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hereby submit this original work as part of the requirements for the degree of:

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Response to chemical cues in male and female Schizocosa ocreata (Hentz) wolf spiders.

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Response to chemical cues in male and female *Schizocosa ocreata* (Hentz) wolf spiders.

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Abstract:

In predatory animals such as spiders, females are often aggressive or potentially cannibalistic, and thus selection has favored males that recognize females and signal their identity and intent to mate at a distance. This often involves recognition of chemical cues or signals (pheromones) from females. Chemical communication is an important part of the mating process in the wolf spider *Schizocosa ocreata*, as cues associated with female silk are sufficient to elicit courtship from males. However, it is not known whether females also respond to chemical cues associated with male silk. Although responses of males to airborne chemical cues from females have been demonstrated in some spider species, this has not been well examined in *S. ocreata*. In this study, I examine how both male and female *S. ocreata* wolf spiders respond to silk-borne and airborne chemical cues from members of the same or opposite sex. I collected silk from mature males and females onto a piece of filter paper and recorded the amount of time that spiders spent in a stimulus chamber containing male silk, female silk, and no silk (control). Males spent more time on female silk, while females spent significantly less on female silk, but neither sex showed a significant difference between male silk and a control. In addition, I conducted studies in an olfactometer in which subjects were exposed to airborne chemical cues from a male spider, female spider, male silk, female silk, or a blank control. I scored the choice made as well as specific male courtship behaviors. Males and females showed no significant directional response to airborne cues from either sex, but males displayed courtship behaviors most often and at higher rates when exposed to airborne cues from females. Furthermore, I compared the rates of male courtship behaviors between airborne chemical cues and silk-borne cues from females and found that silk elicited higher rates of courtship than airborne chemical cues. I conclude that chemical communication in this species is unidirectional and female-originated, and most effective in stimulating courtship when associated with silk.

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Table of Contents:

Abstract ii

Acknowledgements iv

List of Tables & Figures vi

Introduction 7-11

Methods

- Animal Maintenance 11
- Silk-borne Cues Study 11-12
- Airborne Cues Study I 12-13
- Airborne Cues Study II 13-14

Results

- Silk-borne Cues Study 14
- Airborne Cues Studies 14-16

Discussion 16-19

References Cited 20-25

Figures & Tables 26-44

List of Figures & Tables:

Figure 1: Silk-borne Cues Apparatus Design

Figure 2: Side View of Olfactometer I

Figure 3: Side View of Olfactometer II

Figure 4: Mean Total Time Spent on Silk

Figure 5: Choice by Treatment in Airborne Cues Study

Figure 6: Presence/Absence of Courtship Behaviors by Treatment in Airborne Cues Study

Figure 7: Rates of Courtship Behaviors by Treatment in Airborne Cues Study

Figure 8: Comparison of Courtship Behaviors in Silk-borne vs. Airborne Cues Studies

Table 1: 2-way ANOVA of Total Time by Year and Source of Silk in Silk-borne Cues Study

Introduction:

Animal communication is critical to individual fitness, especially in the context of mating and reproduction (Witt & Rovner 1982; Bradbury & Vehrencamp 1998; Alcock 2005). Species-level differences in communication are hypothesized to foster evolution of animal biodiversity (Elgar 1991; Huber 2005). Successful communication consists of one individual (the sender) producing and sending a signal to another individual (the receiver), and the receiver recognizing and responding to the signal (Bradbury & Vehrencamp 1998). There are many different modes of communication utilized by animals, e.g., visual, mechanical, and chemical signals, and many animals use several modes (multimodal signals) simultaneously (Bradbury & Vehrencamp 1998; Hebets & Uetz 1999, 2000; Uetz 2000; Uetz & Roberts 2002; Uetz et al. 2009).

Chemical communication is thought to be one of the most primitive modes of communication and is found in most animal taxa (Tietjen & Rovner 1982; Wyatt 2003; Gaskett 2007; Johansson & Jones 2007). This mode of communication occurs via a relatively simple neural mechanism, in which a molecule or blend of molecules binds to a chemoreceptor and thus activates an action potential. Chemical communication involves a great diversity of "semiochemicals", containing species-specific information, e.g., sex pheromones of arthropods and other animals (Wyatt 2003; Johansson & Jones 2007). From a signaler-receiver perspective, however, a receiver capable of detecting and recognizing information contained in chemicals may gain benefits whether such cues are intended as "signals" or not (e.g., pheromones vs. by-product odors). While in some examples of chemical communication in animals, chemical "signals" benefit both the signaler and receiver, whereas chemical "cues" benefit the receiver, but need not benefit the sender. As a consequence, unless the function of a chemical communication signal is known, "cue" is used as a more conservative term..

Chemical cues may be emitted in a variety of ways. In many animals, chemical cues are secreted by specialized organs or within urine or feces, and deposited directly on a substrate. The chemical may then volatilize and be detected from the air by the receiver, or the receiver may gather information from the chemical cue by direct contact chemoreception. Alternatively, chemical cues may be detected by direct contact with the individual or by an airborne cue that originates from the surface of the individual. In mammals and other vertebrates, it has been demonstrated that the chemical cues associated with excretions and produced by scent glands are important in kin recognition, mate selection, and territory defense (Wyatt 2003; Johnston 2003; Mateo 2003; Johansson & Jones 2007). In particular, the odors that provide information about an individual's major histocompatibility complex (MHC) are important to female rodents when choosing a mate and females prefer males that have different MHC's than their own (Johnston 2003; Penn & Potts 1999). In arthropods, chemical cues that originate from the body surface are known as cuticular hydrocarbons (CHC's) and have been shown to contain information about species identity and/or characteristics of males such as dominance, symmetry, and quality of the immune system, which are important to females when selecting mates (Lawniczak et al. 2006; Howard & Blomquist 2005; Johansson & Jones 2007). While there are a variety of ways in which individuals detect chemical cues, the information carried with the chemical cue and the degree of its importance may vary relative to the source of the cue and the medium through which it is transmitted.

In animals that are ordinarily solitary and territorial, communication becomes important during certain critical times such as when finding and choosing a mate (Shorey 1976). This is especially true in spiders, for which a tendency to be aggressive towards conspecifics and cannibalism are not uncommon. As a consequence, spiders are good models for studying animal communication, because female-biased sexual size dimorphism and the potential for sexual cannibalism create intersexual selection on males to communicate their species identity and intent to mate effectively (Huber 2005; Elgar 1991; Eberhard 2004). Males that are able to distinguish potentially receptive conspecific females from a relatively safe distance based on chemical cues are at a selective advantage over those males that approach unreceptive females. Consequently, mate searching and the stimulation of courtship behaviors in male spiders are often mediated by chemical cues produced by females, which may be airborne, water-borne, associated with silk, or located on the cuticle (Trabalon et al. 1997; Schulz 2004; Gaskett 2007).

In spiders, chemical signals are detected by specialized chemosensory hairs located primarily on the pedipalps (Tietjen & Rovner 1982; Barth 2002; Schulz 2004; Gaskett 2007). Although it has been suggested by some that the tarsal organ may also play a role in the olfaction sense, this organ is primarily hygosensory (Tichy & Loftus 1996; Schulz 2004). While there is some evidence that female spiders can attract males with airborne pheromones, most chemical communication is received via contact chemoreception and is associated with the silk (Searcy et al. 1999; Watson 1986; Tietjen & Rovner 1982; Schulz 2004; Gaskett 2007). Silk is a secretion not found in most other animal taxa and provides a unique medium with which chemical cues may be associated. Like other chemical deposits, the cues associated with silk may be received by contact chemoreception or may volatilize into an airborne component. Most studies of chemical communication in spiders are focused on the chemical cues associated with female webs or dragline silk, e.g., male spiders will initiate courtship display in response to contact with pheromones located on webs or female dragline silk (Ross & Smith 1979; Watson 1986; Suter 1982; Rovner 1977). However, the literature describing the use of airborne olfactory cues by spiders is limited, and the potential for responses is unclear (Schulz 2004; Gaskett 2007).

Studies of spider communication tend to be based upon a common set of assumptions, i.e., that females are the senders of chemical cues and males respond with courtship displays (Schulz 2004; Gaskett 2007). Still, it is important to consider that chemical communication may occur in both directions between males and females, but there have been few studies that have examined whether and how chemical communication occurs from males to females in spiders (Ross &Smith 1979; Roland 1984; Becker et al. 2004; Aisenberg et al. 2010). Furthermore, individuals may detect and respond to chemical cues from members of the same sex, although this is rarely explored (Ayaggari & Tietjen 1986).

The wolf spider *Schizocosa ocreata* (Lycosidae) is a common species found in the leaf litter of eastern deciduous forests in the United States (Dondale & Redner 1978). Male *S. ocreata* use courtship displays consisting of visual and seismic signals, and this species is a well-studied model for multimodal communication (Stratton & Uetz 1981; Uetz & Stratton 1983; Hebets & Uetz 1999; McClintock & Uetz 1996; Uetz 2000; Uetz & Roberts 2002; Uetz et al. 2009). Contact chemical cues associated with female silk alone are sufficient to attract male *S. ocreata* and are critical to the initiation of courtship displays (Stratton & Uetz 1981, 1983), as they contain information about species identity and female reproductive state (Roberts & Uetz 2004, 2005). As chemical cues are potentially important in mate recognition, it is interesting to determine whether and how spiders of both sexes respond to olfactory and contact chemical cues from members of the same or opposite sex.

In *S. ocreata*, as in other species, male courtship displays are costly, as they require energy and may increase the risk of predation (Pruden & Uetz 2004; Roberts et al. 2007; Roberts & Uetz 2008; Cady, Delaney & Uetz, in review). Furthermore, female *S. ocreata* can be aggressive towards conspecific males and sexual cannibalism is not uncommon (Persons & Uetz

2005). Thus, males able to detect a female from a safe distance and begin courtship only when it is relatively likely that a receptive mate is nearby may be at a selective advantage over males that fail to do so. Male spiders typically respond to species-specific information from contact chemical cues located on female silk by initiating courtship, but may also respond to airborne cues from the female or female silk (Gaskett 2007). Tietjen and Rovner (1982) hypothesize that in lycosids, a female hiding under a leaf in litter may gradually accumulate a pheromone "cloud", and as a consequence, males may detect the airborne chemical cues as they walk on or near the leaf. Therefore, airborne chemical cues may, in certain cases, precede detection of cues located on conspecific silk, alerting males to female presence and allowing them to identify themselves with courtship signals in advance. However, within the growing literature on communication and mating in *Schizocosa* species, chemical communication via airborne cues is not well studied, despite its potential role in the mating process, as seen in other lycosids (Tietjen 1979; Searcy et al. 1999).

Roberts and Uetz (2005) demonstrated that the rates of male courtship in *S. ocreata* in response to silk alone vary with female age and potential receptivity. It is important to remember, however, that communication may occur in both directions between signaler and receiver, and the question of female response to chemical cues from males is often raised. Literature on the response of female spiders to male chemical cues, whether directly associated with silk or airborne, is very limited (Ross & Smith 1979; Roland 1984; Becker et al. 2004). Since it has been shown that airborne communication occurs within members of the family Lycosidae (Searcy et al. 1999; Tietjen 1979; Papke et al. 2001, Riechert & Singer 1995), I tested the hypothesis that both male and female *S. ocreata* recognize and respond to contact chemical cues associated with silk and airborne chemical cues of conspecific males and/or females. I predict that spiders will show a preference for airborne cues and contact chemical cues associated with silk from members of the opposite sex, as this attraction may facilitate finding a mate. Furthermore, spiders may avoid airborne cues or contact chemical cues associated with silk from members of the same sex in order to avoid agonistic encounters. The experiments here, conducted with both female and male focal subjects, were designed to investigate the response of *S. ocreata* wolf spiders to airborne chemical cues and contact cues associated with silk within and between both sexes.

Methods:

Animal Maintenance: Spiders were collected as juveniles from the Cincinnati Nature Center Rowe Woods (Clermont Co., OH) and allowed to mature in the laboratory with a 13h:11h light:dark cycle at 22^{o} C and 60-70% RH. As juveniles, spiders were housed individually in translucent cups (7.5 cm diameter) containing soil and Collembola cultures. Spiders were later transferred to individual opaque deli containers (9 cm diameter) and fed one or two 10-day old crickets (*Acheta domesticus*) twice per week with water *ad libitum*. To control for hunger effects, spiders were fed a cricket the night before each trial.

Contact Olfaction: Male and female *S. ocreata* (N=30 per treatment group) were placed in a two-chambered apparatus in which one side was designated the stimulus chamber containing either male silk, female silk, or a blank piece of filter paper (control) (Figure 1). The side of the apparatus that served as the stimulus chamber was randomized among all trials. Silk was collected by placing a male or female spider in a circular arena (15 cm diameter) lined with filter paper overnight. The focal spider was placed in the center of the neutral chamber and allowed to acclimate under a small vial for 2 minutes. The trial began when the vial was lifted and continued for 15 minutes. Trials were recorded using a VHS camcorder (RCA PowerShot) and tapes were later played back for scoring and data collection. Each subject spider was only used once. I measured the total time spent in the stimulus chamber during the 15 minute trial.

When scoring tapes, I recorded a visit to the stimulus chamber as beginning the moment the spider crossed the line separating the neutral and stimulus chamber. Any visit to the stimulus chamber lasting less than 3 seconds was not included in the analysis. In addition, any visit to the stimulus chamber lasting longer than 450 seconds was also not considered when analyzing data. If the focal subject made less than four visits to the stimulus chamber over the entire 15 minute trial that subject was excluded from the data set. These criteria were established by an arbitrary judgment of what constituted a visit. The experiment investigating the preference for silk-borne chemical cues took place over the course of two fall research seasons in 2009 and 2010 which last approximately from December to February. For statistical analysis, I first ran a two-way analysis of variance with year and source of silk as model effects and total time (s) as the dependent variable. I subsequently used a one way ANOVA to test for significance in the total time spent in the stimulus chamber among the three treatment groups.

Airborne Chemical Cues I: In the first experiment investigating airborne cues, focal spiders were placed in the central (neutral) chamber of a three-chambered olfactometer (Figure 2). This olfactometer created airflow via evacuation with an electric fan above a conical chimney located above the central chamber. The rate of airflow was set at the minimum for the fan (0.4 m/s, 1.69 m^3/ min.).

Focal spiders were exposed to directional airflow from the two choice chambers: one chamber contained the stimulus, and the other chamber was left empty. Focal subjects were allowed to acclimate for 3 minutes before release. After release from the acclimation container, focal spider movement into one of the two antechambers was recorded as indicating a response to airborne chemical cues. Initial observations indicated that focal spiders tended to quickly choose a side of the antechamber and then remain there for the rest of the trial. For this reason, the focal subject's choice was designated by the antechamber into which it first entered. A total of 150 male and 150 female *S. ocreata* were assigned to identical treatment groups (e.g., airborne stimuli from a male spider, female spider, male silk, female silk, or a blank control for side bias; 30 individuals per group). Spiders were observed as focal subjects only once, but were also used for silk collection or as a stimulus spider in other trials. In the latter case, spiders were kept in a 15 cm diameter container lined with filter paper overnight to deposit silk. If there was no antechamber entry by the end of 5 minutes, the spider was scored as having made no choice. After each trial, the apparatus was cleaned with a Kimwipe® soaked with 70% ethanol.

Trials were recorded using a VHS camcorder (RCA Powershot) and scored upon video playback. I used a Chi Square analysis to verify that there was no side bias in the apparatus. Focal spiders were scored as choosing the antechamber containing the stimulus (+), the empty antechamber (-), or neither (0). The latency to make a choice as well as the presence or absence of male courtship and specific courtship behaviors were also noted. Chi Square tests were used to examine the likelihood to approach the stimulus chamber among treatment groups. As latency was not normally distributed and could not be transformed to normality, a non-parametric Wilcoxon test was used to test for the latency to make a choice. Chi Square analysis was also used to test among groups in the occurrence of courtship behaviors in male focal subjects. I subsequently narrowed the Chi Square analysis to the occurrence of courtship in trials with different sexes as stimuli (i.e., where males were exposed to either male or female stimuli versus a blank apparatus) in order to better determine the source of variance. A non-parametric Kruskal-

Wallis test was used to examine the rates of specific courtship behaviors among treatment groups.

Airborne Chemical Cues II: A closer analysis of videotapes from the previous study suggested the possibility that mixing air streams within a circular central chamber might affect a focal subject's detection of location of the stimulus source. In order to further investigate the possibility of a directional response to airborne chemical cues, a second olfactometer was designed (Figure 3). An air pump and small fan were used simultaneously to create a more linear airflow through the apparatus at a minimal rate (0.4 m/s; 0.218 m^3/min) as before.

At the start of the trial, a vial was used to introduce the focal subject to the middle of the experimental chamber and the trial was recorded for 5 minutes using a VHS camcorder. Upon video playback, the time spent in the neutral zone, the right arm, and the left arm was recorded for each individual. The same treatment groups from the first airborne cues study were used in studies with the second olfactometer design. Spiders were scored as choosing the arm with airflow from the chamber containing the stimulus (+), the arm with airflow from the empty chamber (-), or remaining in the neutral zone (0). After each trial, the apparatus was cleaned with a Kimwipe® soaked with 70% ethanol.

Statistical analysis consisted of using Chi Square tests to determine if treatment had an effect on the side chosen (+, -, or 0).

Results:

Contact Olfaction: Since year was not a significant factor for male or female subjects (Table 1), I collapsed this effect and subsequently used one-way ANOVA tests when examining the effects of silk source. Total time spent in the stimulus chamber varied significantly among treatment groups (Figure 4). Male focal subjects spent significantly more time in the presence of female silk than a blank control and the time spent in the presence of male silk did not differ from the control. Female subjects spent significantly less time in the presence of silk from another female than a blank control and, while the time females spent in the presence of male silk was intermediate, it was not statistically significant from either group.

Airborne Chemical Cues I: I found no directional bias of the side chosen compared to the side of the stimulus (males: = 0.009, p=0.9719; females: = 0.007, p=0.9327). In trials in which subjects were tested in an empty chamber, there was also no directional bias (males: =3.355, p=0.1868; females: =0.800, p=0.9327). I did not detect any significant directional response by male or female subjects to the four treatment groups in which one of the chambers contained a stimulus (Figure 5; males: =0.743, p=0.8631; females: =2.188, p=0.5344). When analyzing the latency to make a choice, I observed that focal males that chose the stimulus chamber had a significantly longer latency than those that approached the empty chamber (Wilcoxon =5.1837, p=0.0228). Initial observations suggested that this was because of male mate searching and courtship behaviors occurring in the central chamber, which were investigated further below.

I scored the presence or absence of courtship and specific courtship behaviors in male subjects and found a significant difference among treatment groups (Figure 6; Courtship: = 23.349; p = 0.0001; Table 2). However, there was no significant difference among treatment groups in the presence of jerky taps (Table 2). When specifically comparing the occurrence of courtship behaviors between a blank chamber and female stimuli (female and female silk treatment groups) I again found a significant difference in the occurrence of double tap and leg arches, with more of these behaviors occurring when exposed to female stimuli than a blank, but no significance in the occurrence of chemoexplore or jerky taps (Table 2). There was no significant difference in the occurrence of any courtship behaviors when examining males exposed to male stimuli (male or male silk treatment groups) versus a blank apparatus (Table 2). I found a significant difference in the rates of chemoexplore, double tap, and leg arches, but not jerky taps, in response to airborne chemical cues from all five treatment groups (Figure 7; Table 3). In all cases, it appears that males perform more courtship behaviors in the presence of airborne chemical stimuli from females than from males.

Airborne Chemical Cues II: As in the first airborne cues study, I found no significant likelihood to approach the stimulus chamber with respect to treatment for either male or female spiders (Table 4).

I also compared the rates of male courtship observed in the two different experiments above, i.e., where males were exposed to female silk and to airborne cues from females. While such results must be interpreted with caution, scaling behaviors as rates based on a 5 min. trial allows insight about relative effects of these two sources of female chemical cues. I found higher rates of chemoexplore, double tap, and jerky tap in response to chemical cues associated with female silk than to airborne cues (Figure 8: Chemoexplore: Wilcoxon =25.2853, $p<0.0001$; Double Tap: Wilcoxon =14.4159, $p<0.0001$; Jerky Tap: Wilcoxon =12.1299, $p=0.0005$). The rates of leg arches, on the other hand, were significantly greater in response to airborne chemical cues from females than to silk-borne chemical cues (Wilcoxon =3.8674, $p=0.0492$).

Discussion:

In potentially cannibalistic animals such as spiders, males are likely to be under selection to distinguish conspecific females from a relatively safe distance based on chemical cues (Tietjen & Rovner 1982; Gaskett 2007; Rovner 1977; Stratton & Uetz 1981, 1983). However, despite the importance of chemical communication in spiders, studies are primarily focused on how males respond to the contact chemical cues associated with female silk, and literature investigating the response of females to conspecific chemical cues is limited (Becker et al. 2004; Ross & Smith 1979; Roland 1984). While this study confirms the well-established assumption that male spiders respond with courtship and searching behavior to contact chemical cues associated with female silk, it also provides evidence that female *S. ocreata* are not attracted to male contact chemical cues associated with dragline silk or airborne chemical cues. In addition, this study found that male *S. ocreata* respond to airborne olfactory chemical cues from females by displaying courtship behavior, although there was no indication of attraction or searching behavior (i.e., no directional responses).

In olfactory cue studies, a directional response was the only possible behavioral indicator for females, since they showed no other characteristic behaviors (i.e., receptivity displays, other signaling behaviors) that would indicate a response to airborne chemical stimuli. Thus, I feel confident in concluding that there were no female responses to chemical cues from males. Female *S. ocreata* are known to be sit-and-wait predators and are not as active as adult males (Cady 1984). In *S. ocreata*, like many other animal species, females are extremely choosy and do not actively seek mates. These data are consistent with females of this species being solitary and stationary. However, females did spend significantly less time in the presence of conspecific female silk, suggesting they might use contact chemical cues to avoid potential competitors and/or predators. Although males suspend most foraging activity upon reaching adulthood and start searching for mates, female *S. ocreata* during this time, are ravenous and often aggressive towards conspecifics, so contact chemical cues associated with silk may allow females to maintain a maximum distance from one another.

The findings of this study are consistent with previous findings that male *S. ocreata* respond to the contact cues associated with female silk and increase searching behavior and courtship when they detect a nearby female (Uetz & Denterlein 1979; Roberts & Uetz 2005). In this study, males showed no differences in response to contact chemical cues from male silk when compared to a blank control. As with female subjects, it might have been expected that chemical cues associated with conspecific silk is a means by which males maintain maximum distance from one another. However, these results differ from results of a previous study (Ayyagari & Tietjen 1986) that suggested male *S. ocreata* use chemical cues to avoid conspecific males, who are potential competitors. While that study (Ayyagari and Tietjen 1986) used extracts of chemical cues from the silk of multiple male donors (N = 60, 147, or 694), in this study male focal subjects were exposed to chemical cues from only one conspecific male. As a consequence, these studies are not truly comparable, and so the question of male response to cues from other male conspecifics requires further study.

The response of male *S. ocreata* to female silk is well-known, but the response to airborne chemical cues has not been extensively investigated in this species (Tietjen 1979). Furthermore, proposed hypotheses that suggest a potential for olfactory communication in this species, as well as the demonstration of olfactory communication in other lycosids, warrant investigation of the presence and response to airborne chemical cues in *S. ocreata* (Tietjen & Rovner 1982; Tietjen 1979, Becker et al. 2004; Searcy et al. 1999). While subjects showed no directional preference towards airborne cues, the presence of male courtship did indicate a behavioral response to airborne cues from females. Because female *S. ocreata* are potentially cannibalistic towards males, it is important for males to clearly communicate their identity and intent to mate when a female may be nearby. On the other hand, courtship is energetically costly and may make the male more conspicuous to potential predators. Thus, males should begin courting before they enter female detection range, but when it is still likely that there is a female nearby. In spiders, airborne chemical cues are thought to be general long-range attractants that do not necessarily elicit a courtship response from males, but contact chemical cues associated with silk contain more specific information about a female such as species identity, age, or mating status (Gaskett 2007, Roberts & Uetz 2005). For this reason, airborne and contact chemical cues may have different relative importance when locating a potential mate. When I compared the rates of courtship between the two studies, males courted more vigorously in response to direct contact with silk than to airborne cues from females. However, I also found significantly higher rates of wave and arch behavior with airborne cues, perhaps indicating recognition but a more tentative level of communication. Even though results of separate studies must be interpreted with caution, it is clear that male *S. ocreata* recognize and respond to both contact and airborne chemical cues from females, and that contact chemical cues associated with female silk may have a stronger effect on male courtship.

Overall, these findings are important because they demonstrate that while males recognize and respond to female chemical cues carried through various media, they respond differently to airborne (olfactory) cues than to contact chemical cues on silk. Although the exact source of airborne cues (e.g., glandular release, volatilization of cuticular hydrocoarbons) is uncertain, this finding suggests the possibility of a hierarchical response in males to chemical cues that reflect female proximity. However, in contrast, females exhibit no apparent response to any chemical cues from males, either airborne or silk-borne. I conclude that chemical communication in this species is unidirectional and female-originated, and most effective in stimulating courtship when associated with silk.

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Figures & Tables:

Figure 1: Schematic diagram (plan view) of apparatus used when investigating response of male and female *S. ocreata* to contact chemical cues associated with silk. Diameters of the plastic containers are 15 cm. Width of opening between chambers is 7.6 cm.

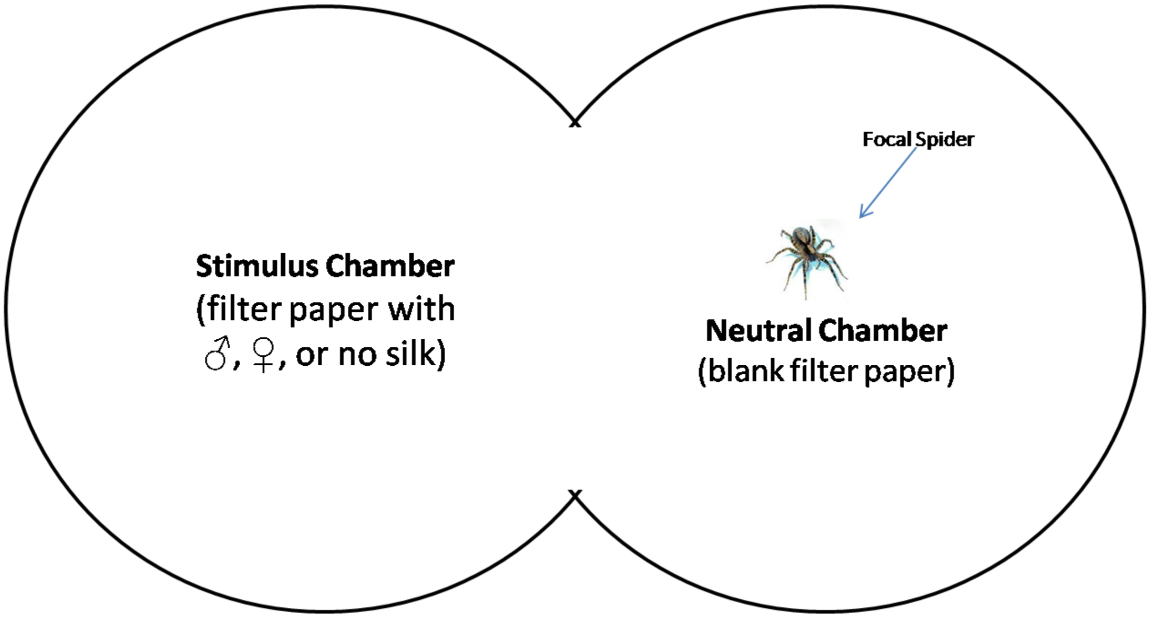
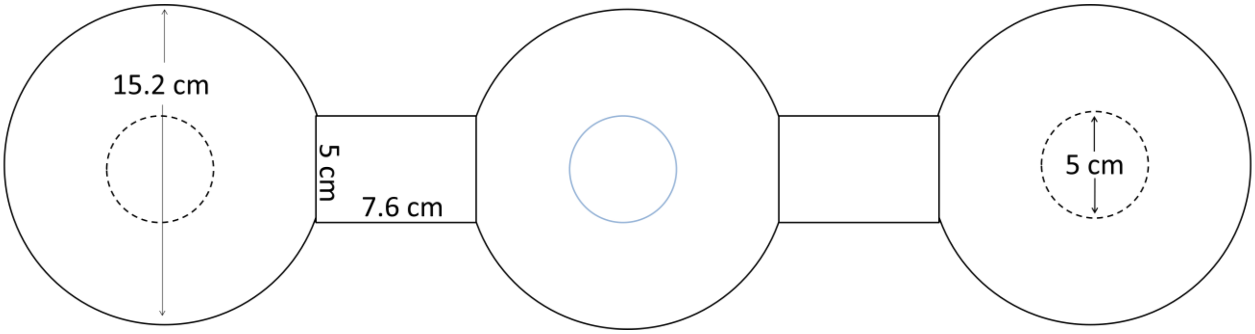

Stimulus Chamber
(filter paper with
♂, ♀, or no silk)
Focal Spider
Neutral Chamber
(blank filter paper)

Figure 2: Schematic diagram (plan and side view) of Olfactometer I: Blue arrows indicate air flow. The focal spider (male or female *S. ocreata*) is allowed to acclimate in the neutral chamber for 3 minutes prior to the 5 min. trial. Foam pads prevent the transmission of vibrations and screens act as visual barriers between the focal spider and stimulus spider.

Plan view:



Side view:

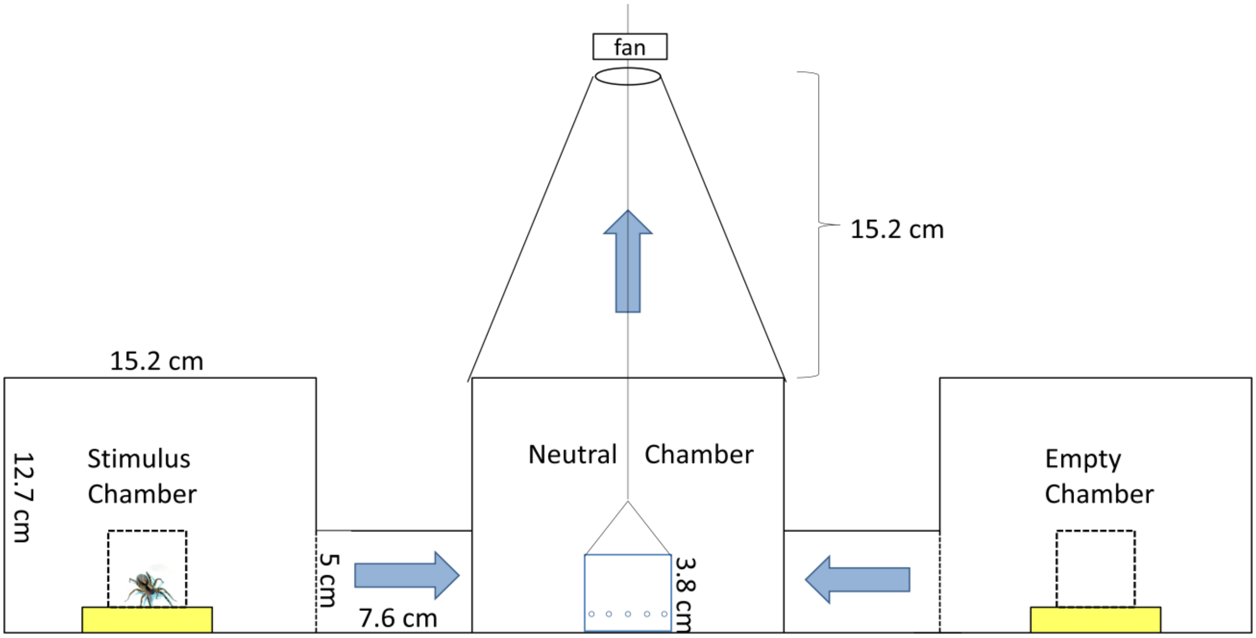
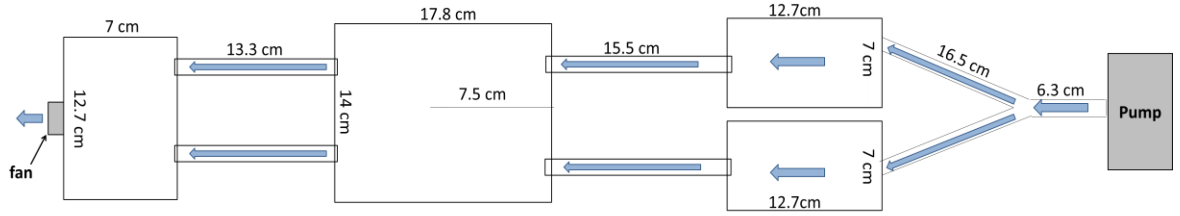


Figure 3: Schematic diagram (plan and side view) of Olfactometer II - Arrows show direction of airflow. Air passes over two stimulus chambers and into an experimental chamber. Air is pulled out of the experimental chamber by a fan located on the opposite end of the olfactometer. The focal spider is introduced as indicated, and observed for 5 min.

Plan view:



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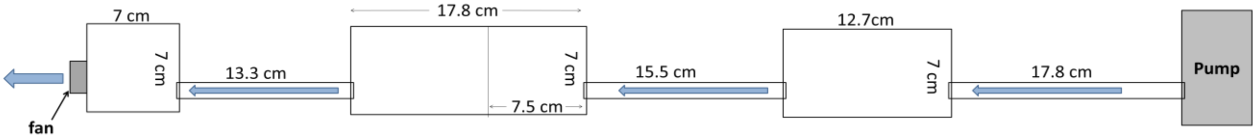
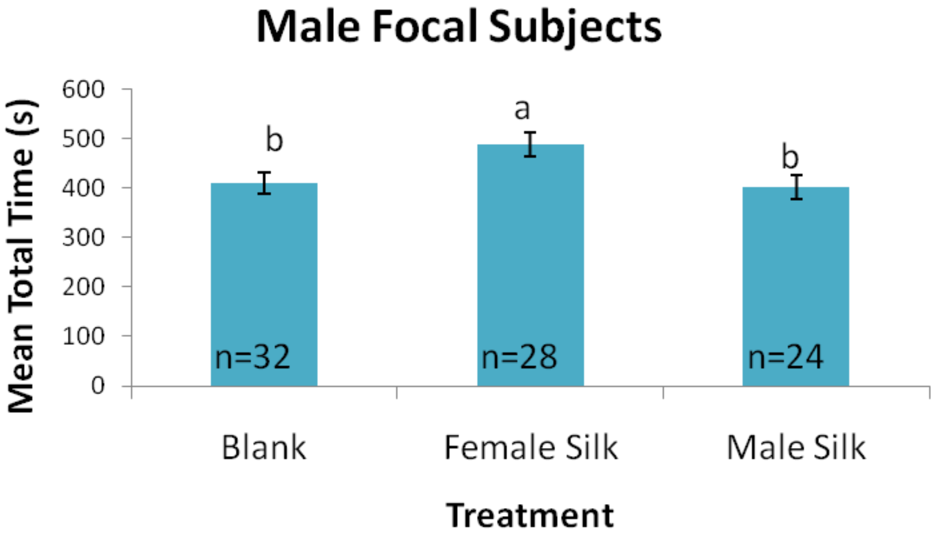
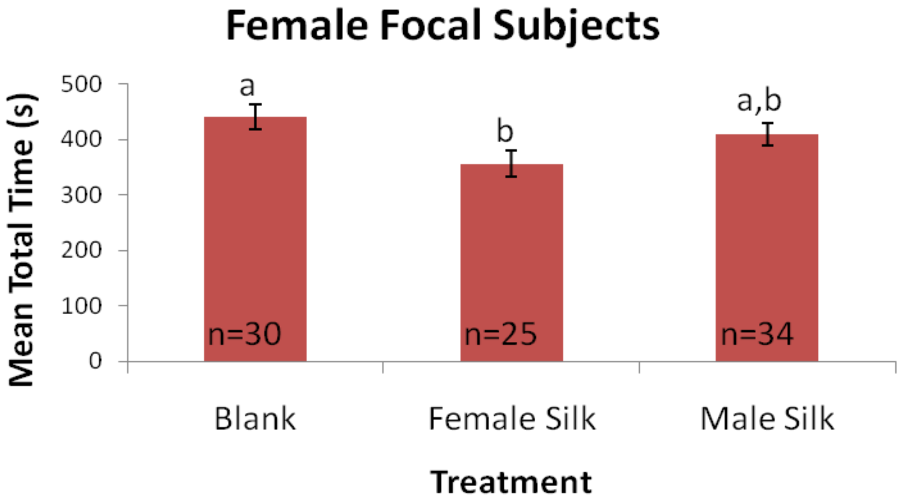


Figure 4: Mean total time (secs) that focal subjects (male or female *S. ocreata* spiders) spent in the stimulus chamber containing male silk, female silk, or no silk. Letters above bars indicate statistically significant differences at $p < 0.05$ based on Tukey HSD *post-hoc* test; bars with the same letter are not statistically different.



Male Focal Subjects



Female Focal Subjects

Figure 5: Likelihood that male or female *S. ocreata* focal subjects approach the stimulus chamber (+, blue) or the empty chamber (-, red) of the olfactometer by treatment. N_{males}=89, $N_{females}$=84; F - female spider, FS - female silk, M - male spider, MS - male silk.

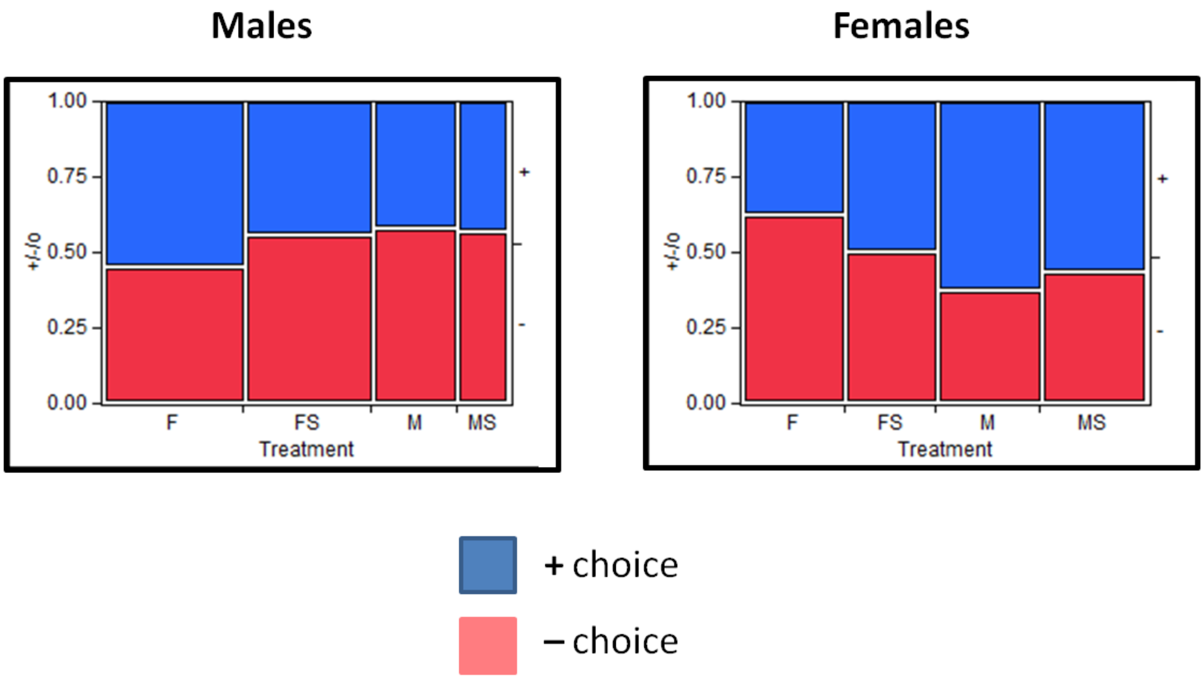

Males
Females
1.00
0.75
0.50
0.25
0.00
+/-/0
+
-
F
FS
M
MS
Treatment
🟦 + choice
🟥 – choice

Figure 6: Proportion of individual male *S. ocreata* test subjects exhibiting presence (+ blue) or absence (- red) of specific courtship behaviors when exposed to airborne chemical cues from different sources (N=118; F - female spider, FS - female silk, M - male spider, MS - male silk).

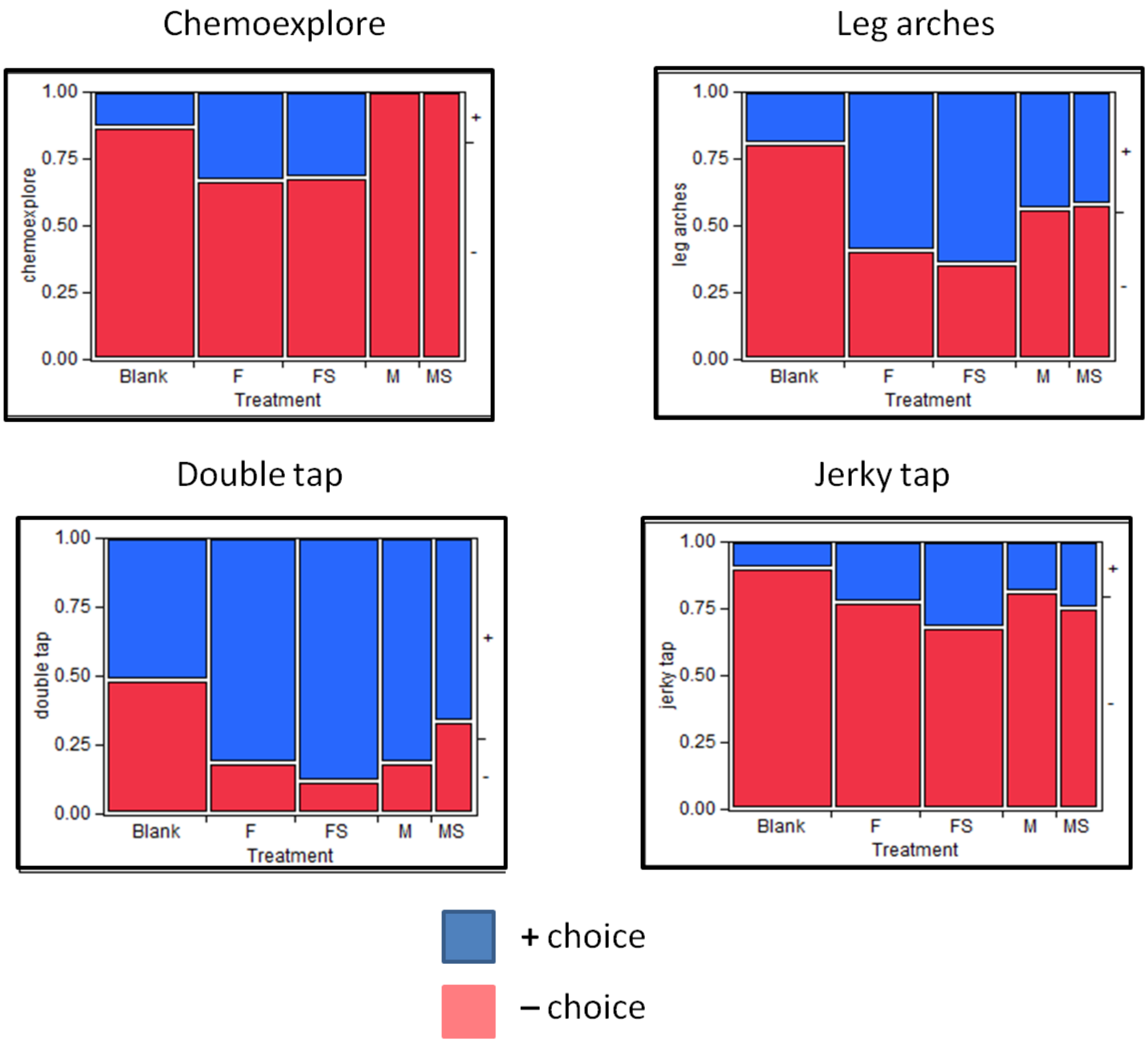

Chemoexplore
Leg arches
Double tap
Jerky tap
chemoexplore
leg arches
double tap
jerky tap
1.00
0.75
0.50
0.25
0.00
Blank
F
FS
M
MS
Treatment
+
-
+ choice
– choice

Figure 7: Mean rates of courtship behaviors of male *S. ocreata* (± S.E.) when exposed to airborne cues from different sources (asterisks beside behaviors in legend indicate significance among treatment groups as determined by Kruskal-Wallis test: * $p \leq 0.05$).

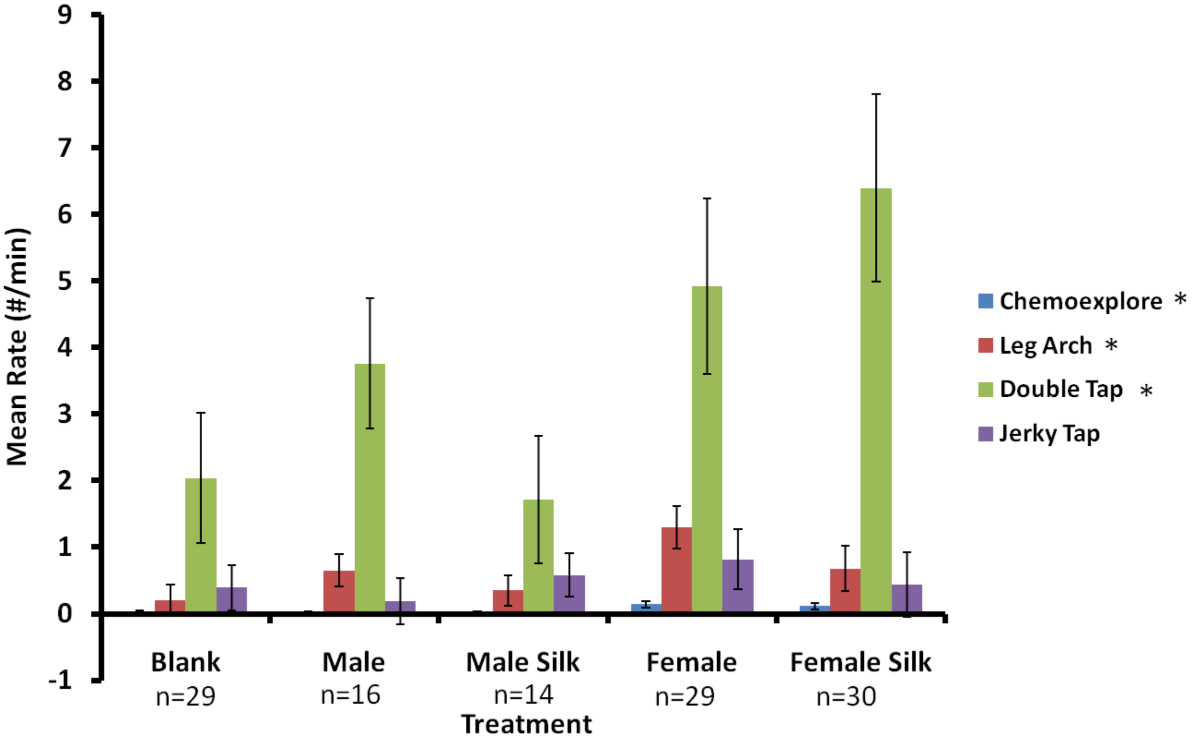

Mean Rate (#/min)
9
8
7
6
5
4
3
2
1
0
-1
Blank
n=29
Male
n=16
Male Silk
n=14
Female
n=29
Female Silk
n=30
Treatment
Chemoexplore *
Leg Arch *
Double Tap *
Jerky Tap

Figure 8: Comparison of mean courtship rates (± S.E.) of male *S. ocreata* exposed to airborne versus contact cues from females (asterisks indicate significance level from Wilcoxon test: * $p \leq 0.05$; ** $p < 0.005$; ***$p < 0.0005$).

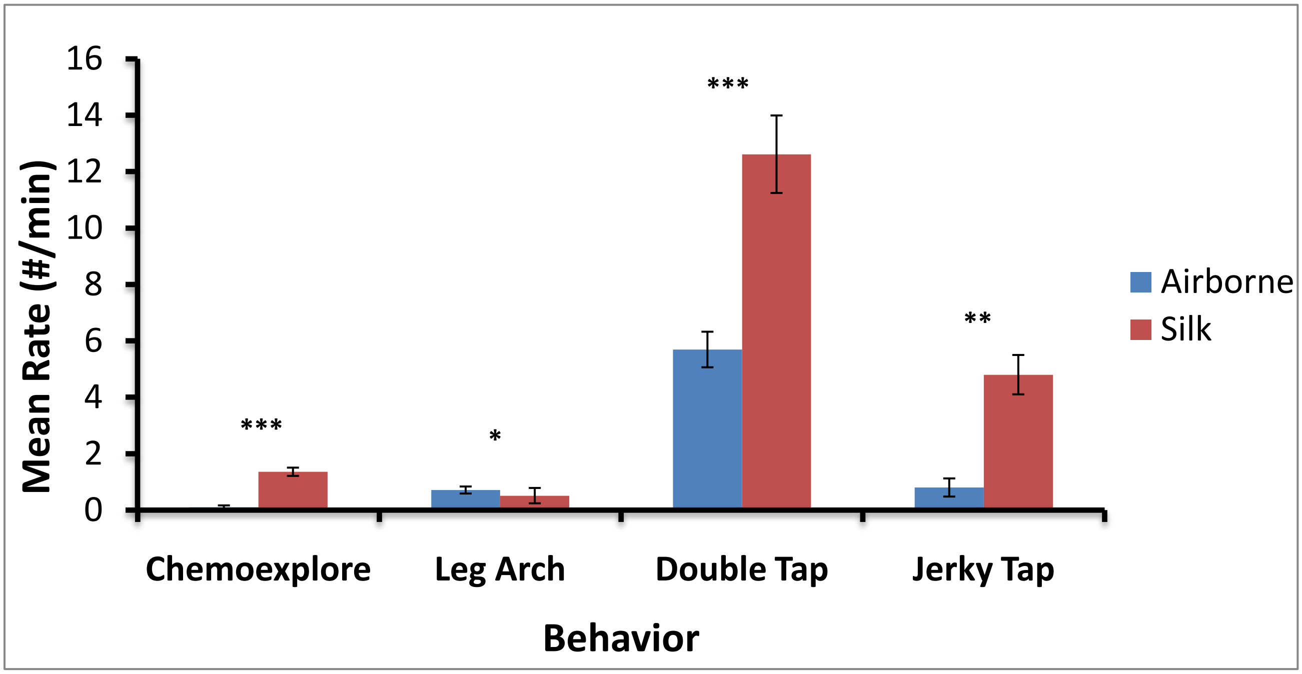

16
14
12
10
8
6
4
2
0
Mean Rate (#/min)
***
*
***
**
Chemoexplore
Leg Arch
Double Tap
Jerky Tap
Behavior
Airborne
Silk

Table 1: Results of 2-way ANOVA for total time spent by focal male and female *S. ocreata* spiders in stimulus chamber with contact cues associated with silk, with year (2009 & 2010) and source of silk (male silk, female silk, no silk) as factors.

Female Subjects					Male Subjects				
	df	Mean Square	F	P		df	Mean Square	F	P
Year	1	7890.557	0.5367	0.4659	Year	1	720.59	0.0464	0.8299
Source of Silk	2	38147.795	2.5945	0.0807	Source of Silk	2	51888.795	3.3448	0.0402
Year x Source of Silk	2	1219.738	0.0830	0.9205	Year x Source of Silk	2	27110.28333	2.6213	0.0789

Table 2: Presence/absence of male courtship behaviors in response to airborne chemical cues in Airborne Cues Study I: CE - chemoexplore, LA - leg arch, DT - double tap, JT - jerky tap.

	All Groups (N=118)				**Female Cues vs. Blank (N=88)**				**Male Cues vs. Blank (N=59)**			
	CE	LA	DT	JT	CE	LA	DT	JT	CE	LA	DT	JT
d.f.	4	4	4	4	2	2	2	2	2	2	2	2
X^2	13.711	14.163	11.823	4.446	4.036	14.401	10.835	4.309	3.876	3.847	4.065	1.779
p	**0.0083**	**0.0068**	**0.0187**	0.3490	0.1329	**0.0009**	**0.0044**	0.1160	0.1440	0.1461	0.1310	0.4109

Table 3: Mean rates of male courtship behaviors (± S.E.) and statistical results in response to airborne chemical cues in Airborne Cues Study I: KW - Kruskal-Wallis Chi Square.

	Blank	**Female**	**Female Silk**	**Male**	**Male Silk**	**KW**	**p-value**
Chemoexplore	0.014916 ±.03400	0.140621 ±0.03400	0.113874 ±0.03343	0 ±0.04578	0 ±0.04894	13.7307	**0.0082**
Leg Arch	0.19705 ±0.23818	1.29519 ±0.23818	0.67782 ±0.23418	0.65058 ±0.32066	0.34826 ±0.34280	12.0360	**0.0171**
Double Tap	2.03765 ±0.9776	4.92405 ±0.9776	6.39949 ±0.9612	3.75730 ±1.3162	1.71309 ±1.4070	9.5235	**0.0493**
Jerky Tap	0.392207 ±0.33673	0.815095 ±0.34269	0.44320 ±0.33107	0.187500 ±0.45334	0.581582 ±0.48464	2.3130	0.6784

Table 4: Results of Chi Square tests examining likelihood to approach the stimulus chamber by treatment group in Airborne Cues Study II.

	Males	**Females**
N	95	84
d.f.	3	3
X^2	1.796	0.665
p	0.6158	0.8813