2005. The Journal of Arachnology 33:76–81

THE EFFECT OF PERCEIVED PREDATION RISK ON MALE COURTSHIP AND COPULATORY BEHAVIOR IN THE WOLF SPIDER *PARDOSA MILVINA* (ARANEAE, LYCOSIDAE)

Abraham R. Taylor and **Matthew H. Persons**[1]: Biology Department, Susquehanna University, Selinsgrove, PA, 17870, USA.

Ann L. Rypstra: Department of Zoology, Miami University, Hamilton, OH, 45011, USA.

ABSTRACT. The wolf spider, *Pardosa milvina* (Hentz 1844), shows effective antipredator responses in the presence of chemotactile cues (silk and excreta) from a larger wolf spider, *Hogna helluo* (Walckenaer 1837). We examined the influence of these substratum-borne cues on male *P. milvina* courtship and copulatory behavior. Forty-one pairs of adult virgin male and female *P. milvina* were placed on substrates with or without silk and excreta from an adult female *H. helluo*. Using behavioral observation software (Noldus Observer® 4.1), we recorded time until courtship, courtship duration, and intensity (leg raise and body shake rates). We also measured the total number of matings, the duration of each mating, and the number and rate of successful and failed palpal insertions. While we found no difference between treatments in mating success, courtship intensity or duration, there were significant increases in time until courtship and significant decreases in palpal insertion rates under predation risk. Males under predation risk also had significantly more failed palpal insertions than males not under risk. Results suggest that predation risk has a relatively minor impact on courtship displays and mating success, but could potentially impact mate searching, sperm transfer efficiency, or copulatory courtship.

Keywords: *Hogna helluo*, kairomone, mate choice, mating, chemical cue

Most wolf spider species engage in conspicuous courtship displays that include leg-waving, stridulating, drumming, tapping or other attention-drawing signals (Kaston 1936; Rovner 1967a, 1968, 1975; Stratton & Uetz 1986; Hebets & Uetz 2000). These visual and vibratory displays often significantly increase mating success of the males (Hebets & Uetz 2000; McClintock & Uetz 1996; Parri et al. 1997, 2002; Rypstra et al. 2003), but may also attract the attention of predators (Kotiaho et al. 1998). Males may mitigate the costs of courtship displays by reducing courtship intensity or duration when predation risk is elevated, however this may compromise species recognition or assessment of male quality by the female and contribute to lower mating success (Kotiaho et al. 1996, 1998).

Male courtship displays may not be the only component of lycosid mating behavior to be compromised by predation risk. Many species engage in prolonged copulation (Stratton et al. 1996) that could lead to reduced vigilance or physical impairment of the ability of either the male or female to quickly escape from a predator. During copulation, wolf spiders may perform a variety of conspicuous behaviors including rapid bouncing or vibrating of the abdomen by the male (Kaston 1936) and abdominal rotations by the female to facilitate pedipalp-epigynal coupling (Stratton et al. 1996). In addition to these movements, the male often scrapes, rubs or taps at the epigynum with his palps and engages in various repositioning movements as the male moves from one side of the female to the other (Kaston 1936; Rovner 1967b; Stratton et al. 1996). If these overt copulatory behaviors also attract attention from predators, pairs may benefit by minimizing their frequency or abbreviating copulation duration when predation risk is high.

Several recent studies have shown that the wolf spider, *Pardosa milvina* (Hentz 1844), is capable of detecting and responding to silk and excreta deposited by a larger syntopic

---

[1] Corresponding author.

wolf spider, *Hogna helluo* (Walckenaer 1837) (Persons & Rypstra 2001; Barnes et al. 2002; Persons et al. 2002). Upon encountering these cues from *H. helluo*, *P. milvina* typically greatly reduce their activity level and show increased vertical movement and substratum avoidance (Persons & Rypstra 2001; Persons et al. 2001, 2002). These behavioral shifts have a probable defensive function since *P. milvina* that reduce activity when encountering *H. helluo* silk and excreta survive significantly longer when confronted with live *H. helluo* than individuals that do not have access to these cues (Persons et al. 2001, 2002; Barnes et al. 2002). If *P. milvina* shows adaptive reductions in activity when encountering cues from *H. helluo*, presumably, the presence of these cues may also alter *P. milvina* courtship and mating behavior. Here we tested the influence of predation risk on *P. milvina* courtship and mating behavior by using *H. helluo* silk and excreta as a proxy for a live predator.

METHODS

Collection and Maintenance.—Between August and October 2002, we collected 82 intact *P. milvina* from corn, soybean and alfalfa fields near Susquehanna University, Selinsgrove, Snyder County, PA. To ensure that all spiders used in our mating experiment were virgins, we collected only antepenultimate and penultimate male and females and reared them to maturity in the laboratory. We also collected adult female *H. helluo* to be used for the deposition of silk and excreta as the source of predator cues. Both species of spider received food and water weekly. Diets consisted of 3–5 adult and subadult house crickets (*Acheta domesticus*) for *H. helluo* and 5–7 one-week-old cricket nymphs (*A. domesticus*) and adult fruit flies (*Drosophila melanogaster*) for *P. milvina*. Housing for *H. helluo* consisted of white round plastic containers (8 cm in height × 11 cm in diameter) with two to three cm of moistened peat moss as a substratum. *Pardosa milvina* were kept in clear round plastic containers of smaller size (5 cm in height × 8 cm in diameter) with one to two cm of the moist peat moss substrate. Spiders were maintained at room temperature (23–25 °C) with a 14:10 L:D photoperiod.

Stimulus Preparation.—We prepared 41 courtship and mating arenas that either did ($n = 20$) or did not ($n = 21$) have silk and excreta from a single adult *H. helluo*. Each arena consisted of a transparent plastic container (Rubbermaid Tortilla Keeper®, 9 cm h × 20 cm diam.). Forty-eight hours prior to testing, a 20 cm diam. circular sheet of white filter paper was placed on the bottom of each arena along with an inverted 15 dram vial lid containing several drops of water. The lid served as a means of providing humidity and a direct source of water to stimulus spiders during cue deposition. A single adult virgin female *P. milvina* was then introduced into each arena and allowed to move freely for a 24 h period. For the no-predator cues treatment, the female was then removed for an additional 24 h prior to being paired with a male. For the predator cues treatment, the female *P. milvina* was also removed for an additional 24 h; but immediately after removal, we introduced a single adult female *H. helluo* into the container where she was allowed to lay down silk and excreta on the filter paper for 24 h. The *H. helluo* was then removed from the arena immediately prior to testing. A different *H. helluo* was used to deposit predatory cues for each male-female *P. milvina* pair in the predator cue treatment. During stimulus preparation, we satiated both the adult female *P. milvina* and the female *H. helluo* by providing constant access to appropriately sized *A. domesticus* 24 h before their introduction into the arena to deposit silk and excreta. We also satiated adult male and female *P. milvina* pairs using the same method before the beginning of the trial. This served to reduce variation in body condition among paired spiders, a possible confounding variable in the female's mate choice decision or male display rates. All *P. milvina* tested had been between four and fourteen days post-final molt and all pairs were alternately assigned to either treatment to control for possible temporal effects on mating or copulatory behaviors between treatments.

Testing Protocol.—Following the removal of *H. helluo*, females were then reintroduced into their respective containers and allowed to acclimate for fifteen minutes, after which the males were introduced into the center of the arena under a clear plastic vial (15 dram). Males were allowed a two minute acclimation period under the vial after which time they were released and allowed to freely interact

Table 1.—Male *Pardosa milvina* behavior with (Predator cues) and without (No predator cues) the presence of silk and excreta from an adult female *Hogna helluo*. Each behavior was analyzed using a two-sample t-test. Mean ± SE for each behavior is reported. * indicates significant difference after a table-wide adjustment to the alpha level (sequential bonferroni).

Behavior	*n*	Predator Cues	No Predator Cues	*T*-value	*P*-value
Leg Raise Rate (/min)	41	13.42 ± 2.84	12.93 ± 2.32	0.135	0.8934
Body Shake Rate (/min)	41	11.14 ± 2.49	12.27 ± 2.14	0.347	0.7305
Time to Court (s)	39	320.72 ± 76.46	117.43 ± 24.98	2.688	0.010*
Courtship Duration (s)	41	799.71 ± 152.68	1033.01 ± 163.47	1.334	0.2010
Copulation Duration (s)	18	860.84 ± 134.27	744.10 ± 107.31	0.135	0.8934

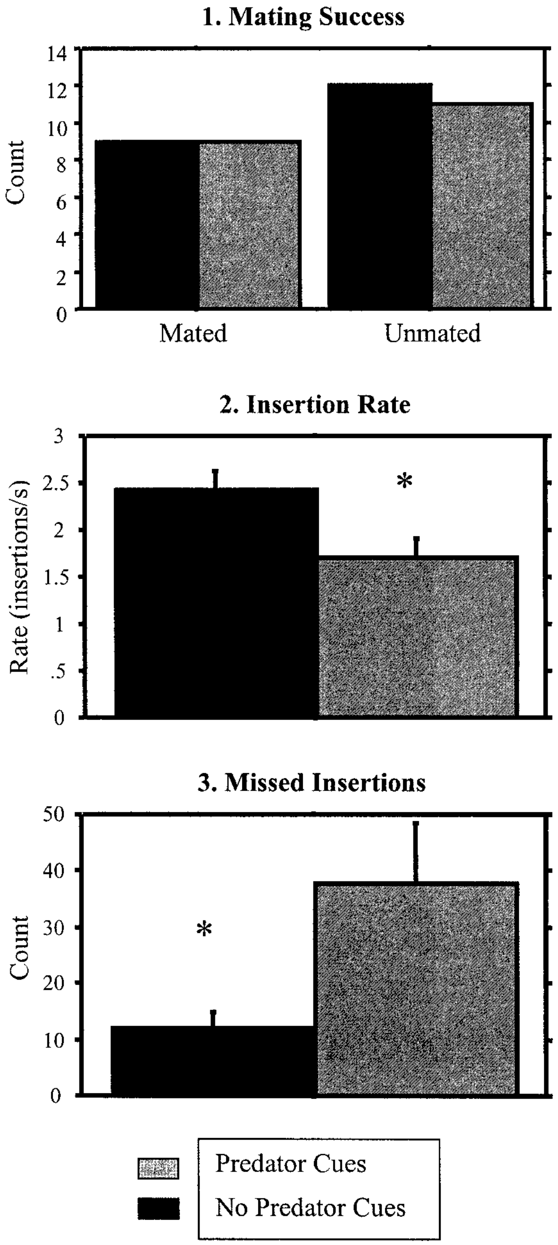
with the female for a thirty minute period. During each trial period we recorded two primary courtship behaviors: leg raises and body shakes (see Montgomery 1903 and Kaston 1936 for a complete description of *P. milvina* courtship behaviors). We also measured (1) time until courtship (the time period from the beginning of the trial to the first body shake or leg raise), (2) courtship duration (the time period from the first body shake or leg raise to a successful mount), (3) time until copulation (the time period from the start of the trial to the first palpal insertion) and (4) copulation duration (the time period from the first palpal insertion to a dismount).

In addition to courtship behaviors and copulation duration, we also measured more specific copulatory behaviors, including the total number of palpal insertions, the rate of palpal insertion per unit of time mounted on the female, and the total number of attempted palpal insertions that failed (general lycosid palpal insertion behavior described in Rovner 1975). For purposes of our study, palpal insertions were recorded as successful only if the extreme proximal end of the hematodocha was observed to visibly expand and the female abdomen concomitantly inflated with this expansion. A palpal insertion was recorded as ‘‘failed’’ only if the pedipalp became decoupled from the epigynum at the time of inflation resulting in the hematodocha expanding external to the female reproductive tract. Because of the difficulty in accurately videotaping copulatory behaviors, all data were recorded live with direct observations using the software package Noldus Observer 4.1®. Each male *P. milvina* was given 30 minutes to mount and begin mating before the trial was terminated. Pairs that were *in copula* after 30 minutes were allowed to continue mating until the male dismounted. Males that failed to mount within the 30 minute period were recorded as unsuccessful. Voucher specimens from this study were deposited in the Museum of Nature and Science, Denver, Colorado.

RESULTS

Most of the conspicuous behaviors exhibited during *P. milvina* courtship displays were not significantly different between treatments (Table 1). The presence of substratum-borne predator cues did not have a significant effect on courtship duration or either measure of courtship intensity (leg raise rates, or body shake rates) (Table 1) but did significantly affect time until courtship (Table 1). In the presence of predator cues, males took more than twice as long to initiate a leg raise or body shake than males without predator cues present (Table 1).

The presence of substratum-borne predator cues did not affect the mating success of *P. milvina* ($X^2 = 0.0191$; $P > 0.90$) (Fig. 1). Mating frequency was 42.8% for the no-predator treatment and 45% for the predator cues treatment. There was also no significant difference in copulation duration between treatments (Table 1). While most courtship behaviors were similar across treatments, we did observe some differences in copulatory behavior. Pedipalp insertion rates were significantly lower for matings under perceived predation risk ($t = 2.620$; $P = 0.0186$; $n = 18$) (Fig. 2), and the number of failed palpal insertions were significantly higher for the predator cues treatment ($t = 2.292$; $P = 0.0358$; $n = 18$) (Fig. 3). The total number of insertions during copulation ranged from a minimum of nine to a maximum of forty (mean = 25.16 ± 2.17 S.E.; $n = 18$) with failed insertion attempts being considerably more variable (range 1–99) (Fig. 3).



Figures 1–3.—1. Male mating success among *P. milvina* pairs with and without *H. helluo* cues present ($n = 21$ for no predator cues, $n = 20$ for predator cues). 2. Mean successful palpal insertion and hematodocha inflation rates (± S.E.) into the female epigynum ($n = 9$/treatment); 3. Total number of failed pedipalp insertions and hematodocha inflations when *H. helluo* cues are present ($n = 9$/treatment). Asterisks indicate significant differences between predator and no-predator treatments based on a two sample t-test ($\alpha = 0.05$).

DISCUSSION

Among measured courtship behaviors, only time until the onset of courtship showed a significant difference between treatments. This difference was likely due to a marked reduction in overall activity level by either the male, the female or both when on substrates containing *H. helluo* cues. During our observation of pairs among control treatments, the male would begin localized searching and chemoexploring immediately after detection of female silk. During this experiment, leg raises and body shakes were observed only after the female had either made some overt movement to draw the male's attention or after the male had made direct physical contact with the female. Males on *H. helluo* cues showed similar behaviors except they exhibited much reduced localized searching and occasionally prolonged periods of immobility. Anecdotally, we observed that females also moved much less frequently on *H. helluo* cues. Although this was not quantified directly in this study, other published studies have consistently documented significant reductions in activity of adult female *P. milvina* when encountering *H. helluo* cues (Persons et al. 2001, 2002; Persons & Rypstra, 2001; Barnes et al. 2002). We believe that reduced female movement rendered them vibratorily and visually cryptic to males and therefore impaired the ability of males to initially perceive females as has been found in other lycosids (Rovner 1996). Male activity could also have been compromised by the presence of *H. helluo* cues. This, in turn, may impair the ability of males to locate female silk or the female directly and delay the onset of courtship. Since we did not directly measure male and female activity in this study, it is difficult to ascertain the extent to which male or female behavior impacts the timing of courtship. Further, it is possible that males could perceive females while under perceived risk to the same degree as males not under risk, but chose to delay courtship because of the possible presence of a predator.

Although the onset of courtship appeared to be affected by *H. helluo* cues, the intensity of male courtship displays and mating success did not differ among treatments. Recent studies have established that male body shake and leg raise rates significantly affect *P. milvina* mating success (Rypstra et al. 2003; Brautigam & Persons 2003) . Since leg raise and body shake rates were the same among treatments, as well as mating success, we can tentatively infer that females do not modify their mate choice criteria with respect to male displays while under risk. It seems likely that males weigh the immediate benefits of mating more highly than the possible predation costs of display. Predator chemical cues, by their nature, only indicate a probability of a predator being in the area rather than confirmation of such a predator. Additional visual or vibratory information about the presence of a predator may be necessary to induce changes in conspicuous components of display that are known to be used in mate choice criteria.

Although predation risk had only a minor impact on courtship behaviors, we found significant differences in important copulatory behaviors. Insertion rates were significantly reduced while under predation risk. Presumably, this was due to a significantly higher number of failed insertions. It remains unclear why failed insertions increased while under risk, however several vertebrate studies indicate that efficiency of complex tasks such as foraging, is compromised by increased predator vigilance (Milinski 1984; Dukas & Kamil 2000, 2001). We suggest that increased predator vigilance during predator encounters may reduce attention paid to complex copulatory maneuvers.

The fitness consequences, if any, of decreased palpal insertion rates are unknown. If sperm is transferred at a similar rate throughout intromission, predation risk may significantly decrease transfer efficiency and possibly limit sperm availability to the female. Alternatively, if most sperm are transferred very early during copulation; as suggested by other spider species (Suter & Parkhill 1990), reduced insertion rates and missed insertions may have a minimal impact on male or female fitness. However, even if all sperm is transferred during the first insertion, continued insertions may still be adaptive. Prolonged intromission by males may serve as a form of copulatory courtship by providing information to the female about body condition, genetic quality, or otherwise serving to convince the female to accept the male's sperm. Continued intromission may also serve as a form of mate guarding, allowing sufficient time for the sperm to capacitate (reviewed in Eberhard 1996). As suggested by Suter and Parkhill (1990), further copulation may function to transfer substances in the ejaculate that may facilitate oviposition or nourish the offspring.

Our results suggest that predation risk may have the greatest impact on mate searching and copulation rather than courtship displays. As such, our study underscores the need to examine not only how conspicuous displays are influenced by predators, but also how copulatory behavior itself is affected. Future studies should address the reproductive consequences of modifed copulatory behavior while under predation risk.

ACKNOWLEDGMENTS

We would like to thank Ashley Shade, Kirsten Wilbur, Ryan Bell, Katie Hess, Valerie Wolfgang, Helen Petre, and Dan Church for their help collecting and maintaining spiders used for this research. This research was funded in part by NSF grant C-RUI DBI 0216776 for M. Persons and C-RUI DBI 0216947 for A. Rypstra.

LITERATURE CITED

Barnes, M.C., M.H. Persons, & A.L. Rypstra. 2002. The effect of predator chemical cue age on antipredator behavior in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). Journal of Insect Behavior 15:269–281.

Brautigam, S.E. & M.H. Persons. 2003. The effect of limb loss on the courtship and mating behavior of the wolf spider *Pardosa milvina* (Araneae: Lycosidae). Journal of Insect Behavior 16:571–587.

Dukas, R. & A.C. Kamil. 2000. The cost of limited attention in blue jays. Behavioral Ecology 11: 502–506.

Dukas, R. & A.C. Kamil. 2001. Limited attention: the constraint underlying search image. Behavioral Ecology 12:192–199.

Eberhard, W.G. 1996. Female control: sexual selection by cryptic female choice. Monographs in Behavioral Ecology. Princeton University Press, Princeton: New Jersey

Hebets, E.A. & G.W. Uetz. 2000. Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). Behavioral Ecology and Sociobiology 47:280–286.

Kaston, B.J. 1936. The senses involved in the courtship of some vagabond spiders. Entomology Americana XVI:97–167

Kotiaho J., R.V. Alatalo, J. Mappes, &. S. Parri. 1996. Sexual selection in a wolf spider: male drumming activity, body size, and viability. Evolution 50:1977–1981.

Kotiaho J., R.V. Alatalo, J. Mappes, S. Parri, & A. Rivero. 1998. Male mating success and risk of predation in a wolf spider: A balance between sexual and natural selection? Journal of Animal Ecology 67:287–291.

McClintock, W.J., & G. W. Uetz. 1996. Female choice and preexisiting bias: visual cues during courtship in two *Schizocosa* wolf spiders (Araneae:Lycosidae). Animal Behaviour 52:167–181.

Milinski, M. 1984. A predator's costs of overcoming the confusion-effect of swarming prey. Animal Behaviour 32:1157–1162.

Montgomery, T.H. 1903. Studies on the habits of spiders particularly those of the mating period. Proceedings at the Academy of National Science and Philosophy 55:59–149.

Parri, S., R.V. Alatalo, J. Kotiaho, & J. Mappes. 1997. Female preference for male drumming rate in the wolf spider *Hygrolycosa rubrofasciata*. Animal Behaviour 53:305–312.

Parri, S., R.V. Alatalo, J.S. Kotiaho, J. Mappes A. Rivero. 2002. Sexual selection in the wolf spider *Hygrolycosa rubrofasciata*: female preference for signal length and pulse rate. Behavioral Ecology 13:615–621.

Persons, M.H. & A.L. Rypstra. 2001. Wolf spiders show graded antipredator behavior in the presence of chemical cues from different sized predators. Journal of Chemical Ecology 27:2493–2504.

Persons, M.H., S.E. Walker, A.L. Rypstra, & S.D. Marshall. 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae: Lycosidae). Animal Behaviour 61:43–51.

Persons, M.H., S.E. Walker, & A.L Rypstra. 2002. Fitness costs and benefits of antipredator behavior mediated by chemotactile cues in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). Behavioral Ecology 13:386–392.

Rovner, J.S. 1967a. Acoustic communication in a lycosid spider (*Lycosa rabida* Walckenaer). Animal Behaviour 15:273–281.

Rovner, J.S. 1967b. Copulation and sperm induction by normal and palpless male linyphiid spiders. Science 157:835.

Rovner, J.S. 1968. An analysis of display in the lycosid spider *Lycosa rabida* Walckenaer. Animal Behaviour 16:358–369.

Rovner, J.S. 1975. Sound production by nearctic wolf spiders: a substratum-coupled stridulatory mechanism. Science 190:1309–1310.

Rovner, J.S. 1996. Conspecific interactions in the lycosid spider *Rabidosa rabida*: the roles of different senses. Journal of Arachnology 24:16–23.

Rypstra, A.L., C. Wieg, S.E. Walker, & M.H. Persons. 2003. Mutual mate assessment in wolf spiders: Differences in the cues used by males and females. Ethology 109:297–306.

Stratton, G.E., E.A. Hebets, P.R. Miller, & G.L. Miller. 1996. Pattern and duration of copulation in wolf spiders (Araneae, Lycosidae). Journal of Arachnology 24:186–200.

Stratton, G.E., & G.W. Uetz. 1986. The inheritance of courtship behavior and its role as a reproductive isolating mechanism in two species of *Schizocosa* wolf spiders (Araneae: Lycosidae). Evolution 40:129–141.

Suter, R.B. & V.S. Parkhill. 1990. Fitness consequences of prolonged copulation in the bowl and doily spider. Behavioral Ecology and Sociobiology 26:369–373.

Manuscript received 9 September 2003, revised 11 January 2004.