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# Dolomedes scriptus (water spider): Male response to females' putative pheromones on perches.

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In fulfillment of the requirements for Society of Westover Fellows

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Sexual signals are commonly used among animals to enhance reproductive success. Chemical signals such as pheromones are commonly used to attract mates. Little is known, though, about how these signals are received or interpreted by the target. I studied the water spider, *Dolomedes scriptus* in the pisaurid family. Most *Dolomedes* species only make webs as a nursery for their young. However, females often create silk drag lines, potentially serving for species recognition. In some Dolomedes species, females who are previously mated are known to cannibalize most approaching males. Males might avoid cannibalism by avoiding mated females, particularly if they can determine her mating status from a distance. Both mature and immature male and female spiders were collected from Claytor Nature Study Center in Bedford, Virginia. Males were exposed to a foam substrate on which penultimate, virgin, and mated females had been housed. Male reaction did not differ significantly between penultimate and mature female substrates. However, males reacted more positively to substrate from virgin females than from previously mated females, suggesting that males can determine a female's mating status before he ever encounters her. This raises some interesting questions about the mechanisms of female mating status and how they have evolved.

Word Count: 200

#### **Introduction**

Animals communicate via several major modalities. Of these modalities, chemical signals are often indicative of species membership and female receptivity (Lindauer 1967). These signals, sent as volatilized chemicals that are intended to influence an external target, (Kevles 1986) are referred to as pheromones. Each pheromone is associated with specific contexts, so the function of the signal is usually obvious to the individual receiving it. There is a constraint in the limitation on the signaler's ability to direct the signal to a specific target (Bradbury and Vehrencamp 1998). Pheromones can be used to manipulate the receiver's behavior. Females of many species signal receptivity, often via pheromones (Lindauer 1967). These might attract males or stimulate courtship behavior (Andersson 1994). For example, some male spiders (*Agelenopsis aperta*) exposed to concentrations of the female sex pheromone reliably produced courtship behavior (Papke et al. 2001).

Spiders also use pheromone signals in silk trails. In this case pheromone trails are received by males as very narrow clouds above a substratum on which the pheromone was released (Tietjen and Rovner 1982). Males trace a female's dragline (that is, the silk line she has left behind her) as they come across and detect airborne molecules (Tietjen and Rovner 1982). Males then use palpal tests to determine if the line is from a female of the same species; if so he will follow the line and begin courtship (Tietjen and Rovner 1982).

There is a risk, however, for males when they display courtship behavior. In many spider species (as well as other invertebrates) females will often cannibalize males

(Arnqvist 1992; Sasaki and Iwahashi 1995; Johnson 2001; Schneider and Elgar, 2001; Moya-Larano et al 2003; Johnson 2005; Persons and Uetz 2005). One hypothesis used to explain this behavior is the adaptive foraging hypothesis (Johnson 2001), predicting that virgin females will see males as potential mates while mated females will see males as potential food items. Johnson's research on the species *Dolomedes triton* (2001) supports this hypothesis, showing that females that had mated previously were much more likely to attack a male prior to copulation than virgin females. In other *Dolomedes* species, females become more aggressive overall and tend to attack males almost immediately after mating (Kissane, personal communication). These data suggest that natural selection might favor male recognition of female mating status prior to initiation of courtship.

I focused on chemical recognition in the water spider, *Dolomedes scriptus*. *Dolomedes scriptus* are in the family *Pisauridae*. Known as nursery web spiders, *Pisauridae* range in body size from 0.5 – 3.7 cm. They are common in the eastern United States and Canada, and they can be found in the riparian zone of streams (Carico 2005). *Dolomedes* males are known for trail-following the females. It is also known that females of this genus engage in sexual cannibalism (Carico 1973). It would be advantageous for males to avoid cannibalism by knowing before an encounter with a female what her mating status is. In my investigations, male *D. scriptus* were exposed to substrate from penultimate (one moult from maturity), virgin, and mated females. I tested whether males' responses to substrate on which females had been housed varied with mating status and maturity of the female.

#### **Methods**

Dolomedes scriptus were collected during the end of June and the beginning of July of 2005 from a section of the Big Otter River running through the Claytor Nature Study Center of Lynchburg College, located in Bedford County, Virginia. Collections started around dusk and ended around 2300. Spiders were located by headlighting. Maturity and sex were examined on site and then again in the lab for a more accurate determination of maturity status.

Spiders were housed in individual round, plastic Gladware<sup>TM</sup> containers (about 945 mL). Each dish contained about 3 cm of water and a piece of  $3 \times 1 \times 3$  cm waterproof, closed-cell camping foam on which the spider might sit (here-after referred to as a perch). Water and perches were changed every other day or daily if the spider moulted or the water turned green. Perches were washed with Dawn <sup>TM</sup> dish soap and rinse thoroughly before being used again. Perches were not cleaned in the 48 hours preceding a trial.

Males were housed in containers with mesh netting covering a hole cut in the top. In these containers, spiders typically rested upside-down from the mesh on the top. To discourage this behavior in females, they were housed in containers with holes poked in the top. In these containers, spiders spend more time directly on the perch. Because of a lack of housing containers, four females were placed in individual dry fish aquaria (32 x  $18 \times 21 \text{ cm}$ ) with a sponge and a paper towel until there were vacancies in the Gladware<sup>TM</sup> dishes. Spiders received one medium sized cricket upon arrival at the lab site and were thereafter fed a medium cricket when the spider's abdomen was smaller in width than the cephalothorax.

Males were not used until they had reached full sexual maturity. Females who were collected at sexual maturity were observed for the formation of an egg sac. If no sac was formed, I assumed they were virgins. Females typically form an egg sac within one week after copulation (Kissane, personal communication).Other females were raised to maturity or penultimate status for use in the experiments.

I measured the spiders immediately before experimental manipulation. This was done by placing them in a tank (32 x 18 x 21 cm) on a piece of floating graph paper with a 0.635 cm square grid. At least two digital photographs of each spider, taken from above the tank were used to measure (using Image J 1.32 software, <u>http://rsb.info.nih.gov/ij/</u>) each leg from joint to joint and the cephalothorax area for each spider. Spiders were then returned to their cages.

Male subjects were chosen as they matured. Each of the 16 males was exposed to two perches: one from a penultimate female and one from a mature female. Male exposure to mating status varied. Among the mature females, four were mated and 12 were virgins. Perch order exposure was randomized. Experiments were conducted in a Pal-Pen<sup>TM</sup> aquarium (32 x 18 x 21 cm) filled with about 3 cm water, three sides occluded by white paper to reduce distraction. No top cover was used because spiders were not able to climb out of the cage. I put each male in the back right corner of the tank on the perch from his home container and allowed 30 minutes for acclimation. After acclimation, males were introduced to the foam perch from the first assigned female. The males were coaxed onto the female perch in order to ensure that some contact would be made and thereafter allowed to roam freely. Each trial lasted for 30 minutes, the second

perch exposure following directly after the first. Behavioral responses were observed and recorded.

Male response behaviors were categorized as either positive or negative. Each behavior is defined and categorized in Table 1.

State:	Definition:	Consistent With:
Scurrying	male runs back and forth in any direction on the water surface	Escape
Climbing	male attempts to climb the tank walls	Escape
Palpal Drumming	male taps the surface of the water with his palps	Courtship
Grooming	male cleans any part of his body using a combination of his legs and mouthparts	Courtship
Waving	male raises his two front legs and waves them in the air	Courtship

Table 1. States of behavior observed and associated definition.

I recorded the time (in seconds) each male engaged in each behavior. The total time spent responding positively to each female perch was calculated as one data set, while the total time spent responding negatively was calculated as another. I calculated the difference in reaction time to each perch by subtracting the negative response time from the positive, referred to here after as overall reaction.

I tested whether male response to female perches varied with female maturity. Paired t-tests were conducted for the time males spent reacting positively, negatively, and overall. I then tested whether male response to perches varied relative to mating status. Paired t-tests were conducted for positive, negative, and overall male response to penultimate versus mature female perches. Two-sample t-tests were conducted for positive, negative, and overall male response to perches from mature virgin versus mated females. Linear regression tested whether female size correlated with male reaction.

#### **Results**

I used t-tests to compare the time males spent reacting to perches from penultimate and mature, and to compare male responses from mated mature to virgin mature females. Male response did not differ relative to the maturity of the females (penultimate or mature) for positive responses (Paired t-test,  $t_{15} = 0.4683$ , P = 0.6463), for negative responses (Paired t-test:  $t_{15} = -0.5069$ , P = 0.6196) or for overall responses (Paired t-test:  $t_{15} = 0.7471$ , P = 0.4665). There was no difference in the negative (Twosample t-test:  $t_{14} = 0.4139$ , P = 0.6852) or overall (Two-sample t-test:  $t_{14} = 2.2254$ , P = 0.0560) response times males exhibited towards mated versus virgin females. However, there was significance in the difference in time males spent responding positively towards virgin and mated perches (Two-sample t-test:  $t_{14} = -2.1675$ , P = 0.0479). Figure 1 presents an illustration of the differences in the average amount of time males spent responding positively to mated and virgin mature females.

Three linear regressions were made using Sigmaplot 9.0, one each for positive, negative, and overall male reaction time as a function of female size. There was no relationship between positive male response to female size (Fig. 2:  $F_{(1,31)} = 3.8401$ ; P = 0.0594), negative male response to female size ( $F_{(1,31)} = 0.2044$ ; P = 0.6544), or overall male response to female size ( $F_{(1,31)} = 2.2088$ ; P = 0.1477).

#### **Discussion**

Males spent more time responding positively to virgin female perches than to mated female perches. The regressions demonstrate that this change in response does not reflect any size differences among the females and that size is not dictating the amount of pheromone in a way that enhances male response. This suggests that males can detect female mate status before ever contacting the female. It also suggests that males will respond differently to mated females than to virgins. By being able to tell which females have already mated, males will be able to court more effectively and may decrease their chances of being preyed upon by mated females.

These data suggest that males may be able to avoid cannibalism by avoiding mated females. Because of the energy advantages that females gain from eating males (Sasaki and Iwahashi 1995), evolution should drive females to become cryptic about their mating status. Advertising will only gives males the upper hand by allowing them to avoid being eaten. Kissane (personal communication) has indicated that female behavior changes following mating in the congener species *Dolomedes* vittatus. The mechanism triggering the changes in chemicals as suggested by these data and behaviors as suggested by Kissane is not understood. These changes could be hormonal or morphological. In some spider species, male sex organs are altered by copulation such that they can only mate once (Berendonck and Greven 2000; Andrade and Banta 2002). It is possible that female sex organs are altered in this fashion in some cases as well. If this were the case, then it would make sense to become aggressive after mating. The energy used for copulating could be utilized instead for preying on males.

My results suggest that males mediate their courtship behavior and potentially their approach behavior with respect to the mating status of prospective mates. However, it is currently unclear how a male will react if he hasn't had time to investigate a female's status. How a male responds when a female is suddenly introduced needs to be investigated. In order to do this it would be important to try to document what exactly courtship is for *Dolomedes scriptus*.

In Kissane's work with *D. vittatus*, she was able to identify and describe completely the courtship behavior of males. Although *D. vittatus* and *D. scriptus* are very similar, there are certainly some unidentified nuances that differ in their communication styles. Knowing these differences would allow researchers to more accurately gauge male response to females of different mate status. Once we have fully described what courtship behavior consists of for *D. scriptus*, we can examine whether a male will automatically begin courting a female he encounters if he has not had time to investigate her or if he will display some other type of behavior that has not been observed yet.

It would also be interesting to know the chemical make-up of the substances that females are leaving behind. Another step in this research would be to extract silk from mated and virgin females and see if there is any chemical difference in the pheromones they are emitting. Maybe there are two completely different pheromones, or maybe there is a difference in the concentration of pheromone for mated females. Papke et al. (2001) were able to isolate a sex pheromone from the spider *Agelenopsis aperta*, but they were not able to determine any difference in concentrations of pheromone presence in individual spiders, and there was no mention of a difference in compounds present in

varying stages of maturity or mating status. Understanding the chemical factors determining male response would enhance our knowledge of communication a great deal.

There are many animal species in which copulation actually changes a female morphologically or hormonally. It is thought that some males use invasion tactics where their ejaculate contains a hormone that enters the female body (Eberhard 1996). These hormones then cause some sort of physical change that enhances his reproductive success with her eggs, such as causing her reproductive tract to contract, bringing the sperm and eggs closer. Other times, she may just become more aggressive towards other males (Eberhard 1996), as in *D. vittatus*. If we can determine what it is that is causing these changes in *D. scriptus* or in other spiders, we may be able to apply those changes to other species as well. Because of the adaptive value of males' recognition of female signals, there are evolutionary consequences involved. If we can determine what is causing these behavioral changes, we may be able to determine how they arrived at this point evolutionarily and thus improve our understanding of the evolutionary mechanisms of chemical communication in the more broad sense as it applies to other organisms.

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### Fig 1. Female mating status vs. Male positive reaction time

Mate Status

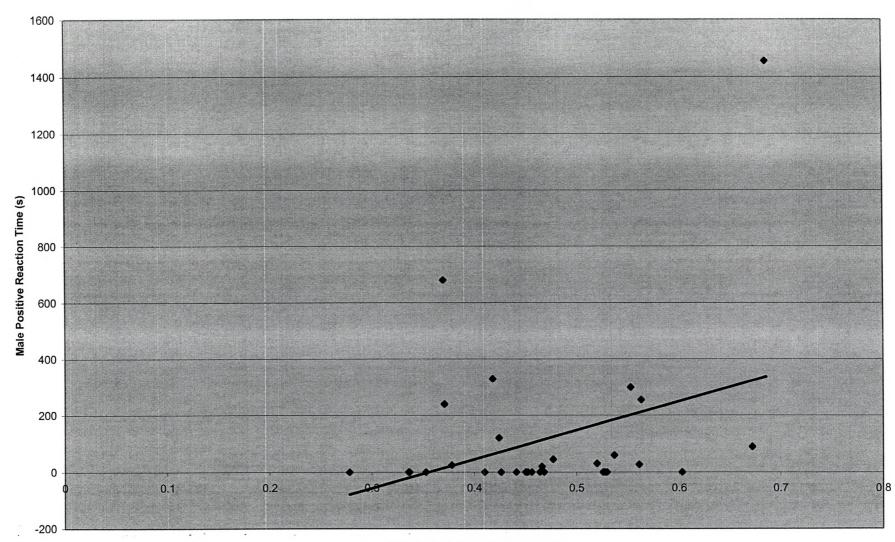


Figure 2. Female cephalothorax area vs. Male positive reaction time

Female Cephalothorax Area (cm2)