# Exploring the effects of *Pseudomonas aeruginosa* on mating behaviors in the wolf spider *Rabidosa punctulata*

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## ABSTRACT

Variation in mating behaviors attempt to maximize fitness individual fitness for each of the sexes across different contexts. Mate choice by females attempts to maximize their fitness either indirectly by enhancing offspring quality, or directly through gains that may enhance their personal investment into survival or reproduction. Choice of mates that minimize the risk of parasite or disease transmission should play a large role in female decisions. Male mating behaviors attempt to maximize their fitness through increasing their likelihood to mate with females they encounter. The wolf spider *Rabidosa punctulata* is a cursorial species, which brings it into contact with a common soil-dwelling arthropod pathogen, Pseudomonas aeruginosa, commonly causing internal infections that initiate costly immune responses. We artificially infected males with *P. aeruginosa* by inoculating their exoskeleton and subsequently investigated its effects on both female and male mating behaviors. The application of bacteria did not increase overall external bacterial load or internal infection load in males. Mating behaviors of neither the females (mate choice or time spent in association) nor the males (mating tactic expression) were influenced by male our male infection treatment. Investigation into any potential transfer of bacteria to females suggested no female costs of this association as females in both the treatment and control had similar external bacterial loads. Overall, this suggests that bacterial infections, both external and internal, may not be a driving source of selection on this wolf spider mating system.

Keywords: *Infection, Mate choice, Pseudomonas aeruginosa, Rabidosa punctulata.*

## INTRODUCTION

In species in which females (but not males) provide investment in offspring, males may be impartial in selecting a mate who is less likely to lose ability to care for offspring because of infectious disease. Additionally, diseased individuals may transmit the pathogen to offspring during care (whether during gestation or postnatal care), further reducing the value of their care to a mate chooser (Tybur and Gangestad 2011).

Mate preferences may operate in part to mitigate the threats posed by infectious disease. Infectious agents, such as bacteria, viruses, fungi, or parasites, are commonly acquired from conspecifics via direct contact with an infected individual, contact with an individual's bodily fluids, air, or other substances excreted or expired, as well as those excretions that encounter food, water, or other matter. Opportunities for transmission through these forms of contact are strong during inter-individual relations. Mating relations are certainly among these, particularly in species in which sexual mates have lengthy periods of close contact.

Because of the potential for transmission of infection to mates, being able to recognize the cues of infection in potential mates may be crucial in not only survival of females, but her offspring as well (DeYoung and Wilgers 2016).

Close physical contact with a conspecific that carries an infectious disease entails a risk of transmission of the pathogen, regardless of that contact being sexual or non-sexual. Sexual contact typically involves the especially close contact required for gamete transmission.

When conceived broadly, condition may be compromised by a wide variety of recognizable diseases and injuries, including current infection, metabolic disease, disease of an organ system, various genetic diseases, injuries, and downstream effects of a previous pathogenic attack.

Preferences for healthy mates benefit an individual by reducing the chance of acquiring an infectious disease. If mating takes place over a discrete, short time, then current disease status is of sole importance. In instances of bi-parental care, in which partners are in close proximity for a mating season or multiple years, one can also benefit from choosing a mate that is not prone to infection, so future infection risk is limited. In such instances, direct benefits via disease avoidance could, in theory, be obtained through preferences for individuals with cues or signals of immunocompetence, independent of current infection. Preferring healthy individuals as mates could yield reproductive benefits, as well. Spiders often identify the health of their potential mates by the status of the laden silk, as well as body condition (i.e. size, energy, proper number of legs, etc.).

Wolf spiders are ideal study species for this line of research because they naturally live in environments that provide many opportunities for exposure to parasites and pathogens (Gilbert et al. 2016). Male

*Rabidosa punctulata* are known to forego courtship by directly grabbing females and often grappling with them for copulation. Direct mounts achieve copulation faster than courtship (DeYoung and Wilgers 2016). This is important to recognize when looking for behavior differences when the spiders are infected. Here we will be investigating how 1) the infection influences the mating tactics used during mating interactions, and 2) the presence of infections affects a male's chances to copulate. In this experiment we will be looking to see if Females will allow an infected male to mate with her. As well, if a female is healthy, would she run the risk of possibly becoming infected and therefore harm her offspring? We will also be addressing an infected male concern about infecting the female he mates with and possibly producing ill offspring. Finally, will either male or female alter mating tactics to accommodate for ailments? Given that the life span of the spider is only about 2 years, which is relatively short, is mating the one and only goal of the spider (Comstock and Gertsch 1956)? Therefore, my hypothesis is stated as: When *Rabidosa punctulata* is infected with *Pseudomonas aeruginosa*, does mating behavior differ?

Infections have an immense effect on all walks of life and by answering the questions of my project we can be one more stride to understanding how infection affects organisms in nature.

## MATERIALS AND METHODS

### Spider Collection and Manipulation
Wolf spiders (*Rabidosa punctulata*) were collected from the field as immatures to be manipulated. Upon collection, the spiders were individually housed in individual plastic containers (8.4 x 8.4 x 11.0 cm) and isolated from others so all individuals would not have come into contact with a mature individual of the opposite sex prior to trials. Spiders were provided with water through a wet cotton swab and were fed 3 body-size matched crickets per week. Spiders were all held in a climate-controlled room (21–24 degrees Celsius) and under a 14:10 light-dark cycle. Spiders were checked every 2–3 days for molts until maturation.
### Experimental manipulation
*Pseudomonas aeruginosa* was purchased from Carolina Biological Supply (Item #155250A) and stored at 36 degrees Celsius. Spiders were infected 2 days prior to trials by external exposure of a 1 ml containing 600,000 colony forming units (CFU) of the solution were applied to the cotton swab using a pipette. The control group of spiders were given a 1ml of sterile water only to mimic the infection procedure.
### *Pseudomonas Aeruginosa* Dilution
With the stock *P. Aeruginosa,* using a Spectronic Genesys 2 spectrophotometer, absorption was measured in comparison to nutrient broth. With the absorption reading, the McFarland standards were used to determine the approximate cell density of the stock bacteria then diluted accordingly using the equation below to determine the correct amount of nutrient broth to dilute the stock to 600,000 CFU:

$$\text{Approximate cell density x } 10^8 (.1 \text{ ml}) = 600,000 \text{ CFU/ml (x)}$$

### Trials
Control trials were run with healthy males and females. Chosen females used were matured between 12-14 days, the prime mating age in female *R. punctulata*, while the readiest male spider was used (Wilgers,D. Personal instructions. 1 Oct. 2018.). Prior to trials, all spiders were weighed to the nearest .001 g on an electronic scale. Spiders were then placed in circular plastic arenas (diameter 1⁄4 20.2 cm, height 1⁄4 7.3 cm) for trials and allowed to acclimate and deposit pheromone laden silk onto filter paper for at least one hour before experiments began. After at least an hour, the males' paper was cut in half, and one half was given to the male's trial partner. The female was observed interacting with the male's half filter paper and was timed on how much time of a 15-minute period she spent on the male's filter paper. After this the spiders are ready for interaction.

Trial durations lasted 15 minutes. The following behaviors were recorded for every trial: tactic used by the male (courtship and/ or direct mount), latency(s) to tactic, number of each tactic, number of attacks by the female, and copulation (yes/no). All individuals were only used once. After each trial, the arenas were cleaned with ethanol to remove silk cues and excreta. 48 hours post trial every spider was tested for infection confirmation or lack thereof. Both external swabs and hemolymph were collected from males and cultured to investigate any association between external bacteria count and internal infection. Hemolymph was collected by removing the tip of a male spider's leg with scissors and collecting the liquid in a micro-capillary tube. This procedure was run on all male spiders to compare bacterial growth levels between infected and control groups. In a capillary tube, at least 5µL of blood culture were collected and plated on an agar plate and run on the male spiders to check for infection. Females were swabbed externally as well to check from potential infection that may have been transferred from the male during trials. Spiders were swabbed with a sterile cotton swab. The swab was then placed in in 1 ml of water and swirled. .2 ml was then extracted and put into another 1 ml of water. .1 ml of that mixture was then extracted and plated making the final dilution of swabbed water x700.

After swab and hemolymph collection, the spiders were put into a deep freezer to stop any bacterial growth. All data was recorded in an excel spreadsheet.

**RESULTS**

A total 27 trials were conducted; 14 trials with infected males and 13 trials with healthy males. 25 trials in total (14 Bacteria and 11 Control) were used for data analysis. No significant differences were found between the treatment groups for male weight ($t$ = 1.20, df =23, $P$ = 0.24), however, males in the bacteria treatment were significantly older than the control males ($U$ = 113.5, df = 23, $P$ = 0.048).

Male mating tactics did not differ between the treatment groups (Figure 1; $X^2$=2.43, df= 3, P=.488). The most common mating tactic used by all male spiders was direct mount with a total of 11 direct mounts, successful gaining copulation 7 times (Figure 2; 63% success; $X^2$=.202, df=1, P=0.65). However, neither of the treatment groups experienced a significantly greater copulation success (Figure 2; Bacteria: 36% success, Control: 27% success). The plated exoskeleton swabs and hemolymph collection showed no differences across the two treatment groups. (Figure 3; Male Exoskeleton Bacteria: t=.695, df=23, P=.494; Male hemolymph Bacteria: t=-.531, df=21, P=.601)
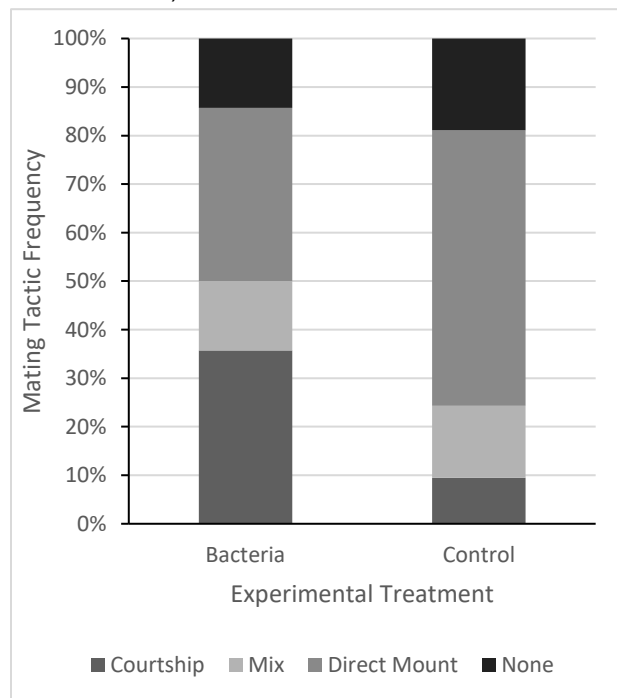


**Figure 1.** Mating tactics used by male treatment groups ($X^2$=2.43, df=3, P=.488).

All female spiders used in our experiments were similar in weight (t=-1.11, df=23, P=0.032) and age (U=64, df=23, $P$=0.48). On average females did not react any differently towards infected males vs. their controlled counterparts. Females did not spend any more time on the male filter paper of either treatment group prior to the mating trial (Figure 4; t=.237, df= 23,

P=.815), indicating that they are not able to indicate whether a potential mate is infected or not by their
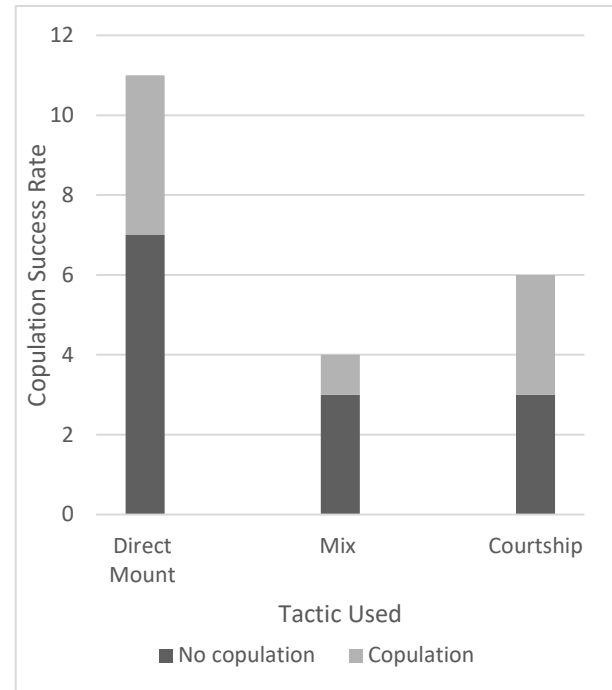


**Figure 2.** Success rate of mating tactic(s) used ($X^2$=.202, df= 1, P=0.65)
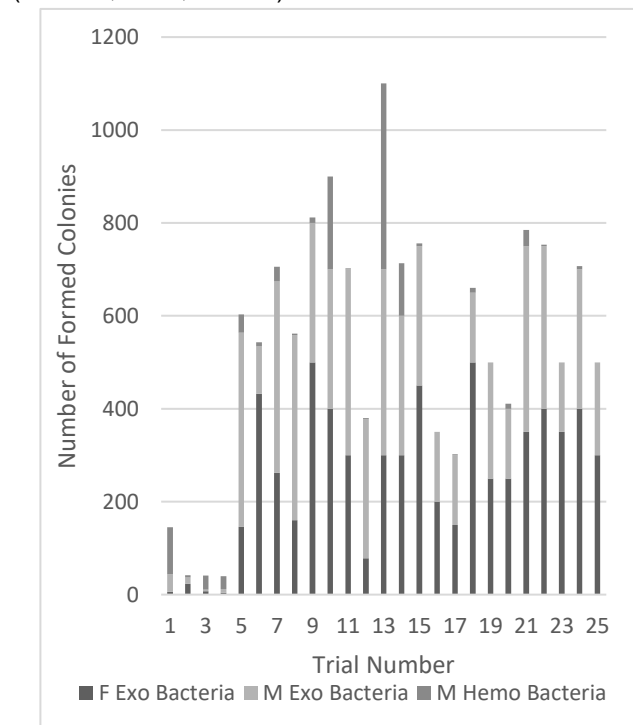


**Figure 3.** Colony forming units collected from each trial. (Female Exoskeleton Bacteria: t=-.296, df=23, P=.770; Male Exoskeleton Bacteria: t=.695, df=23, P=.494; Male Hemolymph Bacteria: t=-.531, df=21, P=.601)

laden silk. Females that interacted with males from the bacteria treatment did not have any significantly elevated bacteria levels (Figure 3; Female Exo Bacteria: t=-.296, df=23, P=.770).
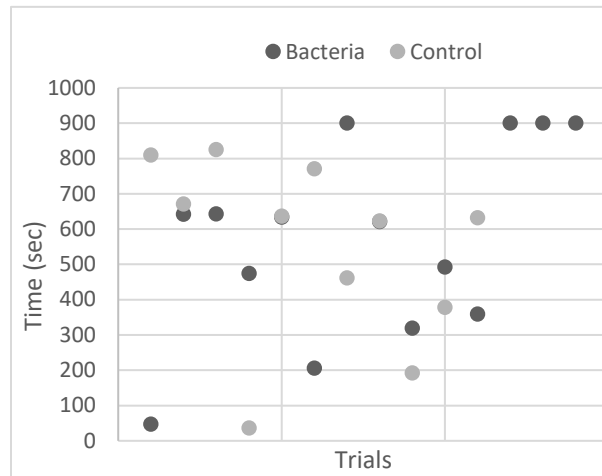


**Figure 4.** Amount of time female spiders spent on male laden silk filter paper (Figure 4; t=.237, df= 23.00, P=.815).

## DISCUSSION

In this study, we found no differing effects of infection on male tactic-specific copulation success in *R. Punctulata*. Males did not alter their mating tactic expression in response to being infected with *P. aeruginosa*. Despite males being significantly older for the bacteria trials, we do not believe this had any effect on the outcome of the study because while the female spider prime mating age is roughly 12-14 days after maturation, male spider mating windows are much larger therefore not affecting our results (Wilgers, pers. obs.).

After further investigation, it was found from a previous study where *P. aeruginosa* was orally ingested by the male spider, peaks in bacteria were found in hemolymph up to roughly 5 hours post ingestion. However, after 7 hours post ingestion, infected males returned to a normal bacteria level in the hemolymph, similar to that of a control group (Gilbert et al. 2016). While *P. aeruginosa* was not orally ingested in this experiment, this information leads us to believe that 48 hours was not an efficient time interval to collect hemolymph. It could have possibly been a premature collection in that the external bacteria had not been given a sufficient amount of time to reach the male internally, or too much time had passed and the spider had a sufficient amount of time to fight off the infection and therefore produce no bacterial growth on the plates.

Future research should investigate time required for external infection to reach a spider's internal systems to improve efficiency for external infection for experimentation. Future studies should also be done to study if female *R. punctulata* alter their interaction behavior when infected. Males of *R. rabida*, another spider species, has been known to be able to assess female mating status through chemical cues in her silk. Females may be able to use this information as well during their mating interactions and if they adjust their mating tactic expression accordingly to maximize fitness. (Young, De, Wilgers, 2016) Females that are able to assess the mating status of males from silk cues could benefit from adjusting their mate choice. It is possible the optimal tactic may not depend on the health of the male and may instead reflect some other factor associated with the mating interaction (e.g., environmental condition, female mating status).

While this paper only followed copulation success due to tactic expression based on fitness, this mating system has many more questions to answer after the observations suggest more promiscuity than once thought. Investigations into the determinants of female and male choice (sperm precedence, size, etc.) may provide considerable insight into the evolution of these mating behaviors.

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