

Effects of Female Mating Status on the Expression and Success of Male Mating Tactic in the Wolf Spider *Rabidosa punctulata*

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ABSTRACT

Mating tactics are used in the mating process to locate a mate, make contact with a mate, copulate, and engage in post-copulatory behavior. The wolf spider *Rabidosa punctulata* will adopt one of two different mating tactics. Courtship in which the male will use visual and seismic signals to attract a mate, or direct mounts, where the male grapples and holds female until copulatory posture is assumed. In this experiment we will be testing the effects of female mating status on the success and expression of male mating tactic. While females of closely related species mate only once and become unreceptive and aggressive, a recent study showed that female *R. punctulata* mates multiply in a short period of time. Here, we explored how female mating status affects male mating behaviors. Mated and unmated females from previous trials were exposed to males and the mating tactics expressed were observed. We found that direct mounts were most commonly adopted and the most successful mating tactic at gaining copulations with already mated females. Males paired with unmated females expressed the courtship tactic most often.

Keywords: *copulation, courtship, direct mount, mating tactic, Rabidosa punctulata.*

INTRODUCTION

Males facing variable reproductive success often adopt different tactics when attempting to acquire a mate. These tactics often lead to increased fitness for individuals that in certain contexts may have failed to mate (Brockmann 2001). Alternative mating tactics are used during all steps of the reproductive process: locating a mate, making contact with mate, copulation, and post-copulatory behavior (Brockmann 2008).

The wolf spider *R. punctulata* has recently been found to express alternative mating tactics (Nicholas 2007). When male *R. punctulata* encounters a female they will adopt one of two different mating tactics: 1) courtship, which uses visual and seismic signals to attract the female or 2) direct mount, where the male grapples and holds female until copulatory posture is assumed. Males that adopt the direct mount tactic want to ensure copulation so they often grab the female and flip her to her back so the female resists less to copulation (Nicholas 2007). Mating tactic expression is condition-dependent, where good conditioned males usually adopt the direct mount tactic, while poor conditioned males adopt the courtship tactic (Wilgers et al. 2009).

Wolf spider mating systems are often characterized by males mating multiply, while females are relatively monogamous (Norton et al. 2005). Females of several spider species are known to mate multiply, store the sperm from each mating and use it later for fertilization of her eggs (Norton 2005). Multiple mating benefits females due to increased genetic diversity of their offspring, but directly increase male competition for fertilizing these eggs. Males that can gain extra copulations with already mated females may increase their fitness,

especially if the copulation is gained using a behavior that may influence the use of their sperm over the males.

Recently, *R. punctulata* females were observed mating multiple times in relatively short trials (Young 2013). This shows that the mating system of this spider is not as simple as one once thought, and introduces the possibility for males to compete for and gain second copulations with already mated females, which could induce sperm competition and cryptic female choice. Males are known to be able to assess the mating status of females through chemical cues in their silk (Robert et al. 2005). If males detect that females have previously mated, they may alter their tactic in order to increase their chances of copulating. Females are less receptive to males after they have already mated once because they are already passing their genes on and they don't feel the pressure to reproduce like unmated females.

We will be testing the effects of female mating status on the expression and success of male mating tactics in *R. punctulata*. I hypothesize that the direct mount tactic will have the most copulatory success because it is not as dependent on the female's behavior.

MATERIALS AND METHODS

We collected 300 *R. punctulata* wolf spiders near Lincoln, Nebraska during the month of August. Spiders were housed in clear plastic containers (8.4 x 8.4 x 11.0 cm) with the sides covered so there was no visual contact between spiders after collection. Spiders were kept in a climate-controlled

environment. The spiders were fed three crickets per a week and supplied with water at all times.

Collected spiders were all immature, except for a few males that were already mature. A few of the mature males were used in the first trial to expose the female to a male. My treatment groups are the females that copulated successfully and the females that didn't gain copulation in the first trial. These treatments groups are important in testing the effect of female mating status on the expression and success of male mating tactics.

The first trial consisted of 59 pairs of mature spiders. We mated the spider pairs 13-15 days after maturity. All spiders were weighed before placed in the arena. The plastic trial arenas were lined with filter paper and sides covered with paper to ensure no visual contact with other spiders. For at least an hour prior to the trial the female were left in the arena alone to allow time for female acclimation and pheromone deposition. Before introducing the male into the arena a clear plastic vial was placed over the female for about a minute to allow the male to acclimate. One male and one female were paired together and chosen at random. All males are only used once to control that all males had not been exposed to a female. The pairs were observed for copulation success mating tactics used, the time the tactic was first expressed, time of copulation, female attacks, overall tactic expressed, and frequency of mating tactics.

The second trial is run 3-5 days after the first trial. The second trial used new virgin males. We again recorded male mating tactic, copulation success, tactic frequency, weight of male and female, age of male and female, time the mating tactic was first expressed, overall mating tactic, time of copulation success and female attacks.. The same procedure is repeated with all spiders until each female has had the opportunity to copulate twice.

To analyze the data I used the statistical analysis program JASP. I ran a T-Test to ensure I used random sampling throughout the experiment. Also contingency tables and chi squared tests to find relationships between data.

RESULTS

A total of two trials were conducted with N=59 in each treatment. In the first trial, 35 of the 59 pairs copulated successfully and 24 of the pairs did not successfully copulate. 36 male spiders expressed courtship as their first tactic, 16 expressed direct mount and 7 males were not active (Figure 1). The majority of the overall tactic expressed was courtship with 32 expressions, direct mount had 16 expressions, 7 males were inactive, and a mixture of tactics (courtship and direct mount) was expressed 4 times out of the 59 pairings. When comparing the tactics that gained copulation, courtship with 18

successful copulations had one more copulation success than direct mount with 17 successful copulations. I found significance with a p value of 0.001 when relating the copulation success and the tactic expressed in the first trial.

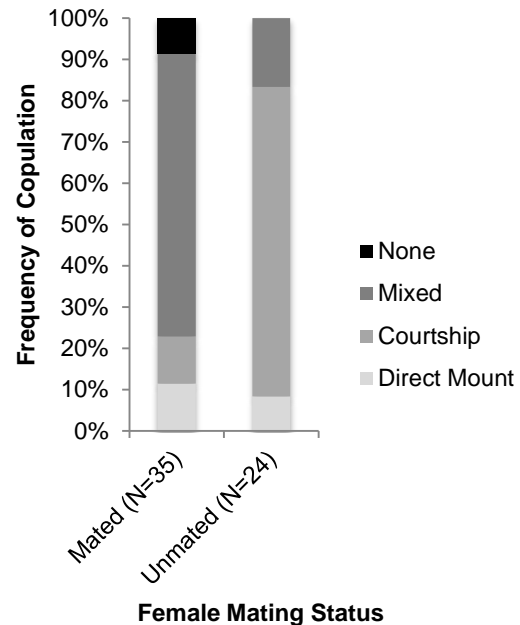


Figure 1. Frequency of mating tactics in the first trial based on copulation success.

During the second trial, 34 pairs out of 59 successfully copulated. Courtship was the most popular tactic expressed first, with 41 males adopting it as a tactic. Direct mount was adopted as a first tactic 15 times and 3 males showed no activity. The most adopted overall tactic expressed was a mix of direct mount and courtship being expressed in 28 of the pairings, then courtship with 22 expressions, direct mount with 6 expressions and 3 males showing no activity. 16 males successfully copulated using the direct mount tactic and 18 were successful using the courtship tactic.

Out of the 35 successful copulations in the first trial and the 34 in the second trial only 12 of the females copulated twice. In 11 of these pairings the male expressed direct mount and in only one pairing the male expressed courtship and was successful in gaining copulation (Figure 2). I found a significant p value of <0.001 when comparing the copulation success in the first trial and frequency of mating tactic in the second trial. There were a total of 22 females that did not copulate in the second trial. Within the unmated pairs about 80% of the time courtship was expressed.

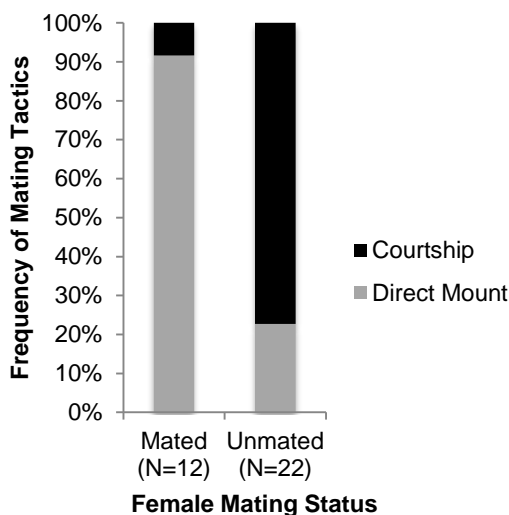


Figure 2. Correlation between mating tactic success in gaining a second copulation and the mating tactic success of gaining a single copulation in the second trial.

DISCUSSION

When observing the 59 mating pairs, males were more successful using the direct mount tactic when the female had previously copulated successfully. In the first trial, males were more successful using the courtship tactic than the direct mount tactic for successful copulation. The Courtship tactic in the second trial was adopted more often than the direct mount tactic when the female had not previously copulated. Courtship was probably more successful in this circumstance because the females may have felt pressure to mate since they previously had not mated and because the male picked up signal cues in the silk. When the female had previously mated in the first trial the direct mount tactic was more successful at gaining copulation success than the courtship tactic.

Only one of the twelve females that copulated twice accepted the courtship tactic in the second trial. Eleven of the twelve females that copulated successfully a second time accepted the direct mount tactic for copulation (Figure 2). This is because the females that had already copulated rarely responded to the courtship tactic. Therefore males that started with courtship changed their tactic to direct mount based on female reaction. Alternatively, males could have detected that females had previously mated through silk cues, and switched to a direct mount for better odds at gaining copulation.

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