SHORT COMMUNICATION

Courtship and the use of pheromones by *Pholcus manueli* (Araneae: Pholcidae)

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Abstract.   Most web spiders use chemical and vibratory cues for intersexual communication prior to mating. Little is known regarding the behavior of the web spider, *Pholcus manueli* Gertsch, 1937. In the laboratory, we examined the behavior of males in female webs. Some webs were washed and allowed to air dry. Both washed and unwashed webs were tested with and without females. Overall, male behaviors were similar to courtship reported for other pholcid species and their frequency was related to male size. Web washing delayed the initiation of activity, reduced abdomen flexes, and increased the likelihood of pedipalp expansion and aggression. The presence of females caused males to initiate movement sooner and to increase silk pulling behavior. These results suggest that courtship is multimodal and that the vibratory dialog between males and females works along with chemical communication in this species. Hopefully, these data will enhance future studies of sexual selection in pholcids.

Keywords: male behavior, chemical communication, mating behavior

Spider silk is a particularly apt vehicle for information transmission and is known to augment visual and airborne signals with complex chemical, vibratory, and tactile information (Gaskett 2007; Uhl & Elias 2011). Many web spiders have poor vision and, as such, rely heavily on their web to help them identify prey, predators, and potential mates (Huber 2005; Gaskett 2007; Uhl & Elias 2011). It is particularly common for female spiders to attach pheromones to the silk, which provides males with information regarding the females' location, quality, and receptivity; often these pheromones are sufficiently potent to stimulate initial courtship activity (Gaskett 2007; Uhl & Elias 2011; Schulz 2013). These early phases of courtship serve to reduce potential aggression or cannibalism while allowing females to assess features of male quality (Schneider & Lubin 1998; Roberts & Uetz 2004; Huber 2005; Schulz 2013). Thus, characterizing the behavior of males in the presence of females and the pheromonal signals that are associated with the webs of females is an important first step in understanding the reproductive biology of any species.

Male and female cellar spiders (Araneae: Pholcidae) engage in a sophisticated dialog that includes air and silk-borne pheromones (Xiao et al. 2009, 2010; Hoefler et al. 2010) as well as body, leg, and pedipalp movements that affect silk tension (Bartos 1998; Uhl 1998; Huber 1999) and auditory signals produced by stridulation (Peretti et al. 2006). The courtship and mating behavior of *Pholcus phalangioides* (Fuesslin, 1775) has been well documented; pheromones in the webs of females elicit male orientation and signaling behaviors (Bartos 1998; Uhl 1998; Hoefler et al. 2010). Specific aspects of these signals along with prior experience, mating status, and body size of both sexes influence mating success (Schäfer & Uhl 2002, 2005; Schäfer et al. 2008; Uhl et al. 2005; Hoefler et al. 2010). Recent work with *Pholcus beijingensis* Zhu & Song, 1999 identifies many courtship behaviors analogous to those of *P. phalangioides* (Xiao et al. 2009, 2010). In addition*, P. beijingensis* is one of the few species where specific details about the structure and function of the pheromones deployed by both females and males are documented (Xiao et al. 2009, 2010). Much less is known about the courtship behavior and potential use of pheromones in other members of the genus *Pholcus*. Specifically, we have not been able to find any published studies on the behavior or ecology of *Pholcus manueli* Gertsch, 1937. The goal of this study was to document male behavior in female *P. manueli* webs in hopes of identifying key courtship activities. In addition, we examined the potential importance of pheromones and body size in influencing male courtship activity in a female web.

Pholcus manueli were collected from various buildings and barns in Butler County, Ohio USA. Once in the laboratory, the spiders were housed individually in translucent cylindrical plastic cups (23 cm diameter, 16 cm high) and held in an environmental room at 25° C on a 13:11 L:D cycle. Adult spiders were fed two crickets, *Acheta domesticus* (each 0.6 cm)*,* per week for a period of at least three weeks prior to testing. Our preliminary observations suggested that both adult males and females mate multiple times and seem to be universally eager to mate when they have been held in isolation for at least two weeks and the females have not produced egg sacs. Each male spider was randomly assigned to a female to populate each of the following treatments: 1) female in a web (n = 15), 2) female in a washed web (n = 12), 3) an empty female web (n = 14), and 4) an empty washed female web (n = 12).

Experimental webs were spun by adult female spiders. One day after feeding, each female was placed into a new cup (23 cm diameter, 28 cm high) lined with a hardware cloth cylinder (16 cm diameter, 24 cm high). The spiders place the bulk of their web attachment points on this cylinder and, as such, we could use it to relocate the web with minimal destruction. After one week, females were removed from webs. We measured the length of the tibia-patella of the right first leg with a digital micrometer attached to a stereomicroscope (0.01 mm accuracy) and held them in vials (1.5 cm diameter, 3 cm high). Males were removed from their webs, measured, and then returned to their home web until experimentation.

For treatments that required washing, we removed the hardware cloth cylinder holding the web and sprayed it six times with 95% ethanol from various angles using a hand-held plant sprayer. The cylinder was then placed in a new cup, covered with a 0.1 cm mesh lid, and placed under a fume hood for 24 h so that the ethanol and any volatile components of the pheromones could evaporate. We moved unwashed webs from container to container in a similar fashion but we did not spray them and we held them sealed cups for the 24 h period to minimize pheromone loss.

For trials with females, the female was reintroduced into her own web and allowed to acclimate for 3 min. Males were then introduced into webs for all treatments, and the experimental container was placed under a video camera in a closed booth that fed to a video recorder

Table 1.—Descriptions of the male behaviors that were quantified and their loadings for the top three principal components.

Male behavior	Description	PC1 (31.0%)	PC2 (17.8%)	PC3 (13.2%)
Web deposits	The male adds silk to the female's web	0.55	−0.30	−0.19
Abdominal flexes	The male raises and lowers his abdomen at a high rate	0.29	−0.29	−0.61
Silk tastes	Male opens his chelicerae and appears to place his mouth on female silk	0.84	−0.23	0.16
Web taps	Male strikes the web or the female's leg repeatedly with one of his legs	0.90	−0.07	0.12
Searches	Male rotates the first pair of legs in large circular swivels	0.55	0.44	−0.30
Silk pulls	Male uses one of his first pair of legs to pull or pluck a strand of silk	0.08	0.86	0.02
Aggression	Male jerks toward the female with chelicerae spread wide	−0.13	−0.43	0.50
Pedipalp extension	The male unfolds one or both pedipalps to full length	0.50	0.28	0.52

located in an adjacent room. The behavior of the male was recorded for 30 min or until copulation or cannibalism started to occur. We reviewed a number of videos and identified eight distinctive behaviors (Table 1). We then watched all videos and recorded the initiation and duration of male activity as well as the frequency with which he performed each of the eight behaviors (Table 1).

We compared the time to initiate and the duration of male activity using two-way ANOVAs with female presence or absence, web washed or not, and the interaction as factors. The rest of the behaviors were combined in a Principal Components Analysis (PCA). Principal Components (PCs) with eigenvalues greater than one were retained and compared using two-way ANOVAs. For the 27 trials that included both males and females, we determined whether they differed in size using a matched pairs t-test. We also examined the relationship between size of the male or female and their behavioral repertoire by calculating Pearson product-moment correlation coefficients (r) between male or female size and each of the retained principal components.

All males initiated activities in trials but it took males significantly longer to start moving when they were in washed webs with no female present (Table 2, Fig. 1A). In all cases, males stopped activity before the 30 min trial was over but their activities lasted significantly longer when a female was present (Table 2, Fig. 1B). The patella-tibia length of males (7.95 ± 0.10 mm), our indicator of body size, was significantly larger than that of females (7.17 ± 0.08 mm) (t = 6.69, P <0.0001).

Three PCs with eigenvalues greater than one emerged from the PCA and together they explained 62.1% of the variation in behavior (Table 1). Male size was correlated with PC1 (r = 0.28, P = 0.04), which captured 31% of the variation in the data. There were strong loadings of web tasting and web taps (>0.75) and moderate loadings of web deposits, searches, and pedipalp extension with PC1 (>0.50) (Table 1). PC1 was not affected by the presence of the female or silk washing (Table 2). Neither PC2 nor PC3 was correlated with male or female size (all P >0.5). PC2 captured 17.8% of the variation in behavior; it reflected high levels of web pulling (the only item with a loading >0.75) (Table 1). Female presence but not silk washing affected PC2 (Table 2, Fig. 1C). PC3 captured 13.2% of the variation in behavior; it incorporated the likelihood of male aggression and pedipalp extension and was inversely related to the frequency with which males performed abdominal flexes (Table 1). Both female presence and silk washing were related to PC3 (Table 2); PC3 was highest in washed webs with females and lowest in unwashed webs without females (Fig. 1D).

Spiders use a variety of sensory channels to communicate and our results verify that communication in *P. manueli* is multi-modal (Hebets & Papaj 2005). While males delayed activity in empty washed webs, they responded quickly when females were present (Fig. 1A), presumably because they detected females through vibratory communication. In addition, reducing pheromones by washing the webs increased the

likelihood of aggression and the unfolding of pedipalps (PC3, Fig. 1D). The union of these two behaviors in one PC seems counterintuitive because pedipalp extension is considered a precursor to copulation in *P. beijingensis* (Xiao et al. 2009) but aggression would seem to signal mate rejection, the opposite response. This particular combination may reflect the male's uncertainty as to the exact status and receptivity of the female precisely because the pheromones are altered by our washing treatment. Notably PC3 values for males in washed and unwashed webs converge when females are present (Fig. 1D), which suggests that females' signals clarify the situation for males.

The array of activities we observed in male *P. manueli* is similar to courtship behaviors reported for other *Pholcus* species. Specifically female presence had a strong impact on PC2 which reflects the frequency of silk pulls, a behavior featured in the courtship repertoire of both *P. phalangioides* and *P. beijingensis* (Bartos 1998; Xiao et al. 2009). Thus, it would seem that this behavior plays an important role in sexual communication prior to mating. Specifically, this silk pulling seems to initiate a conversation with the female and the subsequent exchange that occurs between the two spiders may ultimately reveal the receptivity of the female. The abdomen flex, negatively associated with PC3, is another behavior featured in the courtship of both *P. phalangioides* and *P. beijingensis* (Bartos 1998; Xiao et al. 2009). The fact that PC3 was lower (i.e., that abdominal flexes were higher) in unwashed webs provides evidence that our washing treatment

Table 2.—Statistical analyses for courtship and principal components derived from the behaviors recorded. Non-significant interactions were eliminated from analyses.

| Model features	Df	Test statistic	P
Initiation of activity			
Model	3,48	F = 3.84	0.0153
Female presence		t = 2.22	0.0314
Silk washing		t = 1.77	0.0832
Female * washing		t = 2.03	0.0483
Duration of activity			
Model	2,50	F = 3.28	0.0461
Female presence		t = 2.15	0.0367
Silk washing		t = 1.39	0.1694
PC1			
Model	2,50	F = 0.44	0.6464
Female presence		t = 0.77	0.4420
Silk washing		t = 0.52	0.6085
PC2			
Model	2,50	F = 4.40	0.0174
Female presence		t = 2.97	0.0046
Silk washing		t = 0.09	0.9257
PC3			
Model	2,50	F = 9.59	0.0003
Female presence		t = 3.11	0.0030
Silk washing		t = 3.13	0.0029
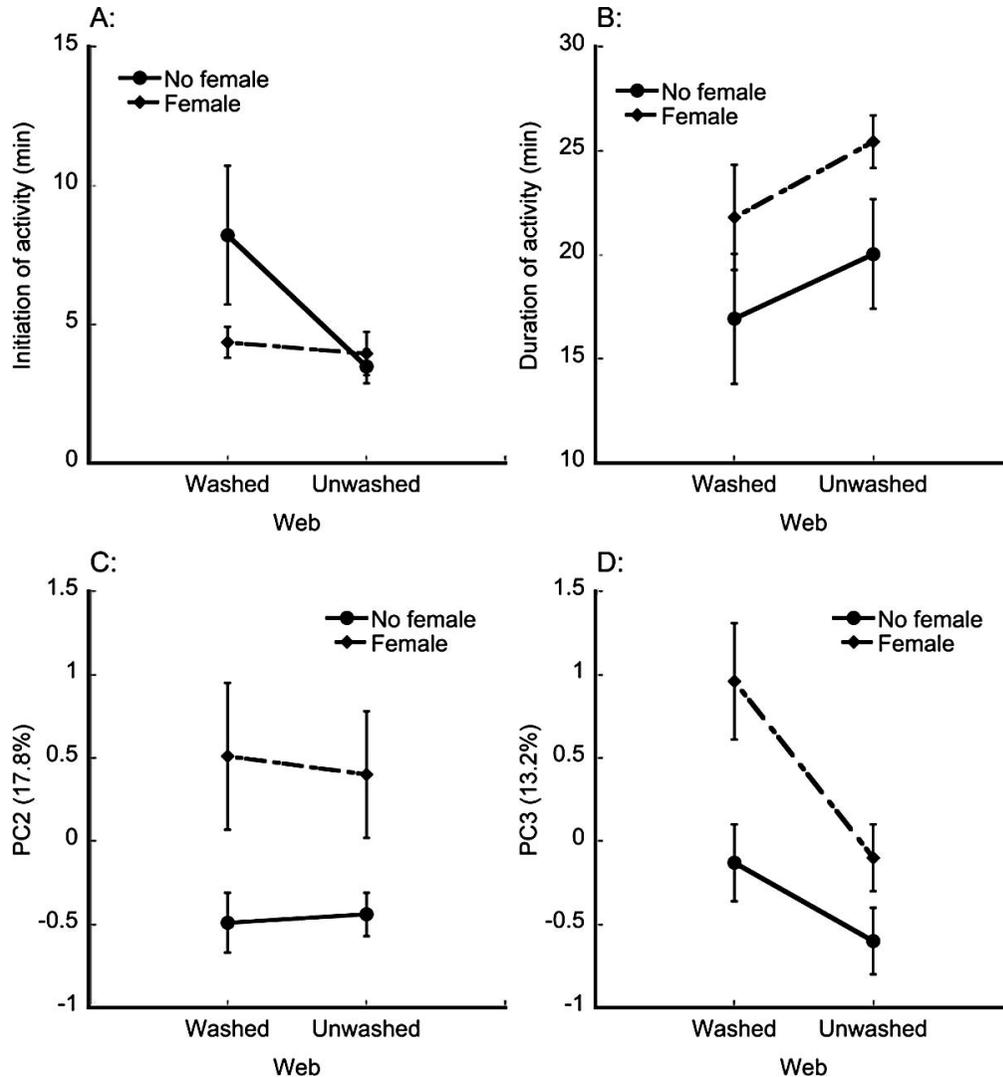
Figure 1.—Male behavioral metrics that were affected by either web washing, the presence of a female, or both. A: Time to initiate activity, B: Duration of activity, C: PC2 (see Table 1 for loadings), D: PC3 (see Table 1 for loadings). Statistical analyses are presented in Table 2.

influenced the normal course of courtship interactions in *P. manueli*. Males also seemed to engage in this behavior more intensely when no female was present (Fig. 3D). Thus, this activity may be an early courtship signal that helps males find females within the web, especially in situations where the chemical information is not clear. Males, with uncertain information in washed webs, may be more anxious to communicate to female web owners that they are potential mates and not prey items.

Intriguingly, many of the behaviors were unaffected by the presence of a female or web washing; PC1 captured most of the behavioral variation that we observed and was tied to the greatest diversity of behaviors. High values of PC1 indicate that males engaged in frequent silk tastes and web taps, behaviors that suggest animals added to their sensory input through gustatory (tastes) and vibratory (taps) channels. In addition, PC1 was moderately associated with web deposits, which would be a means by which males could communicate with females via pheromones (Xiao et al. 2010). PC1 was also associated with searching behavior, where males rotate their legs widely in a manner that expands their sensory range. Taken together, the suite of behaviors reflected by PC1 seems to be general information gathering. However, as males spend more time in the web, they are able to gather more evidence regarding

the location, size, and receptivity of females, all of which are important to mate solicitation and mating success. In webs with no females, males cease this activity sooner (Fig. 1B), but when females are present, they continue longer (Fig. 1B) and alter their behavior patterns (Fig. 1C, 1D).

Interestingly, the activities captured by PC1 increased with male size but we did not uncover any impact of female size on male behavior across treatments. If our female *P. manueli* responded to larger males with more encouragement, then those males may have been stimulated to intensify aspects of courtship captured by PC1. On the other hand, pre-copulatory sexual cannibalism is a much higher risk for small males (Wilder & Rypstra 2008) and, as a result, small males may approach more cautiously than their larger counterparts when on the webs of strange females. Only further study will clarify the details of the early dialog between males and females of this relatively unknown species as they enter courtship and attempt to mate.

The pheromones of *P. beijingensis* have two components that work primarily when they are present in a 2:1 ratio (Xiao et al. 2009). Our results suggest that *P. manueli* is not likely to be as sensitive to a specific blend of compounds. The ethanol and air treatment that we deployed affected male activities but did not eliminate distinctive

behaviors that are key features of courtship documented for other *Pholcus* species. If a precise ratio of compounds were necessary for *P. manueli*, we would have expected our web washing to disrupt that balance and, even if our approach did not remove the pheromones, the disruption would have resulted in larger impacts of web washing on male behavior. It is the case that we had hoped to use more comprehensive washing technique for these initial experiments with a greater diversity of solvents but the webs were too fragile to withstand repeated treatments and we wanted the integrity of the webs to remain so that the females were housed in relatively normal intact webs during trials. Nevertheless, these results set the stage for the more sophisticated behavioral and chemical assays necessary to demonstrate the specific nature of the chemical signals.

Here we provide documentation of the array of behaviors performed by male, *P. manueli,* in females' webs. Our data suggest that both chemical and vibratory communication are important as males and females interact prior to mating. This study identifies a few behaviors that are more or less sensitive to chemical signals as well as those that seem to be directed at actual females. Further study of this species may help us generalize the detailed studies that have been conducted on congeners.

ACKNOWLEDGMENTS

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