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Silk Production in Mature and Penultimate Female Dolomedes scriptus

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Abstract:

Male *Dolomedes scriptus* are able to determine female maturity status by silk alone, as demonstrated by increased courtship in the presence of silk from mature females (Roach and Benson, unpublished). However, the mechanism by which the males determine female maturity status is unknown. It is possible that there are pheromonal signals indicating reproductive status. Specifically, I test whether mature female *D. scriptus* produce a different amount of silk than penultimate females, particularly if mature females produce more than immatures. If mature *D. scriptus* females produce more silk than penultimate females, male *D. scriptus* may determine maturity status of females via the amount of silk or amount of silk chemical. However, I fail to detect a significant difference in silk output between these two female maturity statuses. Therefore, there is further support that other factors, such as pheromones in the silk, may be responsible for male's ability to determine maturation status of female *D. scriptus*.

Introduction:

Reproductive signaling allows an organism to attract potential suitors. Thus, signaling reproductive availability to a potential mate can increase an individual's fitness. Reproductive signaling is accomplished in different ways by different species. For example, birds are commonly known to use songs in order to attract mates (Nowicki and Searcy 2006). Some organisms use visual signaling in order to attract mates, such as the oval squid *Sepioteuthis lessoniana* which changes the color of its skin in order to attract a mate (Lin et al 2017). Another form of signaling utilized by some species is chemical signaling. The females in African *Loxodonta africana* and Asian *Elephas maximus* elephants release a chemical into their urine prior to ovulation (Rasmussen & Schulte 1998). Similarly, the males secrete chemicals from their temporal glands and release a chemical into their urine during an annual period of elevated testosterone, considered to be their breeding period (Rasmussen & Schulte 1998). Similarly, several spider species have been found to use chemical pheromones in their silk for reproductive signaling (Schulz 1997). Some species have been observed using vibrations in order to attract potential mates (Barth 1998).

It is likely that mature female *D. scriptus* secrete a chemical into their silk signifying their reproductive maturity, supported by the male's lack of courtship behavior in the presence of the silk of penultimate females compared to their courtship behavior response when in the present of the silk of a mature female (Roach & Benson unpublished). Despite these observations, reproductive signaling in *D. scriptus* is undocumented. However, multimodal signaling (stridulatory and visual) have been observed in lycosid spider species (Hebets and Uetz 1999). *Dolomedes scriptus* is a species of fishing spider indigenous to the south-east portion of North America (Carico 1973). These fishing spiders hunt for food by positioning themselves partially on a surface adjacent to the water, often with the first two pairs of feet on the water and anchored to the shoreline with some dragline silk. In the event of water levels rising, they can use their silk to tether themselves to shore. *Dolomedes scriptus* molts in order to mature, and spiders can only mate after they molt to maturity (Carico 1973). The females, at times, cannibalize the males after mating, as demonstrated throughout the Dolomedes species (Arnqvist 1992, Johnson 2001). Penultimate females may cannibalize a male *D. scriptus* if given the opportunity (Johnson 2001). As observed in *Dolomedes triton*, a male could still be cannibalized prior to copulation with a mature female, but they are at least afforded the opportunity to court and potentially mate (Johnson and Sih 2005). The risk of males being cannibalized by penultimate females, with whom they cannot mate, creates an incentive to avoid them. The male *D. scriptus* possesses the ability to distinguish whether a female is mature or penultimate by their silk alone (Roach and

Benson, unpublished), but the way in which they discern the maturity of the female by her silk alone is unknown. Here, I am seeking to determine how they are able to discern the maturity of a female by her silk. It has been shown that spiders can produce pheromones in their silk to signal to potential mates (Schulz 1997, Gaskett 2007, Holler & Persons 2009). However, no pheromones have yet been isolated from *D. scriptus*. Due to the complexity of searching for unknown chemical pheromones, I chose to investigate another variable that may be a reproductive signal. The objective of this study is to measure and quantify the silk output of both mature and penultimate *D. scriptus* and compare the silk output.

Methods:

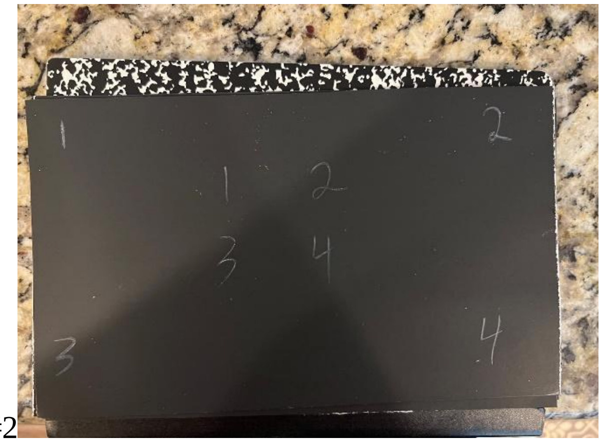
Collection and Housing

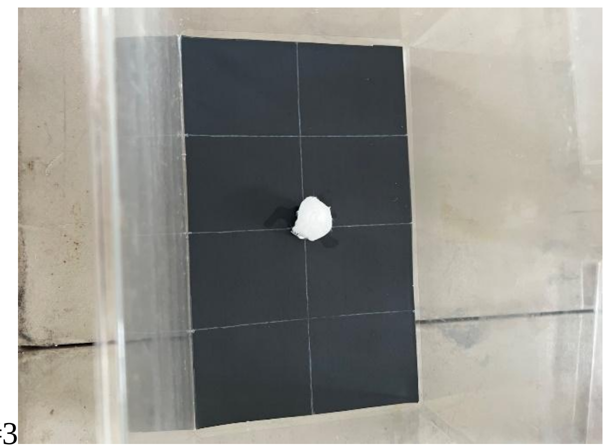
A total of 54 *D. scriptus* spiders were collected from the Big Otter River, at the Claytor Nature Study Center of the University of Lynchburg, VA. Of these, 24 were male and 30 were female. Plastic containers (~10cm x ~10cm x ~10cm) with 2-3 small holes drilled in the lids with a small amount of water in the bottom (~1cm deep) and a makeshift raft (~3x3cm) of closed-cell foam housed the spiders after collection. Their water was changed out as needed. The spiders were offered a single small cricket every 2-3 days, except during silk production observations. If a spider missed a feeding time due to silk production observation, it was offered a cricket immediately upon extraction.

Silk Production Chambers

Plexiglas Pal Pens ™ were lined with a black poster board cutout adhered to the bottom using mounting putty. This "arena" housed the spider during the silk assay. The inside of one of the Plexiglas Pal Pen ™ containers was measured using a tailor's measuring tape. The same measuring tape was then used to measure cutting marks on poster boards. One piece of poster board was cut out and test fitted and set to the side to be used as a template for the remainder of the experiment.

#1 

#2 

#3 

Figures #1-3: These photographs depict the poster board preparation for the spider silk assay arena.

A dot was marked on both ends notating the 7.62 mm mark (half the width of the posterboard), and the two marks connected, dividing the poster board in half. Four equal squares were marked via white coloring pencil on either side of the cutout, for a total of eight squares altogether (see Figures 1-3). These squares were designated one through four for the end squares and one through four for the center squares. One center and one end square would each be randomly chosen for the silk assay.

Care in Chambers

The grid papers were secured to the bottom of the silk collection chambers using 4 small pieces of tacky in the corners. A damp cotton ball on a piece of aluminum foil provided the spider with water (Figure 3). Any leakage is promptly removed via paper towel.

Assay Details

Spiders remained in the silk production chambers for ~48 hours. To quantify silk output, two of the squares on the black poster board were randomly selected from each container, one corner and one adjacent to the water source. After 48 hours of silk deposition, the female was removed. After female removal, a mature male was placed into the assay for 3 minutes while their behavior was observed and video recorded. Following the male's removal, the number of glue spots of silk and total number of silk strands were counted and recorded for each of the randomly selected squares. After the squares were counted, the chamber was prepared for another spider. To clean the arenas, paper towels were used to wipe down the interior of the arena with rubbing alcohol and then water in order to clean the inside of the container and ensure the complete removal of any silk and possible pheromones. After being cleaned, a new spider was placed into the arena. This process was repeated for each assay. Once a penultimate, females were not tested again until maturation. Maturation was determined by development of sex organ structure on the underside of the female's abdomen. Upon maturation, female spiders were placed into a cleaned and prepared terrarium and their silk output recorded again. The counts of glue spots and silk strands were summed and organized in relationship to the spiders age and virginity status, log transformed (since the data was count-data), and a statistical analysis performed using a two sided Welch's t-test calculated using R statistical software (R Core Team 2017).

Results

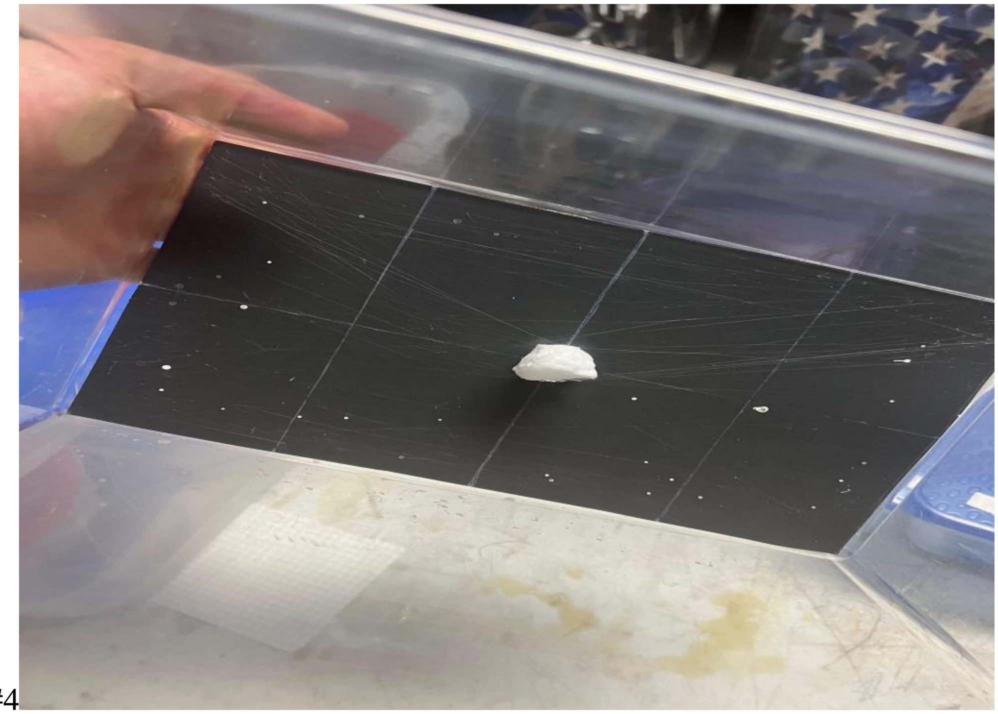
#4 

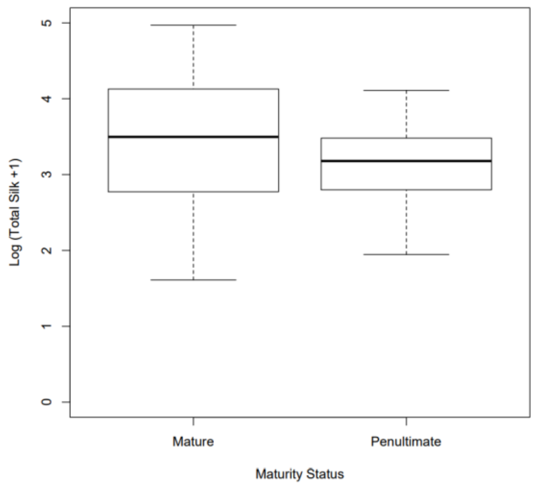
Figure #4: This figure shows an example of the assay arena after removal of a female. Note that the white spots are not glue spots. The white spots are feces. The glue spots of silk are seen when viewed more closely in person.

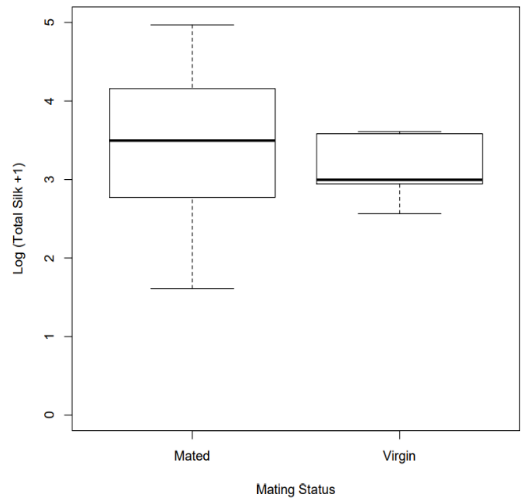
After the assay arena was completed, the data (number of glue spots and silk strands) were compiled into a table. The data was log transformed and the data was analyzed for the appropriate comparisons. The mean numbers of the silk strands and glue spots for the penultimate and mature females was calculated and compared. The mean value for mated/mature females tested is 42.41, with a minimum value of 4 and a maximum value of 143.. The mean value for penultimate females tested is 26.14, with a minimum value of 6 and a maximum value of 60. The mean value for mature virgin females tested is 24, with a minimum value of 12 and a maximum value of 36.

	Virgin	Mated/Mature	Penultimate
Min.	12	4	6
Max.	36	143	60
Average	24	42.41	26.14

Table #1: This table shows raw data (before log transformation) in regards to the sums of silk strands and glue spots for the penultimate, mated/mature, and virgin female *D. scriptus*. The values include the minimum, maximum, and average for each category as notated on the left.

A Welch's t-test found no significant difference in silk production between penultimate and ultimate females ($t_{12.83}$= 0.70, P = 0.49). A second Welch's test failed to detect a difference ($t_{15.34}$ = 0.083,P= 0.41) for the comparison of silk production between virgins and mated. A box plot displays the difference between mated and virgin *D. scriptus*. There is significant overlap between the amount of glue spots and silk strands produced by penultimate and mature females. There was also significant overlap in a similar boxplot the amount of glue spots and silk strands produced by mated and virgin females.





Figures #5 (Left) and Figure #6 (Right): The images above depict the boxplots derived from the data as computed by R software (R Core Team 2017). It shows significant overlap between silk production values for mated and virgin *D. scriptus*. It also shows significant overlap between silk production values for mature and penultimate *D. scriptus*. In these plots, the boxes show 95% confidence intervals. The lines in the boxes depict the median values. The dashed lines notate the maximum and minimum ranges outside of the 95% confidence intervals.

<u>Discussion:</u>

In this experiment I failed to detect a significant difference in silk production between mated and virgin or penultimate and mature as may be expected if silk quantity is used for reproductive signaling. These data do not suggest that the male response to virgin female silk reported by Roach and Benson (unpublished) is due to any differences in quantity of silk. It should be noted that, given the small sample size of this study, there may be a difference in silk output in mature versus penultimate *D. scriptus* that I was unable to detect in this study due to my sample size. A follow-up to this study similar in procedure but involving more subjects would aid in confirming the results of this study. Another suggestion for future study is to harvest more penultimate subjects. This way, their virgin/mated status is known. Known virgin female *D. scriptus* can then be mated and their silk quantity measured to determine if virginity status affects silk output. A larger study would either further support the hypothesis of chemical presence in mature female *D. scriptus* silk, or a difference in silk production could emerge. Emergence of a difference in silk production in a large study would be considered unexpected, given the significant overlap in silk production values found in this study. An alternative follow-up to this study, would be an attempt to isolate the chemical(s) present in the silk.

The lack of difference in silk output in mature and penultimate *D. scriptus* strengthens the hypothesis that chemical pheromones are present in their silk. This is consistent with research showing other spider species, in which chemical pheromones have been isolated (Gaskett 2007, Schulz 1997). The chemicals found in the silk of spiders in previous work with other spider species would serve as a prudent place to begin isolating the chemical responsible for reproductive signaling in *D. scriptus*. However, any putative chemicals for *D. scriptus* this far are highly speculative.

If no chemicals are able to be isolated from the silk of mature female *D. scriptus*, it is evident that there is another method of reproductive signaling at play yet to be discovered. It is clear that the males are detecting signaling in order to more essentially seek out receptive partners (Roach and Benson unpublished). Male response to this unknown signal begins when the female *D. scriptus* reaches maturity, which highly suggests the signal is a reproductive signal (Roach and Benson unpublished).

The likelihood that the signal is chemical, is further supported by the nature of this particular species, *D. scriptus*. The use of long range chemical pheromone signaling may allow the female *D. scriptus* to passively attract males, which are highly active during the breeding season (Andersson 1994, Carico 1973). Given the nocturnal nature of *D. scriptus*, utilizing a visual reproductive signal would be less efficient, as well as more costly for the males which are likely to be cannibalized if they mistakenly approach a penultimate female (Andersson 1994, Johnson and Sih 2005).

Further research into signaling in the *D.* species will provide valuable insight into how reproductive signals are used in nature. If they do in fact use chemical signaling, it will help us better understand how chemicals can be used by organisms. Understanding how *D. scriptus* uses chemical signaling will broaden our understanding of how chemical signaling is utilized and provide further insight into when and how chemical signaling is selected over other forms of signaling. If the method of signaling employed by *D. scriptus* proves to be a form other than chemical, it will further sharpen our understanding of how and why specific forms of reproductive signaling are used under different circumstances.

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