Female mating status affects male mating tactic expression in the wolf spider *Rabidosa punctulata*

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Abstract

Males and females have conflicting interests on the frequency and outcomes of mating interactions. Males maximize their fitness by mating with as many females as possible, while choosy females often reduce receptivity following copulation. Alternative male mating tactics can be adaptive in their expression to a variety of mating contexts, including interactions with a relatively unreceptive mated female. Male *Rabidosa punctulata* wolf spiders can adopt distinctive mating tactics when interacting with a female, a complex courtship display, and/or a more coercive direct mount tactic that often involves grappling with females for copulation. In this study, we set up female mating treatments with initial trials and then paired mated and unmated females with males to observe both female remating frequencies and the male mating tactics used during the interactions. Males adopted different mating tactics depending on the mating status of the female they were paired with. Males were more likely to adopt a direct mount tactic with already-mated females and courtship with unmated females. Already-mated females were considerably less receptive to males during experimental trials, although they did remate 34% of the time, the majority of which were with males using a direct mount tactic. While males adjusting to these contextual cues were able to gain more copulations, the observation of multiple mating in female *R. punctulata* introduces the potential for sperm competition. We discuss this sexual conflict in terms of the fitness consequences of these mating outcomes for both males and females.

Key words: Lycosidae, alternative mating tactic, female multiple mating, sexual conflict

While males and females have some shared interests in terms of offspring success, the optimal fitness outcome of any mating interaction is achieved in different ways between the sexes. The common sex-specific mating strategies witnessed across the animal kingdom involve choosy females looking to maximize offspring quality and promiscuous males looking to maximize mating opportunities (Andersson 1994), however, it is clear that many taxa vary considerably from these sex roles norms (review in Green and Madjidian 2011). Both sexes are active participants in mating interactions and respond to one another in ways that attempt to maximize their individual fitness. Regardless, the mating strategies expressed by each sex often conflict with each other with regards to optimal outcomes of mating interactions (e.g. mating frequency, rejection, coercion, sexual cannibalism), perpetuating an arms race that has resulted in tremendous variation in sex-specific mating strategies evolving in response to one another (Arnqvist and Rowe 2005).

Females attempt to maximize individual fitness by improving offspring fitness. They are generally fecundity-
Bunch and Wilgers: Female mating status affects male mating tactics

limited due to costly gamete production and in many systems control the outcome of mating interactions through mate selection. Mate choice based on some aspect of male quality, can either directly benefit herself and her ability to raise offspring or provides her offspring with good genes (Andersson 1994). Female mating decisions are often plastic, as females are known to adjust choosiness, preferences, and receptivity dependent on a variety of intrinsic and extrinsic factors (reviewed in Jennions and Petrie 2007). Depending on their life history, choosy females can either mate once with a preferred male and then become unreceptive and aggressive towards subsequent male suitors (i.e. monandry), or mate multiply (i.e. polyandry, polygamy). Multiple mating is beneficial to females, through increased direct benefits from mates (e.g. parental care, nuptial gifts, feeding territory access) or increased offspring genetic diversity (Arnqvist and Nilsson 2000; Fedorka and Mousseau 2002). While these mating behaviors optimize a female’s individual fitness, they often result in either male rejection or increased sperm competition, which directly contrast and even inhibit mating outcomes that are in a male’s best interest (Shuster and Wade 2003).

Males maximize individual fitness by increasing the number of female eggs they fertilize (Andersson 1994). Males are generally female-limited and face intense selection on traits that help acquire copulations (e.g. female choice, male-male competition) and increase fertilization success (e.g. sperm competition; Shuster and Wade 2003). In response to intense selection from choosy females and competing males, males across many taxa, rather than adopting a single mating strategy, have evolved alternative mating tactics, whose expression is responsive to variation in intrinsic and extrinsic factors across different mating encounters (review for Arachnids: Christenson 1984; Insects: Brockmann 2008; Fish: Taborsky 2008; Reptiles: Calsbeek and Sinervo 2008; Birds: Krüger 2008; Mammals: Wolff 2008). Males adopt a variety of different tactics (e.g. satellite males, sneaking, female mimicry, coercion), whose expression is adaptive as it maximizes male fitness by increasing a male’s reproductive success and/or minimizing costs associated with the mating interaction (reviews in Gross 1996; Brockman 2001; Taborsky et al. 2008). Many alternative mating tactics achieve their success by circumventing the selection favoring other males by adaptively adjusting mating tactic expression in response to male-male competition (e.g. Alcock 1997; Jirotkul 1999; Shine et al. 2005; Auld et al. 2015), variation in male condition or size that may disadvantage them (e.g. Christenson and Goist 1979; Thornhill 1981; Howard 1984; Leary et al. 2005; Shine et al. 2005), and a variety of factors that may influence female receptivity and choice, including predators that may influence female behaviors (e.g. Koga et al. 1998; Evans et al. 2002; Wilgers et al. 2014), female age (e.g. Lubin 1986; Sentenska et al. 2019), female receptivity (e.g. Dеноël et al. 2001) and female mating status (e.g. Guevara-Fiore et al. 2008). While these male tactics may improve male fitness they are often in direct conflict with female fitness goals, as many male mating behaviors (e.g. mating plugs, compounds in seminal fluid, coercion) have direct and negative impacts on female mating behaviors and her lifetime fitness (Brown et al. 1997).

Wolf spider (Araneae: Lycosidae) mating systems are ripe with sexual conflict. Mating interactions pair polygynous males performing elaborate courtship displays to choosy females that are monandrous (Norton and Uetz 2005; Wu et al. 2008; but see Fernández-Montraveta and Cuadrado 2003 for examples of female multiple mating) and often cannibalistic (Wild and Rypstra 2008; Wu et al. 2008; Wilgers et al. 2009). While strong selection from male-male competition is not evident (Kotiaho et al. 1997; Delaney et al. 2007; De Young and Wilgers 2016; but see Hoeffer et al. 2009), females are highly selective based on a variety of aspects of male courtship displays including vibrational displays (Kotiaho et al. 1996; Rundus et al. 2010; Wilgers and Hebets 2012a) and the degree of ornamentation on male forelegs (Scheffer et al. 1996; Hebets 2005; Stafstrom and Hebets 2013; Wilgers and Hebets 2012a). Once mated, female wolf spiders often reduce receptivity and increase aggressive behaviors towards courting males (González and Costa 2008; Wu et al. 2008); however, some males use coercive tactics to mate with less receptive females often resulting in female injury (Johns et al. 2009).
Rabidosa punctulata (Hentz 1844) is a medium-sized wolf spider found in eastern and central United States. During mating interactions, female R. punctulata are choosy, as they are more likely to mate with courting males that are in good condition and more often cannibalize poor condition males (Wilgers et al. 2009). Upon acceptance of the mate, females approach receptively and settle for male mounting as seen in other wolf spider species (Rovner 1968; Wilgers pers. obs.). Recent studies have provided evidence of females accepting copulations from multiple males, however, this receptivity was over short time periods (De Young and Wilgers 2016) and even simultaneous double matings (Persons 2017). During interactions with females, male R. punctulata express distinctive alternative mating tactics that appear to have differential levels of circumvention of female control of the mating outcome. Males can adopt 1) a complex multimodal courtship display, consisting of substrate-borne vibrations and visual leg wave displays and mount only following female receptivity, 2) a direct mount, consisting of a male mounting an unreceptive female (i.e. no receptivity displays) which often includes the pair grappling and the male flipping females onto their back, limiting female resistance to copulation (Nicholas 2007), or 3) a mixture of the two tactics, including a switch at some point during the interaction. The expression of these mating tactics is known to depend on male condition, but not female condition, where larger males in better condition are more likely to adopt the more coercive direct mount tactic (Wilgers et al. 2009). The presence and behaviors of competing males in the environment does not influence male mating tactic expression, although males that adopt direct mounts with competitors in the area are more likely to gain copulations (De Young and Wilgers 2016). Males are known to assess and respond to silk cues from heterospecific predators and adjust their tactics to limit potentially costly detection of courtship by predators (Wilgers et al. 2014). The expression of alternative mating tactics in R. punctulata appears dynamic and adaptive, although there are many other cues that may be of consequence to males during these interactions related to the female they are attempting to mate with.

In this study, we examine female receptivity to multiple mating and whether males can use any cues available during interactions to assess the mating status of females and then optimally adjust their tactic expression to maximize their reproductive success. We would expect if females are less receptive to mating multiply, that males would be more likely to adopt the coercive direct mount tactic in order to increase chances of copulation.

Materials and Methods

Spider Collection and Maintenance

We collected 300 R. punctulata wolf spiders in Lancaster county, Nebraska in August 2016. Upon collection, spiders were housed in clear plastic containers (8.4 × 8.4 × 11.0 cm) with visual barriers. Spiders were kept in a climate-controlled environment (≈ 21–24° C) and kept on a 14:10 light-dark cycle. The spiders were fed three crickets per a week and supplied with water ad libitum.

All female spiders were collected as immatures from the field. Seven males were mature when collected, these mature male spiders were only used in the first trials of the experiment to set up the female mating status treatments.

Mating trials

In order to manipulate female mating status, we ran females through an initial mating trial to set up female mating status treatments. One hour prior to each trial, we placed the virgin female, age 13–15 days post maturation, in a circular plastic trial arena (diameter 20.2 cm) lined with white filter paper (Whatman #1, 185mm) and surrounded by white paper walls for visual barriers. During this time, the female acclimated to the arena and deposited pheromone-laden silk on the substrate. Conspecific interactions between wolf spiders are often contextualized by this silk that is deposited on
the substrate as they move through their habitat (Tietjen 1977; Roland 1984; Gaskett 2007). The chemical compounds in this silk are sex-specific (Tietjen 1979), and are known to initiate male courtship displays during mating interactions as well as providing males with information on female species (Persons et al. 2001; Roberts and Uetz 2004), female hunger (Moskalik and Uetz 2011), and female mating status (Rypstra et al. 2003; Roberts and Uetz 2005) known to alter male mating behavior. 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. Treatment trials lasted 5 minutes after the male acclimation period. The pairs were observed for male mating tactic, latency to courtship, latency to direct mount, number of courtship bouts, male mating tactics expressed, copulation success, time of copulation, and female attacks. Copulating pairs from the treatment trial were allowed to mate for a minimum of one hour, which is similar to mating times reported in closely related species (Stratton et al. 1996) and has been shown to be sufficient time for adequate insertions and sperm transfer in other wolf spiders (Costa and Toscano-Gadea 2003; Szirányi et al. 2005). All spiders were weighed just prior to being placed in the arena. Male and female pairings were chosen haphazardly. All males were only used once during our experiment to ensure that males used during the experimental trials were naïve.

Following the treatment trials, females, both mated and unmated were placed in experimental trials with new, naïve males to examine how males responded to female mating status with respect to male mating behaviors adopted during the interaction. Experimental trials were performed 3-5 days after the treatment trial. Experimental trials were performed in the same manner as above, except trials lasted 30 minutes. There were no differences between the female groups (mated vs. unmated) in the experimental trials for female mass ($t_{57} = 0.47, P = 0.64$), female age (days post-maturation; Mann Whitney $U = 362, P = 0.35$), male age (days post-maturation; $t_{57} = 1.31, P = 0.20$), or male mass ($t_{57} = 0.56, P = 0.58$).

All statistics were performed in JASP (v0.14.1). Non-normal data were analyzed using non-parametric statistics. All results are reported as means ± SE.

**Results**

During the 59 treatment trials, the majority of males adopted only the courtship tactic during their interaction with females, while some males used a direct mount during the interaction alone or mixed with courtship during the interaction (Figure 1). A total of 7 males did not attempt a mating tactic during their interaction with the female. Overall, 35 females mated (59%), while 24 remained unmated (41%). The male mating tactics that directly acquired copulation during these treatment trials were similar across those females that mated (courtship: $N = 18, 51%$; direct mount: $N = 17, 49%$).

During the subsequent experimental trials ($N = 59$), the mating tactic that males adopted when interacting with females depended on the mating status of the female as a result of the treatment trial (Likelihood ratio; $X^2 = 28.26, P < 0.001$). Males were significantly more likely to use a direct mount during their mating interactions with previously mated females than they were with unmated females (Figure 1). Of those males that adopted a mixed tactic, the majority of males attempted courtship first in each of the treatments (Mated: 16/24; Unmated: 4/4), while males in the mated treatment were the only ones to adopt a direct mount first (8/24). Across all males, the frequencies of first male tactics each male expressed during the trials differed significantly between the treatment groups (Likelihood ratio; $X^2 = 10.12, P = 0.006$). Males paired with unmated females used courtship first in 92% of trials (8% direct mount), while males paired with mated females used courtship first in only 57% of trials (34% direct mount, 9% no tactic). Male latencies to first courtship were significantly longer in trials with mated females (Table 1). In addition, once males started courting, they spent longer amounts of time courting and performed significantly more courtship bouts in trials with mated
Bunch and Wilgers: Female mating status affects male mating tactics

females (Table 1). Male latency to adopt a direct mount tended to be longer in trials with mated females, but this
difference was non-significant (Table 1).

In the experimental trials, female mating frequency was significantly different between the mated and unmated
female groups (Likelihood ratio; \(X^2 = 21.64, P < 0.001\)). While, 92% (22/24) of the unmated females mated, only 34%
(12/35) of the already mated females mated again. The tactic used to directly gain copulation differed across the two
female treatment groups (Likelihood ratio; \(X^2 = 16.55, P < 0.001\)). Already mated females were less receptive in the
experiment trials, as less than 10% of females showed receptivity to a courting male that resulted in copulation. Mated
females accepted copulation with males that used a direct mount tactic to mount, whereas as males paired with unmated
females were more likely to use courtship to gain copulations (Figure 2). For those males that copulated, the latency to
mounting the female was longer during mated female trials than unmated female trials, but this difference was not
significant (Mated: \(N = 12, \bar{x} = 592.5 \pm 145.7\) sec; Unmated: \(N = 22, \bar{x} = 340.3 \pm 66.1\) sec; Mann-Whitney \(U = 167.5, P = 0.21\))

There were no cannibalism events in the experimental trials and only one female aggressive attack on a male in the
unmated female group.

Discussion

In this study, we found that male \(R. punctulata\) were able to assess the mating status of females during mating
interactions and respond by altering their mating tactic expression. Males were more likely to adopt the more coercive
mating tactic when paired with mated females. This adjustment in tactic provided them a mating advantage over males
that used a pure courtship tactic when paired with female \(R. punctulata\) that will mate multiply but are considerably less
receptive during subsequent interactions.

Alternative male mating tactics are an adaptive response that increases a male’s fitness across a variety of contexts
by reducing potential costs and maximizing mating opportunities (Gross 1996). Previous studies have shown male \(R.
punctulata\) to be respond and adjust their mating tactic appropriately to both internal cues related to their own body
condition (Wilgers et al. 2009), as well as external environmental cues indicating the presence of predators (Wilgers et
al. 2014). In our study, it appears that male \(R. punctulata\) were able to perceive and respond to multiple cues from the
female that provided context for the mating interaction: silk and female behavior. First, male \(R. punctulata\) were
exposed to female silk that was deposited during her acclimation prior to the interaction. Chemical communication has
been proposed as the most basal of spider signaling modalities, and the pheromones found in female silk are known to
be important in spider mating interactions (reviewed in Tietjen and Rovner 1982). Changes in these silk cues due to
female mating status has been shown to influence male mating behaviors in other spider species, typically increasing
latency to court and reducing courtship activity (e.g. Rypstra et al. 2003; Roberts and Uetz 2005; Stoltz et al. 2007).
While this adjustment may reduce costs associated with the interaction (i.e. wasted energy expenditure, cannibalism,
etc.), it also reduces potential reproductive benefits in those species. Male \(R. punctulata\) clearly adjusted their mating
behaviors in response to these cues, while they waited longer to court mated females, males were more likely to use a
direct mount tactic initially which helped maintain mating success. Second, some males adjusted their tactic in response
to female behaviors during the mating interactions. Once mated, female wolf spiders express significantly fewer
receptivity displays to courting males (Meyer and Uetz 2019). Female feedback behaviors (e.g. orientation, approach,
settle) have been shown to influence male mating behaviors, typically increasing male courtship activity (Sullivan-
Beckers and Hebets 2011). Over 50% of males paired with a mated female initially adopted courtship as their mating
tactic. The vast majority of these males adjusted their strategy during the interaction and attempted to more aggressively
direct mount females without her receptively settling. In this study, males that courted these less receptive females often adjusted their strategy, eventually attempting a more coercive tactic. The plasticity expressed by males in this context may have been simply in response to female receptivity and not to female mating status. Future studies should examine the expression of this mixed tactic and its success in response to females that are unreceptive in other contexts, such as age or condition (example in R. rabida; Wilgers & Hebets 2012b). Males that attempted a direct mount in either of these strategies adjusted their mating behaviors in a way that maintained or increase reproductive success when paired with mated females, as less than 10% of copulations with these females were associated with courtship. The most effective male mating strategies in this study were clearly dependent on the mating status of the female in the interaction. Males able to assess the context of the mating interaction and express the proper tactic accordingly will likely experience greater lifetime fitness, thus favoring the evolution of male sensitivity to cues related to female mating status and the corresponding context-dependent expression of mating tactics.

The sexual conflict in this mating system was further enhanced by the observations that female R. punctulata mated with multiple males during separate interactions. In a previous study, females were found to mate with multiple males in triad scenarios after the female became receptive to mating initially (De Young and Wilgers 2016; Persons 2017). While female receptivity to courting males was significantly reduced, females eventually accepted copulation with males directly mounting them. This eventual receptivity to remating in subsequent and separate interactions contrasts the monandry observed in closely related species (Norton and Uetz 2005; Persons and Uetz 2005; Jiao et al. 2011). In fact, not only do females of other wolf spider species become less receptive to males after mating, they often become highly aggressive and cannibalistic (Persons and Uetz 2005; observed in R. rabida, D. Wilgers unpub. data). Subsequent matings were often a result of a direct mount tactic. An interesting possibility is that female R. punctulata are not being coerced into these copulations, rather females use these interactions as a more direct assessment of male quality. Females are larger than males and often eject and escape a males’ aggressive mount as seen in this study. Females in previous studies have been observed to even cannibalize males attempting a direct mount (Wilgers pers. obs.). Evidence from other polyandrous species suggest unwanted and coercive matings rarely result in paternity benefits and that females are receptive to extra matings as a way of trading up potential fathers (East et al. 2003; Pitcher et al. 2003). The condition-dependent nature of the mating tactics in this species suggests larger, better conditioned males tend to adopt the direct mount compared to courtship (Wilgers et al. 2009). If body condition has a genetic component, males that express direct mounts during mating interactions could be providing sperm of higher genetic quality to compete with her first mate’s sperm (Wilgers & Hebets 2014).

Multiple matings by females coupled with their distinctive spider reproductive system introduces a distinctive twist to the sexual conflict in R. punctulata via sperm competition (Austad 1984; Eberhard 2004). In some spider species, the structure of the female reproductive system allows females to store sperm from different copulations separately and utilize this sperm for fertilization independently (Useta et al. 2007). In species where females mate with multiple males, there is considerable variation across species as to the order of mates and sperm precedence (Elgar 1998; Snow and Andrade 2005; Schäfer and Uhl 2002). Sperm precedence in R. punctulata is unknown, although first sperm precedence is predicted in wolf spiders and other entelegyne spiders (Austad 1984). Sperm competition has resulted in adjustments to a variety of male copulatory behaviors in attempt to increase fertilization success, including longer copulation durations (e.g. Andrés and Rivera 2000; Garcia-González and Gomendio 2004) and copulatory courtship (Eberhard 1991). While determinants of sperm usage in female R. punctulata is unknown, any mating adjustment that increases sperm transfer through additional matings would likely be favored among males. Future studies examining the male fertilization success of multiple matings based on order, male quality, and mating tactic expression could shed light on actual male reproductive success, the nature of female assessment and cryptic choice, and its impact on the outcome of...
sexual conflict in this species.

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Figure 1. Male mating tactics expressed during the treatment trial (left) and the two experimental female treatment groups, mated and unmated (right). In the experimental trials, male mating tactic frequencies differed significantly ($P < 0.001$) between those males paired with already mated females ($N = 35$) and unmated females ($N = 24$).
Figure 2. Mating tactic used to directly gain copulation with females differing in mating status. The frequencies of mating tactics males used to gain copulations differed significantly ($P < 0.001$) between mated females ($N = 12$) and unmated females ($N = 22$).
Table 1. Variation in *R. punctulata* male mating behaviors in response to female mating status

| Male Mating Behavior       | Mated            | Unmated         | P-value\(^1\) |
|----------------------------|------------------|-----------------|---------------|
| Courtship Latency (sec)    | 247.9±59.1 (N = 28) | 71±29.9 (N = 22) | 0.001         |
| Time Spent Courting (sec)  | 1256.7±104.3 (N = 28) | 421.2±109.3 (N = 22) | < 0.001     |
| # Courtship Bouts          | 24.5±3.1 (N = 28)      | 11.2±2.4 (N = 22)   | < 0.001     |
| Direct Mount Latency (sec) | 417.7±70.7 (N = 28)    | 189.3±93.2 (N = 6)    | 0.18         |

\(^1\)P-values are reported from a Mann-Whitney U test comparing each variable across female mating status.