of the spider. Field caught *P. milvina* males and *T. helluo* females were maintained in the laboratory for at least two weeks prior to experimentation to allow them to acclimate to the conditions and to reduce any differences in hunger.

Experimental feeding regimes.—We standardized the condition of *P. milvina* males by providing them with three crickets, 24 hrs prior experimentation. We standardized the condition of the *T. helluo* that were to provide predator cues by feeding them four crickets 24 hrs before cue deposition began. This feeding level followed a protocol deployed in other studies that used *T. helluo* chemotactile cues to test the effects of predation risk on *P. milvina* (Persons et al. 2001, 2002; Taylor et al. 2005; Rypstra et al. 2016).

For both experiments, we manipulated the prey provided to *P. milvina* females for three weeks, starting two days after they molted to adulthood. Females in the food-limited regime were fed two crickets during the first week, and one cricket during the second week. They were not fed in the final week before experimentation. We provided females under the well-fed feeding regime two crickets four times per week for the three weeks prior to experiments. At each feeding, we verified that at least one of the crickets had been killed and consumed. We also removed any living crickets, and all cricket remains before introducing additional prey. Twenty-four hours before experimentation, we provided the four crickets to the well-fed females and observed them to feed for 1.5 h before removing any remaining carcasses or living prey.

The carapace width and abdomen width of all female *P. milvina* were measured directly after their participation in the experiment using an ocular micrometer accurate to 0.1 mm. The carapace cannot change in adult spiders, but the abdomen will expand as the spider feeds. Together these measurements can verify that our feeding regimes had translated into body condition differences. We compared the abdomen widths of well-fed and food-limited females using an ANCOVA, with carapace width as the covariate (Jakob et al. 1996; Garcia-Berthou 2001).

Cue collection for both Experiments 1 and 2.—Both experiments involved presenting animals with *P. milvina* female chemotactile cues, consisting of silk, feces, pheromones, and other excreta, that were deposited on filter paper (Whatman #1). The filter paper lined the bottom of a plastic cylindrical arena, 19 cm in diameter with 6 cm walls. We included a 1.5 cm plastic disc with a damp cotton ball on top of the filter paper in the middle of the arena to ensure that the cue spider had access to water. The female was released into the container and the lid was secured. We left the female to roam freely for a 24-hour cue deposition period. The plastic disc was removed, the cotton water source was discarded, and the filter paper was used in experiments within 5 mins.

We collected *T. helluo* cues separately but simultaneous to the collection of *P. milvina* female cues. We placed 9 filter paper discs (each 3.5 cm in diameter) on the bottom of a circular 12 cm arena with 5 cm walls in a non-overlapping pattern. We included 1.5 cm plastic disc with moist cotton for moisture. We released a single female *T. helluo* and secured the lid. We allowed these female predators to deposit their chemotactile cues for the same 24 hr period as the female *P. milvina* that were involved in the same experiment.

At the commencement of the experiment, the lids were removed from both the container with the *P. milvina* and the container with the *T. helluo*. The spiders were corralled under opaque vials while the arenas were prepared. The 9 discs containing *T. helluo* cues were placed in a non-overlapping pattern on top of a larger piece of filter paper lining the test arena (19 cm in diameter), which either contained female cues or was blank (see fig. 1 in Rypstra et al. 2017). Nine plain filter paper discs with no predator information were positioned in the same manner for control trials. After this period of cue deposition, any *P. milvina* not involved in the experiment and the *T. helluo* used for cue deposition were returned to their home container in the environmental chamber and the water source was discarded.

Video recordings for both Experiments 1 and 2.—We recorded the behavior of the spiders in both experiments. After the arena was prepared, it was positioned under an overhead camera (Sony Hi8 Handy cam model CCD-V101) located in an isolated booth in a separate environmental room. The *P. milvina* under investigation were introduced into the test arena and sequestered under vial(s) (2 cm in diameter) for a two min acclimation period. We then removed the vial(s) to release the spider(s) and recorded their activity for 60 min.

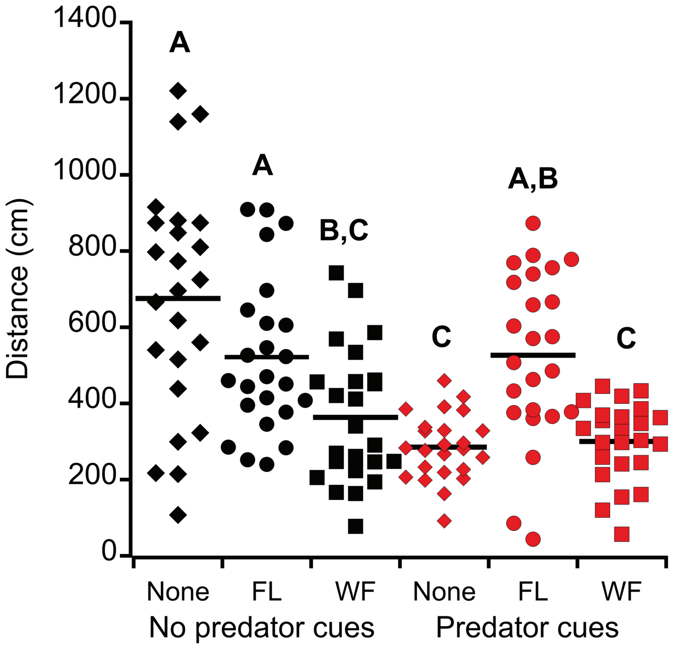

Figure 1.—The distance traveled (cm) by *P. milvina* males in containers with no female chemotactile cues (None), cues from food-limited females (FL), or cues from well-fed females (WF), with and without additional cues from the predator, *T. helluo*. The horizontal lines indicate the means. Treatments indicated with different letters are significantly different by Tukey HSD.

Experiment 1: Male activity on female cues.—In this experiment, we tested the hypothesis that predator information affects the activity of *P. milvina* males when they are presented with chemotactile cues from well-fed or food-limited females. The arenas were prepared with either blank filter paper or filter paper with female cues covering the bottom; the smaller discs were used to present predation risk (as in fig. 1 from Rypstra et al. 2017). Because males readily court virgin females and mate multiple times (Rypstra et al. 2003), we used a mixture of males collected from the field and males that matured in the laboratory. This approach allowed us to have a sample size of 24 for each of six different combinations of chemotactile cues: (1) blank filter paper with blank filter paper discs, (2) blank filter paper with discs containing predator cues, (3) filter paper with cues from food-limited females with blank filter paper discs, (4) filter paper with cues from food-limited females with discs containing predator cues, (5) filter paper with cues from well-fed females with blank filter paper discs, and (6) filter paper with cues from well-fed females and discs with predator cues. We recorded the activity of males for 60 min. The automated digital data collection system (Videomex-V, Columbus Instruments, Columbus, OH, USA) extracted the distance traveled (cm), time spent moving (s), and average speed (cm/s) of the males from the recordings. We used a Generalized Linear Model (GLM) against a Poisson distribution with the log as the link function to evaluate treatment differences. We ran the models with female body condition, predator cue presence, and their interaction. We included male type (field caught or laboratory reared) as a factor in initial analyses but we eliminated in situations when it was not significant since it was not germane to our hypothesis. Follow-up Tukey Honestly

Significant Difference (HSD) pairwise comparisons were conducted to evaluate specific treatment differences.

Experiment 2: Courtship and mating.—In this experiment, we tested the hypothesis that female body condition affects courtship behavior and mating success and that the presence of predator information modulates those impacts. Prior to trials for all treatments, female *P. milvina* deposited cues on the large filter paper that lined the entire arena in advance of trials. Each female was tested on their own cues. All the males for this experiment were collected as immatures and completed their final molt in the laboratory. We documented courtship behavior and mating success in four situations: (1) food-limited females with their cues and blank filter paper discs ($n = 24$), (2) food-limited females with their cues and discs with predator cues ($n = 22$), (3) well-fed females with their cues and blank discs ($n = 21$), and (4) well-fed females with their cues and discs containing predator cues ($n = 18$).

After the cue deposition period, the female was corralled under an opaque vial while the filter paper discs were added to the arena. Once the discs were in place and the arena was positioned under the camera, we introduced the male under a separate opaque vial. The vials were lifted after a 2 min acclimation period and the interactions between the pair were recorded for 60 min. Upon review of the video recordings, we documented the number of courtship bouts and summed their times to obtain the total duration of courtship. We also counted the number of leg raises and body shakes that the male performed during the five min period during which we observed the most vigorous courtship. We also noted the number of times the female lunged or chased the male and categorized these aggressive acts as either low (≤_5 lunges or chases) or high (> 5 lunges or chases). We noted whether or not the male was injured (i.e., leg loss), and whether or not copulation occurred. We quantified courtship intensity as body shakes/min and, separately, leg raises/min (as in Rypstra et al. 2003). We transformed these measures of courtship intensity and courtship duration in order to approximate normality and used the transformed variables in subsequent analyses.

We first determined whether mating success overall was related to courtship duration, body shakes/min, or leg raises/min by comparing these values for all mated and unmated males in separate t-tests. We used logistic regression to determine if female status, predator cues, or their interaction affected mating success. We categorized aggression as either low (≤_5 attacks or chases) or high (> 5 attacks or chases) and tested for the effects of female status, predator cues, or their interaction using the logistic regression. We also used logistic regression to assess whether male leg loss was related to female status, predation risk, or their interaction. We used GLM against a Poisson distribution with the log link function to determine if there were effects of our treatments on the number of courtship bouts performed by males. We ran ANOVAs to examine treatment effects on courtship duration or either of our metrics of courtship intensity. We calculated Spearman's ρ between each of our measures of courtship intensity and the length of the courtship period to determine if intense activity on the part of the male was associated with longer or shorter courtship times. All analyses were conducted using JMP Pro® Version 17.0.0 (2022) (SAS Institute Inc, Cary, NC, USA).

RESULTS

Verification of feeding differences.—Our feeding regimes were successful in differentiating well-fed and food-limited females in both experiments. Female abdomens were significantly wider for well-fed females in both the male activity experiment (ANCOVA, df = 2,80, F = 16.61, $P < 0.0001$), and the mating experiment (ANCOVA, df = 2,93, F = 17.34, $P < 0.0001$).

Table 1.—Results of Generalized Linear Models against a Poisson distribution with the log-link function that evaluated the effect of female cues, predator cues and their interaction on male activity data from Experiment 1.

Model features	Df	Test statistic	P
Time spent mobile (s)			
GLM	5,138	$\chi^2 = 4306.94$	<0.0001
Female cues		1957.23	<0.0001
Predator cues		812.40	<0.0001
Female * Predator cues		1841.39	<0.0001
Distance travelled (cm)			
GLM	5,138	$\chi^2 = 6230.41$	<0.0001
Female status		1978.86	<0.0001
Predator cues		1664.56	<0.0001
Female * Predator cues		2376.72	<0.0001
Speed (s/cm)			
GLM	5,138	$\chi^2 = 61.35$	<0.0001
Female status		7.94	0.0189
Predator cues		0.05	0.8165
Female * Predator cues		48.69	<0.0001

Experiment 1: Male activity on female cues.—Male type (field caught vs laboratory reared) did not have a significant effect in any of the models run for this experiment (all $P \geq 0.1$) and so it was not considered in the models reported. The presence of female cues, predator cues, and the interaction between the two significantly affected the distance that males travelled (Table 1). Males did not walk as far on cues from well-fed females compared to food-limited females (Fig. 1). The inclusion of predator cues reduced the distance travelled when no female cues were present (Fig. 1). Predator cues and female status were important to the time that the males spent engaged in locomotion and the model uncovered a significant interaction between them (Table 1). With no predator cues included, males spent more time walking when female cues were present but the predator cues reduced this activity metric when they were combined with cues from a well-fed female (Fig. 2). Female body condition, but not predator cues, affected the walking speed of the males and there was a significant interaction between the two stimuli in the model (Table 1). Males walked fastest when no cues were present and slowest when presented with cues from well-fed females alone, but predator cues eliminated those differences (Fig. 3).

Experiment 2: Courtship and mating.—The natural log was sufficient to normalize courtship duration and both courtship intensity measures that we documented (body shakes/min and leg raises/min). Mating occurred after 4.2 ± 0.9 min of courtship whereas unsuccessful males did not stop courting until 9.9 ± 2.1 min had passed (t = 2.55, $P < 0.0133$). However, there were no significant differences in the number of body shakes/min or leg raises/min performed by successful vs. unsuccessful males (body shakes: t = 1.2, $P = 0.2535$; leg raises: t = 0.47, $P = 0.6388$).

Mating success was not affected by predator cues but food-limited females were less likely to mate than their satiated counterparts (Tables 2, 3). On average, males engaged in 1.9 ± 0.2 courtship bouts and there were no differences in this metric

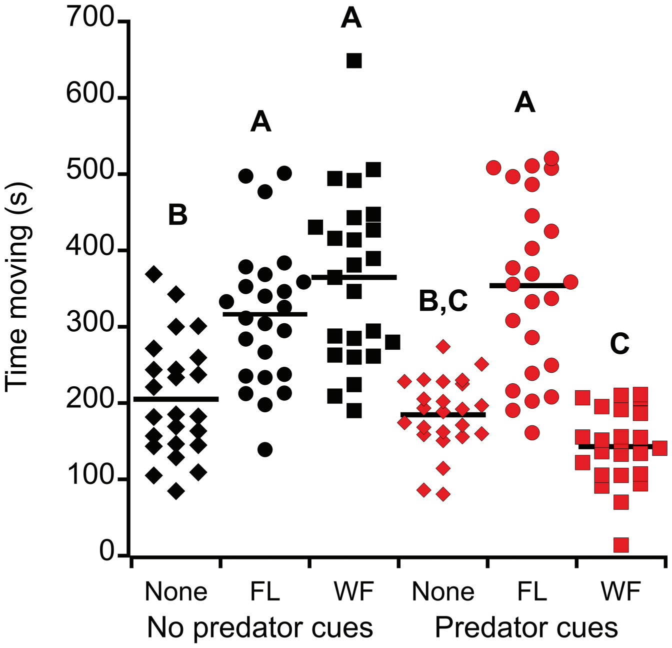

Figure 2.—The total time spent in locomotory activity(s) by *P. milvina* males in containers with no female chemotactile cues (None), cues from food-limited females (FL), or cues from well-fed females (WF), with and without additional cues from the predator, *T. helluo*. The horizontal lines indicate the means. Treatments indicated with different letters are significantly different by Tukey HSD.

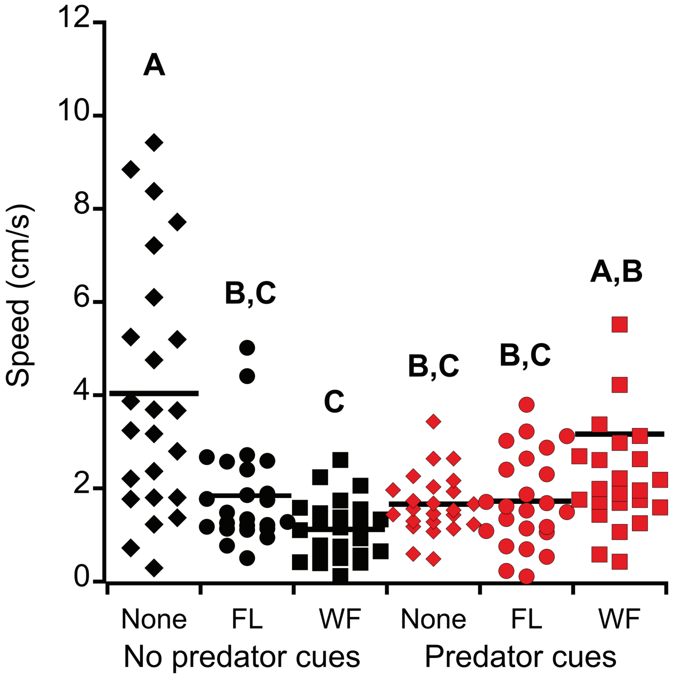

Figure 3.—The average speed (cm/s) that *P. milvina* males moved in containers with no female chemotactile cues (None), cues from food-limited females (FL), or cues from well-fed females (WF), with and without additional cues from the predator, *T. helluo*. The horizontal lines indicate the means. Treatments indicated with different letters are significantly different by Tukey HSD.

among treatments (Table 2). Males spent more time courting before mating or giving up when they were paired with food-limited females as compared to well-fed females but the presence of predator cues had no effect on mating duration (Tables 2, 3). Neither female body condition n-or predator cues affected courtship intensity as documented by the rate the male performed leg raises or body shakes (Tables 2, 3). However, courtship duration was positively correlated with both the rate at which the male performed body shakes ($\rho = 0.28$, $P < 0.0153$) as well as the rate at which he raised his legs ($\rho = 0.51$, $P < 0.0001$). Hungry females were more aggressive toward males than well-fed individuals but predator cues only affected the aggressive tendencies of well-fed females (Tables 2, 3). Males were much more likely to be injured by food-limited females, especially in the absence of *T. helluo* cues (Tables 2, 3).

DISCUSSION

Male *P. milvina* detect the body condition of females from their silk and excreta alone and the changes in behavior that we observed are also reflected in the strong effects of female condition on the likelihood of mating. Specifically, males were much less likely to mate with aggressive females in poor condition regardless of whether predator cues were present. Interestingly, the addition of substrate-borne chemotactile cues from a *T. helluo* predator affected the male's activity in the presence of cues from well-fed females, but their only impact on direct male-female interactions was to decrease the aggressiveness of well-fed females.

Overall, males slowed down, but spent more time walking, when female cues were presented to them. The "alerting hypothesis" suggests that once one sensory modality is stimulated, the sensitivity to input from other modalities should be heightened, which would allow the receiver to garner more detailed information about the specific location and status of the signaler (Rowe 1999). In spiders, pheromone-laden dragline silk serves to ready the male for the initiation of courtship (Barth 2002). In the situation considered here, once males identify females through their chemotactile cues, their sensitivity to visual and vibratory sensory input should increase in an effort to find and further assess the receptivity of the female (Rypstra et al. 2017). Thus, the behavioral shift that we observed in the presence of female cues can be interpreted as moving more cautiously while the males switched to a localized search allowing them to scrutinize the area. Indeed, those males presented with well-fed virgin female cues were likely to have been engaged in some preliminary courtship activity in an attempt to lure females out of hiding, and that activity would be read by our software as slower locomotion while covering less ground. Since silk is energetically costly (Prestwich 1977; Tanaka 1989) and *P. milvina* females adjust the pattern of silk deposition in response to environmental stimuli (Sweger et al. 2010; Havrilik et al. 2015; Khan & Persons 2015), it seems likely that well-fed individuals deposit more and different cues that contain detailed information as to their condition and readiness to mate. Presumably the difference in silk pattern, along with other chemical cues, causes the male's reduction in speed and the distance traveled to be more pronounced with satiated females than it was with cues from hungry individuals.

The results from our first experiment verify that males can identify the female's body condition from information in the silk and other excreta that were deposited as she moved through a barren area, however, in our second experiment, the females were

Table 2.—Results of statistical analysis evaluating the effects of female condition, predation risk and their interaction on courtship and mating behaviors of *P. milvina*. The natural log was taken of courtship duration, body shakes / min, and leg raises / min prior to statistical analysis. Aggressive acts were categorized as low (<5 during the interaction) or high (>5 during the interaction).

Model Features	Df	Test statistic	*P*
Mating success			
Logistic	3	$\chi^2 = 18.47$	0.0004
Female status	1	16.31	<0.0001
Predator cues	1	1.24	0.2662
Female * predator cues	1	0.84	0.3588
Courtship bouts			
GLM (Poisson distribution)	3	$\chi^2 = 0.20$	0.9771
Female status	1	0.01	0.9094
Predator cues	1	0.19	0.6614
Female * Predator cues	1	0.00	0.9745
Courtship duration (min)			
ANOVA	3,71	F = 5.62	0.0016
Female status	1	16.72	<0.0001
Predator cues	1	0.04	0.8367
Female * Predator cues	1	0.01	0.9083
Body shakes/min			
ANOVA	3, 71	F = 0.49	0.4921
Female status	1	0.98	0.3250
Predator cues	1	0.34	0.5514
Female * Predator cues	1	0.12	0.7254
Leg raises/min			
ANOVA	3,71	F = 0.53	0.6605
Female status	1	1.36	0.2464
Predator cues	1	0.18	0.6734
Female * Predator cues	1	0.03	0.8757
Aggressive acts			
Logistic	3	$\chi^2 = 24.03$	<0.0001
Female status	1	22.91	<0.0001
Predator cues	1	4.24	0.0396
Female * Predator cues	1	2.66	0.1027
Male injuries			
Logistic	3	$\chi^2 = 20.72$	<0.0001
Female status	1	18.50	<0.0001
Predator cues	1	0.00	0.9998
Female * Predator cues	1	0.00	0.9998

likely continuing to communicate with the males through additional silk cues. For example, female *P. milvina* increase silk dragline and attachment disc production when they are in the presence of silk from a courting male (Khan & Persons 2015), and they reduce the deposition of cord silk when a male is visible but not courting vigorously (Havrilak et al. 2015). Thus, female silk is a medium of sexual communication that can indicate her receptivity and encourage the performance of males and may, in part, drive the increase in courtship intensity we observed over the duration of courtship. Indeed, the extensive use of chemotactile cues by *P. milvina* may explain why we have been unable to identify a clear behavioral receptivity display on the part of females. While Brautigam & Persons (2003) were able to link distinctive female leg taps that were aimed in the direction of the male to mating success, we were unable to detect this or any other receptive posture or activity from our recordings.

Male courtship behavior in spiders communicates their quality and reproductive intentions, while at the same time, reducing the predatory tendencies of the female (Herberstein et al. 2014; Uetz et al. 2016). In our experiment, males spent more time courting food-limited females than they did in their performances for more attractive and less aggressive well-fed females. In addition, the duration of courtship was positively correlated with the intensity with which they performed leg raises and body shakes. As noted, these males may have been encouraged by silk receptivity signals from the females, however in nature, the animals could move away from one another in order to avoid an encounter. With no option to leave the vicinity of the female in our closed laboratory arenas, this continued and intensified courtship behavior may be the male's primary defense against female aggression.

Sexual cannibalism is rare in *P. milvina*. Wilder & Rypstra (2008) report only five instances (14% of trials) and all of those occurred after copulation was complete. None of the females in the present study killed the male even though they had lower body condition and were contained together in the closed arena for a longer period of time than the couples in Wilder & Rypstra (2008). Nevertheless, hungry females presented a danger to males in our mating experiment. Indeed, 32% of the males paired with food-limited females lost one or more legs during the experiment. Leg autonomy is not uncommon in *P. milvina*; field censuses found that up to 35% of individuals captured across the season were missing legs (Brueseke et al. 2001; Brautigam & Persons 2003). However, in laboratory experiments, the loss of one or two anterior legs had no impact on mating success for male *P. milvina* (Brautigam & Persons 2003). In the congener, *P. valens* Barnes, 1959 (Araneae: Lycosidae), males with missing legs experience lower survival than intact males (Brown et al. 2018) and in the wolf spider, *S. ocreata*, females display reduced receptivity to visual and vibratory information from males with missing legs (Fraser et al. 2020). Thus, the interactions with hungry females have the potential to compromise the reproductive success and long-term fitness of males even though such costs have not yet been documented for *P. milvina*.

In both experiments, the addition of predation risk only impacted spider actions with well-fed females or their chemotactile cues. When predation risk was added to chemotactile cues from well-fed females, the male's activity mimicked their reactions to predation risk alone. In a previous study, *T. helluo* posed a greater risk to females than to males during mating interactions, and the authors argued that the increased visual and vibratory sensitivity and heightened awareness instigated by substrate-borne chemical cues elicited antipredator behavior that protected the male while it rendered the female more vulnerable (Rypstra et al. 2017). In contrast, the attention required for females to identify and accurately assess male quality from their appearance and activity might have distracted the female's attention from extraneous environmental stimuli and increased her vulnerability. Nevertheless, the well-fed females in our mating experiment reacted to predation risk by reducing their aggression toward the male which is an activity that could attract the attention of an eavesdropping predator.

Our results are consistent with those in an earlier study of the impact of *T. helluo* cues on *P. mivina* courtship duration, courtship intensity, and mating success (Taylor et al. 2005). However, Taylor et al. (2005) found a significant reduction in pedipalp insertion rate and significantly more failed insertions when predator cues were present. Although we uncovered no effects of predation risk as represented by *T. helluo* cues on mounting success or courtship, it is possible that copulatory courtship and sperm

Table 3.—Courtship and mating metrics for each predator and female treatment in experiment 2. For the number mated, the number of highly aggressive trials, and the number of trials with male injuries, the percent of trials each category is included in parentheses. For courtship duration, body shakes / min, and leg raises / min, we report the mean (± S.E.). Results of statistical analyses are provided in Table 2.

Metric	Female status			
	Food-limited		Well-fed	
	No cues n = 24	Predator cues n = 22	No cues n = 21	Predator cues n = 18
# successfully mated (%)	7 (29.2%)	6 (27.3%)	17 (81.0%)	11 (61.1%)
Courtship duration (min)	9.0 ± 2.2	11.3 ± 3.4	3.8 ± 1.3	3.4 ± 0.1
Body shakes/min	5.0 ± 0.9	3.5 ± 0.6	4.8 ± 0.9	4.6 ± 0.8
Leg raises/min	7.4 ± 1.0	8.2 ± 1.3	6.4 ± 1.1	5.3 ± 0.4
# with >5 aggressive acts (%)	13 (54.2%)	10 (45.5%)	3 (14.3%)	0 (0.0%)
Male injuries (%)	9 (37.5%)	5 (22.7%)	0 (0%)	0 (0%)

transfer were compromised. Thus, we might expect that those effects would be exacerbated when females were in poor condition.

Neither of our measures of courtship intensity was predictive of mating success. Evidence for a link between male courtship activity and mating success is mixed for *P. milvina*. Explicit connections between these metrics and female selection have emerged in some studies, especially for leg raises/min (Brautigam & Persons 2003; Rypstra et al. 2003; Hoefler et al. 2009a) but other investigations reveal added layers of complexity to male-female interactions. For example, courtship in *P. milvina* males is highly repeatable except when good condition males have successive encounters with low quality females (Hoefler et al. 2009a). Additionally, when the legs that are raised during courtship are asymmetrical, males increase courtship intensity in the presence of *T. helluo* cues whereas those males with symmetrical legs reduce that behavior (Rypstra et al. 2016). Here we observed longer courtship periods for food-limited females which could be due to the female's reluctance to accept the male. Likewise, the length of courtship was positively correlated with the intensity of the male's performance, a result that suggests he may be intensifying his activity as the lack of interest and aggressiveness of hungry females became clear.

We satiated *T. helluo* prior to cue deposition in order to ensure that they produced a consistent layer of silk and other excreta. We surely would have seen some differences in the reactions to *T. helluo* cues, if the animal that produced them had been fed differently; in a prior study female *P. milvina* reacted differently to chemotactile cues from hungry vs. satiated *T. helluo* (Bell et al. 2006). However, we chose to treat the cue spider representing predation risk as in previous studies of the activity and mating behavior of *P. milvina* (Persons et al. 2001, 2002; Taylor et al. 2005; Rypstra et al. 2016). Additional studies should explore the effects of the experience and motivation of *T. helluo* on the activity and mating interactions of male and female *P. milvina* as this would help clarify the trade-offs that occur in a natural setting.

The distribution, body condition, and sex ratio of *P. milvina* are all influenced by *T. helluo,* its larger intraguild predator (Marshall et al 2000; Rypstra et al. 2007, 2017). While we have determined that males recognize and react to chemotactile information regarding the female's quality and the risk of potential predation by *T. helluo*, the condition of the female had larger impacts on the interactions between the sexes than the threat of predation. In some natural situations, females may be able to move away until they encounter a male in a low-risk environment, however others may have few options but to proceed with courtship and mating when they encounter a suitable mate. This study provides some insight into how males detect and respond to females that differ in body condition and how they manage the trade-off between mating and predation risk.

ACKNOWLEGMENTS

Financial support for this project came from a grant from the American Arachnological Society (to AMS), NSF grants 0216776 and 0216947 (to MHP & ALR), the Department of Zoology of Miami University, and the Hamilton Campus of Miami University. We are grateful for the contributions to this project made by E Heltzel, N Gordon, CD Hoefler, M Koper, J Riem, JA Schmidt, A Salazar, H Shannon, K Waisanen, SM Wilder, M Yazdani, K Voris, and H Voris. These individuals provided indispensable support and assistance throughout the study and the preparation of this manuscript.

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Manuscript received 4 July 2024, revised 27 January 2025, accepted 31 January 2025.