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Spiders, microbes and sex: Bacterial exposure on copulatory organs alters mating behaviour in funnel-web spiders

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Abstract

Environmental microbes have the potential to be involved in nearly all behavioural processes. For example, mating systems where males use intromittent organs to transfer sperm to females represent a means by which environmental microbes collected by males can breach entry into females' body cavities during mating. However, the degree to which the acquisition of environmental microbes onto important sex structures alters courtship behaviours remains unknown. Here, we collected bacteria from the copulatory organs of *Agelenopsis pennsylvanica* funnel-weaving spiders in situ to test whether exposure to bacteria on copulatory organs can alter hosts' courtship behaviour, reproductive success and survival. We used a standardized assay to repeatedly measure each spider's aggressiveness, a behavioural component of both male courtship and female sexual receptivity. Then, we experimentally altered the bacteria present on male and female spiders' copulatory organs with an application of either (a) a mixture of bacteria collected from conspecifics to increase bacterial presence, (b) an antibiotic to reduce bacterial presence or (c) a procedural control. Each spider was paired with a size-matched spider of the opposite sex whose copulatory organs were unaltered, and we measured the latency until the onset and the duration of courtship. Spiders were then isolated, and we measured each individual's time until death and female fecundity over the next 40 days. We found that female exposure to bacteria had multiple effects on mating dynamics. Males took over four times longer to begin courting females that had been exposed to bacteria compared to unexposed and antibiotic-treated females. Only when courting these bacteria-exposed females, males began courtship sooner when females were more aggressive. Lastly, females whose mate had been exposed to bacteria experienced reduced survival. These data suggest that bacteria present on animals' copulatory organs can alter courtship behaviours, female survivorship, and may potentially play a role in mating dynamics.

KEYWORDS
Agelenopsis, aggression, bacteria, courtship, signalling, spider

1 | INTRODUCTION

The most common ecological interaction with which any animal is confronted is undoubtedly with the diverse milieu of microbes present in their environment (Ezenwa, Gerardo, Inouye, Medina, & Xavier, 2012; McFall-Ngai et al., 2013). Associations with environmental microbes can affect not only host physiology and behaviour (Parks et al., 2018), but can also shift the outcome of interactions with predators

(Ramirez & Snyder, 2009), competitors (Grosholz, 1992) and potential mates (Worden, Parker, & Pappas, 2000). In the context of sexual behaviour, microbes encountered via contact with mates can potentially threaten future performance. Exposure to sexually transmitted parasites is often cited as a potential cost of mating in diverse animal species (Lockhart, Thrall, & Antonovics, 1996; Sheldon, 1993; Smith & Dobson, 1992), and exposure to even non-pathogenic microbes can have drastic consequences due to the activation of costly immune cascades (Gendrin, Welchman, Poidevin, Hervé, & Lemaitre, 2009). Unfortunately, little is known about how exposure to environmental microbes can alter the behaviours that influence the outcome of mating events, such as male courtship and female receptivity.

The arthropod cuticle is the primary barrier against the entry of environmental microbes, though mating systems where sperm are transferred to females via males' intromittent organs present opportunities for environmental microbes collected by males to breach entry into females' body cavities. Although studies on sexual transmission of environmental microbes are rare, examples from birds show that cloacal and seminal fluid samples usually contain benign microbes (Hupton, Portocarrero, Newman, & Westneat, 2003). However, potentially harmful microbes can be acquired by males from the environment and then transmitted to females during mating (Kulkarni & Heeb, 2007), which can alter the composition and diversity of female bacterial communities (White et al., 2010). For example, male bed bugs accumulate bacteria on their intromittent organs and transmit these to females during traumatic insemination, with drastic consequences for egg production and survival (Reinhardt, Naylor, & Siva-Jothy, 2005, 2003). Thus, female receptivity to mating may be reduced when encountering males harbouring large bacterial loads, or males may increase courtship effort in order to capitalize on immediate mating opportunities if acquired microbes reduce their likelihood of future mating. These changes in male courtship may be particularly apparent in species where mating opportunities are infrequent.

North-American spiders of the genus *Agelenopsis* (Araneae: Agelenidae) live primarily solitary lives in individual funnel-shaped webs in bushes and understory vegetation. In some species, female *Agelenopsis* remain in their webs while males roam to find mating opportunities. Females also exhibit pre-copulatory sexual cannibalism, so individuals may encounter only a few potential mates in their lifetime (B. Cusack & J. N. Pruitt, unpublished data; Riechert & Singer, 1995). In *Agelenopsis pennsylvanica*, receptivity to mating can be dependent on female temperament, where more aggressive females are more likely to cannibalize potential mates (Berning et al., 2012; Pruitt et al., 2014). Males use structures on the front pair of accessory appendages called pedipalps for sperm transfer to females (Figure 1b). These structures are also used during courtship in *Agelenopsis* (Singer et al., 2000), and for stridulation or visual signals in other spiders (Elias, Mason, & Hoy, 2004; Fernández-Montraveta & Simó, 2006), where bacteria could be acquired from the environment and transferred into the female body cavity during sperm transfer. Environmental microbes do occur on the cuticles of *A. pennsylvanica* and have been shown to alter host behaviour (Parks et al., 2018). However, the degree to which exposure to environmental

microbes can alter male courtship effort or female receptivity remains unknown.

Here, we use the funnel-weaving spider *A. pennsylvanica* to test the hypothesis that an experimental addition of bacteria on animals' copulatory organs can alter courtship behaviours and the outcome of mating events. We isolate and identify bacteria collected from the sex organs of spiders in situ, re-expose spiders' copulatory structures to a mixture of these bacteria, and measure the courtship behaviours of mating pairs in the laboratory. To test whether an alteration in bacterial load in either direction (+/−) can alter these mating outcomes, we also treated spiders' copulatory organs with a broad-spectrum antibiotic to reduce bacterial abundance. Further, by measuring spiders' behavioural phenotypes (aggressiveness) prior to mating, we aim to identify whether individuals' behavioural tendencies play a role in the outcome of mating encounters after bacterial exposure.

2 | MATERIAL AND METHODS

2.1 | Animal collection

We collected funnel-web spiders, *A. pennsylvanica* as penultimate juveniles along urban hedgerows in Oakland, Pittsburgh, PA, in August 2014. Spider collection involved dropping a 2-week-old domestic cricket in the spider's capture web and scooping up the spider with a plastic cup as it left its retreat to attack the prey item. Spiders were immediately transferred into sterile 50 ml falcon tubes and maintained individually in the laboratory in sterilized round plastic containers (radius = 9.4 cm, height = 7.6 cm). We fed spiders ad libitum directly after transport into the laboratory to reduce the likelihood of pre-copulatory sexual cannibalism during our mating observations (Berning et al., 2012), and then for the rest of the experiment, we fed spiders with a diet of one 2-week-old cricket twice weekly and water provided with a spray bottle.

2.2 | Experimental overview

We used repeated behavioural assays to assess spiders' aggressiveness. Then, spiders' sex organs were exposed to one of three experimental treatments: a bacterial cocktail to increase the representation of naturally occurring bacteria, an antibiotic to decrease bacterial presence, or a procedural control treatment. Each individual was paired with a control spider of the opposite sex (no bacterial treatment or handling) in staged mating bouts, where we measured the onset and duration of courtship. After staged mating encounters, we monitored the reproductive output of females and the survival of all spiders.

2.3 | Bacterial collection and identification

Bacteria were collected from male spiders by CO_2-euthanizing two adult males and immediately removing their pedipalps (the male intromittent organ) with microdissection scissors (Fisher Scientific, catalog # 08-953-1B). The detached palps were transferred into 1 ml of sterile LB broth for 24–48 hr at 24°C. Although we did not observe

any haemolymph leak out of the excision point, it is possible that some bacteria came from the haemolymph and not from the outer surface of the pedipalp. Bacteria were collected from two female spiders by CO_2-euthanizing adult females and using a sterile conical cotton swab to sample the ventral abdomen near the epigynum (the female sex orifice) and plating directly onto LB agar. The bacteria were grown for 24–48 hr at 24°C. Individual bacterial colonies were isolated and re-cultured repeatedly and then identified using 16S ribosomal RNA gene sequencing and MicroSeq® BLAST Software (SeqWright Genomic Services). We isolated two species of bacteria from the ventral abdomens of two females: *Staphylococcus warneri* and *Staphylococcus epidermidis*, and four species of bacteria from the left and right palps of two males: *Micrococcus luteus, Micrococcus yunnanensis, Curtobacterium flaccumfaciens* and *Arthrobacter pascens* (Table 1). Since the arthropod cuticle does not represent a particularly favourable environment for many bacteria (Brooks, 1963), these bacteria were likely acquired recently by spiders from their environment. Bacterial stocks were frozen in 25% glycerol and stored at −80°C while behavioural assays took place and were resurrected onto LB agar plates directly before application onto experimental animals.
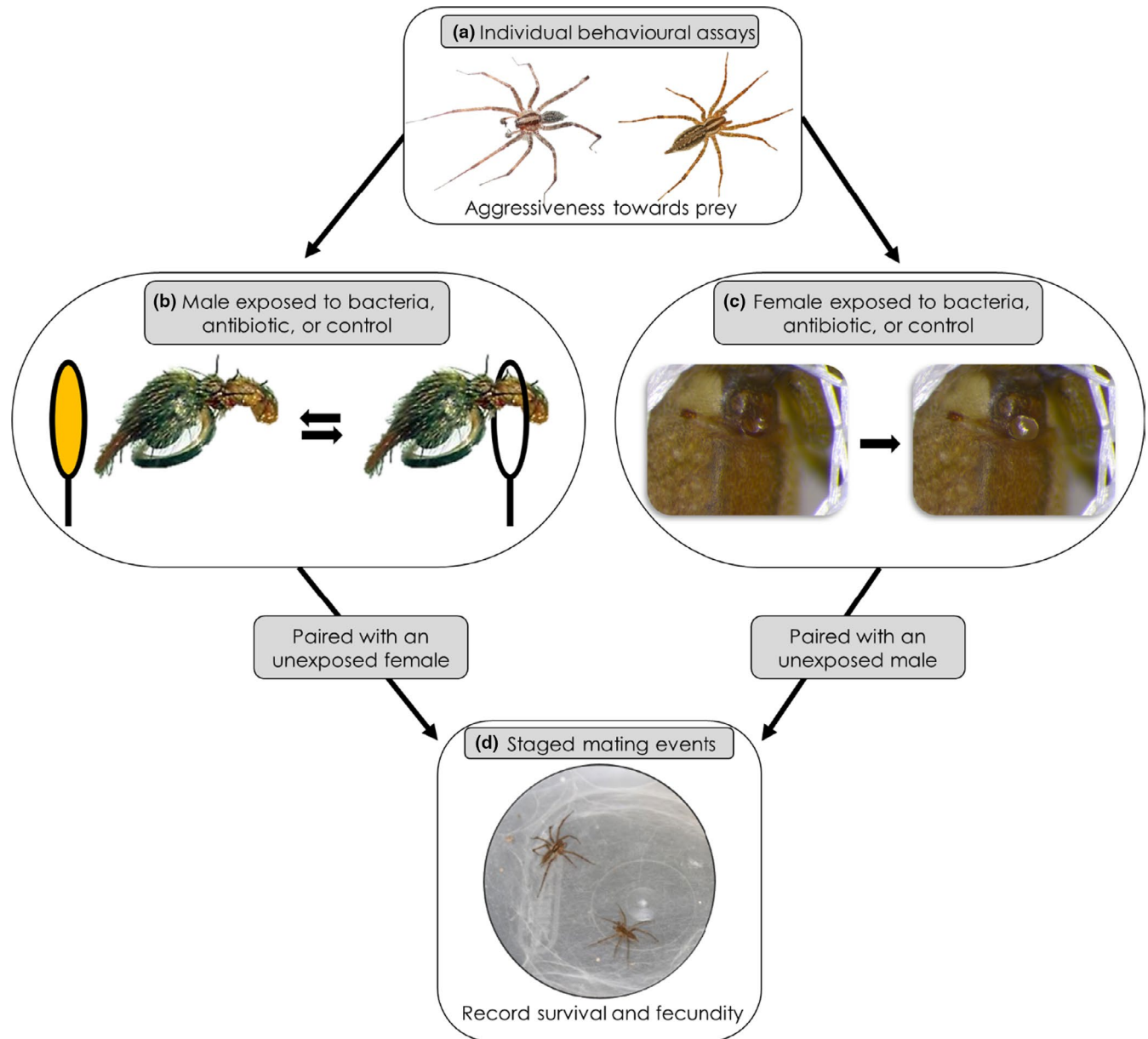

FIGURE 1 Schematic diagram of our experimental design. (a) We tested adult male and female *Agelenopsis pennsylvanica* for their aggressiveness towards prey three times each. (b) Males were secured under mesh and exposed to bacteria or antibiotics using a sterile 1 μl inoculating loop that was passed the over both of the males' palps three times each. (c) Females were exposed to bacteria or antibiotics using a 10-μl Hamilton syringe to insert 2 μl of the solution directly into their epigynum. (d) Experimentally exposed spiders were then paired with an unexposed spider in a staged mating event, after which we measured each spider's survival and females' fecundity. Photographs by Judy Gallagher, Patrick Edward Moran, Andrew Bateman, and Michelle Elise Spicer. The palp pictured here is that of a male *Agelenopsis potteri*

TABLE 1 Collection sources and characteristics of spider cuticular bacteria used in this study

Bacteria	Source	Characteristics	Reference
Staphylococcus warneri	♀ ventral abdomen	Gram-positive, common commensal bacteria of skin microbiota	Barigye, Schaan, Gibbs, Schamber, and Dyer (2007), Schleifer and Kloos (1975)
Staphylococcus epidermidis	♀ ventral abdomen	Gram-positive, common resident of skin microbiota	Fey and Olson (2010)
Micrococcus luteus	♂ left palp	Gram-positive, saprotrophic, common environmental bacterium	Madigan and Martinko (2006)
Micrococcus yunnanensis	♂ left palp	Gram-positive, sometimes isolated from plant roots	Zhao et al. (2009)
Curtobacterium flaccumfaciens	♂ right palp	Gram-positive plant pathogen causing bacterial wilt disease	Tegli, Sereni, and Surico (2002)
Arthrobacter pascens	♂ left palp	Gram-positive soil bacterium	Chaplin (1957)

2.4 | Individual behavioural assays

Prior to bacterial exposure and staged mating trials, we measured each spider's aggressiveness towards prey once a day for three consecutive days. The onset of behavioural assays occurred 2 days following a routine feeding event, in attempts to standardize spiders' hunger level and motivation to forage. Individual aggressiveness, especially that of females, is associated with a number of important behaviours in *Agelenopsis*, including foraging, antipredator behaviour, territoriality and mating (Berning et al., 2012; Pruitt & Husak, 2010; Riechert, 1978). We used a prey capture assay which represents a common and repeatable measure of aggressiveness in spiders (Hedrick & Riechert, 1989; Pruitt & Husak, 2010). Aggressiveness assays occurred in spiders' home containers by removing the container's lid, providing a 30 s acclimation period and then placing a single 2-week-old cricket in the centre of the capture web. We recorded the latency for spiders to attack the cricket, where more aggressive spiders attack the prey more rapidly. Spiders were given 300 s to attack the prey item, after which the assay was terminated (205/1,081 assays were terminated in this way). Latency to attack in terminated assays was recorded as 300 s; spiders were still included in the analyses (Lichtenstein et al., 2016; Pruitt & Keiser, 2014).

2.5 | Bacterial and antibiotic exposures

Using a random number generator, spiders were randomly assigned to one of three experimental groups: (a) exposure to the bacterial cocktail, (b) exposure to the antibiotic, or (c) a procedural control. The bacterial cocktail consisted of an equal-parts mixture of liquid cultures of the six bacteria collected from *A. pennsylvanica* spiders, each grown to an optical density (OD_{600}) of approximately 1.0 (Table 1). Previous experiments have verified that this topical exposure protocol can drastically increase bacterial load present on the spider's body surface (Keiser, Wright, & Pruitt, 2016). The antibiotic treatment consisted of 10 ng/µl tetracycline hydrochloride, a broad-spectrum antibiotic (Sigma Aldrich; product # T7660).

We CO_2 anesthetized male spiders and immobilized them under mesh. Then, we dipped a 1 µl inoculating loop into the bacterial cocktail (*n* = 20) or antibiotic (*n* = 15) and passed the loop over both of the males' palps three times each (Figure 1b). This coated the outer surface of the palps with the solution. Female CO_2-anesthetized spiders were secured under mesh, and we used a 10-µl Hamilton syringe (Model 701; Hamilton Company) to insert 2 µl of either the bacteria cocktail (*n* = 10) or the antibiotic (*n* = 11) into their epigynum (Figure 1c). Females were observed under a dissecting microscope for 2 min after exposure to ensure that the application was absorbed into the epigynum. Spiders were placed back into their housing containers for 24 hr before the onset of staged mating trials. To account for the stress of experimenter handling, control spiders were handled similarly but were not exposed to either solution. In a separate experiment, we tested for the effects of the sterile LB solvent used to administer bacteria and antibiotics by exposing male (*n* = 19) and female (*n* = 12) spiders to sterile LB broth using the same methods, and then pairing them with a control spider of the opposite sex for a mating trial. Thus, we had 66 experimental pairs and an additional 31 LB solvent pairs for a total of 97 pairs of spiders. Final sample sizes were constrained by availability of size-matched males and mortality throughout the time from field collection to experiment completion.

2.6 | Staged mating trials

Each spider that had been exposed to bacteria or antibiotics was paired with a size-matched control spider of the opposite sex. We also formed 10 pairs of control individuals. We placed the housing containers into larger open-top plastic bins (30 × 18 × 12 cm), introduced males into females' housing containers and allowed the spiders to interact with the lids off for 3 hr. Males were free to exit the housing container and move to the plastic bin at any time throughout the mating trial. During this time, we measured (a) the latency until males began courtship displays (drumming the silk with their legs and performing abdomen sways) and (b) the latency between the onset of courtship and the onset of catalepsis with a stopwatch in real time. Catalepsis is an important stage in the mating sequence of *Agelenopsis* where males subdue females with pheromones into a quiescent state before copulation (Becker, Riechert, & Singer, 2005; Singer et al., 2000). This value thus represents the total amount of

TABLE 2 Results of general linear models predicting the latency until the onset of courtship and the duration of courtship (time from onset of courtship until catalepsis)

Effect	df	F-value	p-Value
Latency until onset of courtship			
Treatment	4,58	6.07	.0005*
Female aggressiveness	1,58	1.21	.28
Male aggressiveness	1,58	0.04	.85
Treatment × Female aggressiveness	4,58	5.28	.001*
Time from courtship to catalepsis			
Treatment	4,58	2.64	.05
Female aggressiveness	1,58	0.23	.64
Male aggressiveness	1,58	0.71	.41
Treatment × Female aggressiveness	4,58	1.82	.14

Note: Significant p-values are marked with an asterisk, compared to a Bonferroni-corrected alpha value of .006.

time males spent courting before mating occurred (i.e., courtship duration). After successfully mating, males departed the web before females awakened to avoid sexual cannibalism. We then separated males and females into their own housing containers and monitored their survival for the next 40 days. We also estimated females' reproductive output by weighing each egg case they produced over this time period using a digital scale.

2.7 | Statistical analyses

We verified the repeatability of spiders' aggressiveness using one-way ANOVA to partition the total variance to within- and among-individual components, with spider ID as an independent variable and trial number as a random effect. The ratio of the variance among individuals to the total phenotypic variance represents the estimate of repeatability (Boake, 1989; Falconer & Mackay, 1996). For all further analyses, we used the mean value from spiders' three assays as representative of their aggressiveness. For mating behaviours, we used general linear models (GLMs) to predict (a) the latency until courtship began and (b) the courtship duration. We used linear regression to verify that these two response variables were not autocorrelated ($F_{1,51}$ = 0.61, p = .44). The GLMs contained the following independent variables: treatment pairing (female bacteria, female antibiotic, male bacteria, male antibiotic, control), female aggressiveness, male aggressiveness and the interaction terms between treatment and male/female aggressiveness (Table 2). Non-significant interaction terms were removed for model simplification. Post hoc tests were performed using Tukey's HSD. To control for multiple testing and false discovery rate, we compared p-values to a Bonferroni-corrected alpha value of .006. We analysed survivorship for males and females separately, due to natural sex differences in longevity, using log-rank Mantel–Cox survival analyses with treatment pairing as the predictor. We tested for differences in egg case weights among

the treatments with a GLM with the same independent variables as above. All analyses were performed in JMP version 12.1.

2.8 | Ethics statement

This research was not reviewed by an institutional or governmental regulatory authority because the study subjects are invertebrates that do not fall under the confines of these institutions. However, we took precautions to adhere to the ASAB/ARRIVE Guidelines for the Use of Animals in Research to minimize unnecessary stresses or harm to the spiders. A recent study showed that a similar application of cuticular bacteria to *A. pennsylvanica* was not detrimental to survival (Parks et al., 2018).

3 | RESULTS

Agelenopsis pennsylvanica spiders exhibited temporally consistent individual differences in aggressiveness ($F_{133,394}$ = 1.58, p = .0009, repeatability .40), which is consistent with several other studies on this species (Berning et al., 2012; Sweeney et al., 2013). Fifty-eight
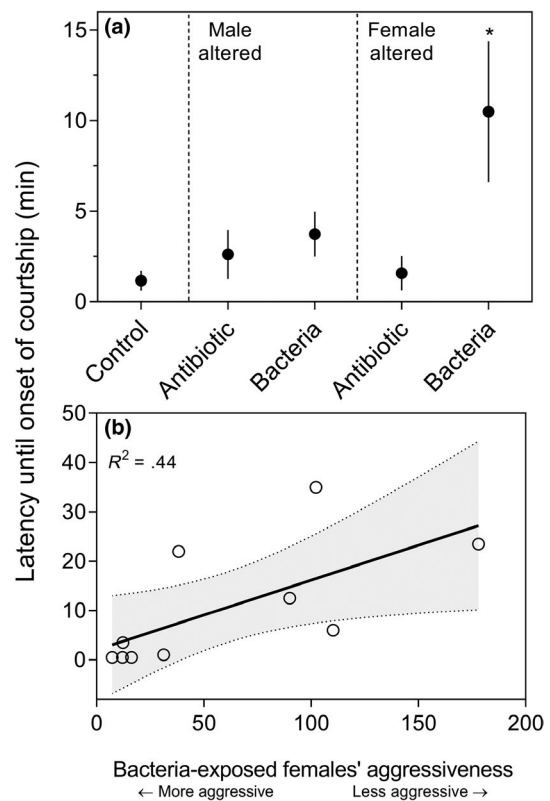

FIGURE 2 (a) Pairs in which the female was exposed to bacteria showed an increase in the latency until males began courtship compared to all other treatment pairings. Vertical lines represent standard error of the mean; significant differences among groups based on the Tukey's HSD test are denoted with an asterisk. (b) Only in mating pairs where females were exposed to bacteria, males began courtship sooner when females were more aggressive. Grey area around the regression line represents the 95% confidence intervals
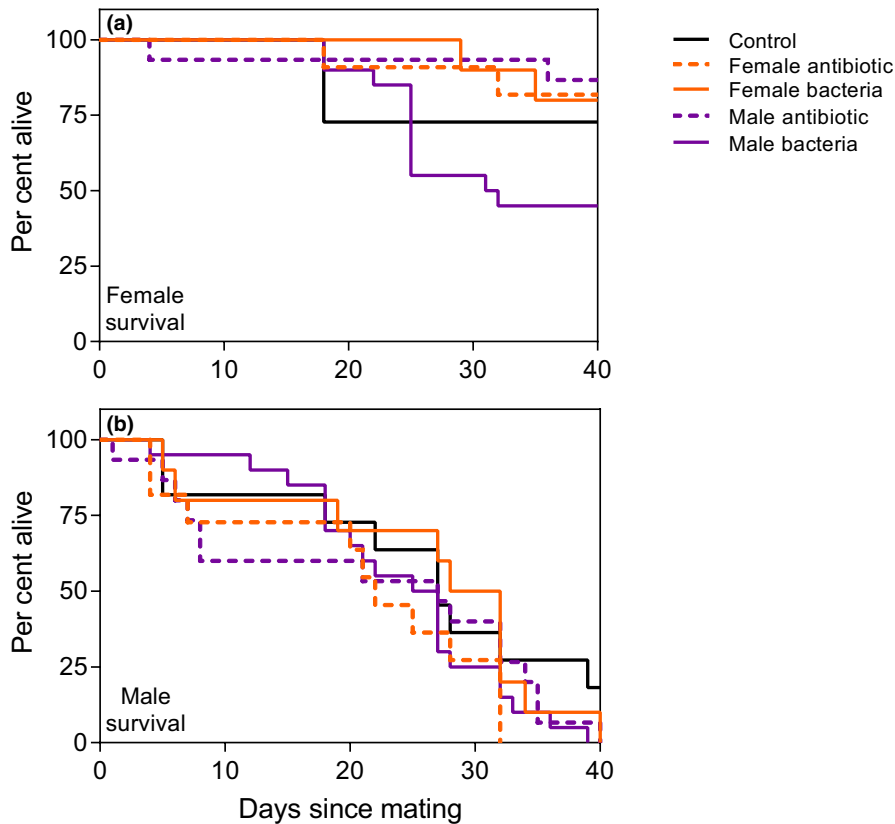
FIGURE 3 (a) Females that mated with a male exposed to bacteria experienced reduced survival. (b) Exposure to bacteria, antibiotics or altered females had no effect on male survival

of the 66 experimental pairs mated (88%), and two males were eaten in post-copulatory sexual cannibalism. When females were exposed to bacteria, males took 4.6 times as long to begin courtship compared to all other treatment groups ($F_{4,58}$ = 6.07, p = .0005; Table 2; Figure 2a). There was also a significant interaction between exposure treatment and female aggressiveness in predicting the latency for males to begin courtship ($F_{4,58}$ = 5.28, p = .001; Figure 2a,b). Only in pairs where females were exposed to bacteria, males began courtship sooner when females were more aggressive. No other experimental treatment was associated with such a trend, nor did any independent variable predict the duration of courtship (all p > .05; Table 2). In separate control trials where males and females were exposed to sterile LB broth, we found no differences between their latency until courtship and duration of courtship (all p > .11), suggesting that there were no solvent effects on courtship behaviour (Dirienzo, Mcdermott, & Pruitt, 2015).

Lastly, females whose male mating partner had been exposed to bacteria experienced reduced survival (χ^2 = 11.51, df = 4, p = .02; Figure 3a). Median time to death for females who mated with bacteria-exposed males was 31.5 days, whereas median time to death for the other treatment pairs was between 53 and 67 days. However, exposure to bacteria, antibiotics or female treatments had no effect on male survival (χ^2 = 4.50, df = 4, p = .34; Figure 3b). We detected no effects of spider behaviour or experimental treatment on the mass of egg cases produced (i.e., fecundity) after mating (mean egg case mass across all groups was 0.027 ± 0.02 SD; all p > .24).

4 | DISCUSSION

Courtship and mating represent potential routes for horizontal transmission of microbes acquired from the environment, especially for microbes that colonize intromittent organs. Exposure to microbes could also alter mating receptivity in females or courtship effort in males. Here, we used funnel-web spiders to test whether exposing male and female copulatory organs to environmental microbes influences their mating behaviours, fecundity and survivorship. We found that males took over four times longer to begin courting females that had been experimentally exposed to bacteria, while neither male nor female exposure to antibiotics altered male courtship. Further, only when courting females that had been exposed to bacteria, males began courtship behaviours sooner when paired with more aggressive females. Females whose sexual partner had been exposed to bacteria experienced reduced survival. Thus, the bacteria present on animals' copulatory organs can play a notable, though nuanced, role in mating success.

Exposure to microbes can impact the expression of host sexual behaviours. We observed delayed courtship behaviours when females were exposed to bacteria. Latency to begin courtship/mating is considered to reflect a component of mate choice (Shackleton, Jennions, & Hunt, 2005) though the underlying mechanisms here remain unknown. We present two non-mutually exclusive hypotheses: (a) males may detect cues of the bacterial load of their potential partners and adjust their behaviour accordingly (Gendrin et al., 2009), or

(b) females may reduce sexual receptivity after experiencing an increased bacterial load, altering cues associated with their receptivity, so males delay the initiation of courtship. Although courtship duration is most likely a product of male courtship effort and female receptivity, the initiation of courtship is most likely a property of male behaviour based on assessments of female receptivity. We found no effect of exposing males to bacteria on their courtship behaviour (as has been found in other host–parasite systems: Abbot & Dill, 2001; McLachlan, 1999), perhaps because this was a topical application while female exposure was an internal application. Although similar topical applications of cuticular bacteria have been found to influence spider behaviour (Keiser, Shearer, et al., 2016; Keiser, Wright, et al., 2016; Parks et al., 2018), more detailed experiments are needed to illuminate the mechanistic underpinnings shaping the relationship between bacterial exposure and sexual behaviour.

We found that males began courting females more quickly when the female was more aggressive *only* in cases where females were exposed to bacteria. Such rushed mating strategies are most commonly associated with male efforts to minimalize sexual cannibalism (Albo, Winther, Tuni, Toft, & Bilde, 2011; Wilder, Rypstra, & Elgar, 2009). Aggressive females of many species are significantly more likely to kill or attempt to kill their mates prior to, during, or after courtship (Johnson & Sih, 2005; Lawrence, 1992; Schneider & Elgar, 2002), though cannibalism is also dependent on male quality (Kralj-Fišer et al., 2012). Under such circumstances, it stands to reason that males should benefit from minimizing time spent with a potentially lethal female to reduce his likelihood of cannibalism, and minimize his own exposure to potentially harmful bacteria. Since males took longer to court bacteria-exposed females on average, it appears that the rushed courtship towards aggressive females to avoid cannibalism may override males' potential avoidance of females exposed to bacteria. Signalling may also play an important role in the interaction we observed among female aggression, exposure to bacteria, and courtship; males may be able to detect potential female receptivity to courtship, or exposed aggressive females may signal their condition.

Internal fertilization represents a means by which environmental microbes can gain access to potential hosts. This is particularly evident in the case of traumatic insemination, for example, where the presence of bacteria on male bedbugs' intromittent organs can reduce egg production and cause female mortality (Reinhardt, Naylor, & Siva-Jothy, 2003). We found here that when females mated with males who had been exposed to bacteria, females experienced decreased survival, even compared to females that had been inoculated *directly* with bacteria. Thus, sperm transfer via males' pedipalps appears to be a viable mechanism for the additional transport of bacteria, and a potentially efficacious mechanism at that. A study on the wolf spider *Schizocosa ocreata* found that pathogenic bacteria consumed by males can be transmitted to females during copulation (Gilbert & Uetz, 2016). However, because we did not verify transmission from males to females, nor did we verify that the bacteria were isolated to the epigynum, several other mechanisms may be at play. Future physiological and microbiome research is needed to understand how the modification of bacterial communities in and on the body can alter behaviour. In *S. ocreata*, like in *A. pennsylvanica*, sexual partners also appear to be able to detect the presence of bacteria (pathogenic or otherwise) in their prospective sexual partners (Gilbert, Karp, & Uetz, 2016; Gilbert & Uetz, 2016, 2019). We found no evidence that treatment with antibiotics altered courtship behaviour or survivorship, though future studies could ascertain whether antibiotic treatments reduced the presence of beneficial microbes that might play a role in spider mating.

Together, the data presented here suggest that bacterial exposure on animals' copulatory organs can alter courtship behaviours, female survivorship, and play a nuanced role in mating dynamics. Perhaps differences among individuals in their history of interactions with microbes may explain some of the discrepancies between studies of female temperament, courtship and sexual cannibalism (Kralj-Fišer, Schneider, & Kuntner, 2013). Given the ubiquity and diversity of microbes in nearly all environments, and our increasing awareness of the importance that microbes play in animal ecology (Ezenwa et al., 2012; McFall-Ngai et al., 2013), studies like this echo the sentiment that animal behaviour and microbial ecology are intimately linked (Ezenwa et al., 2016). Spiders are becoming an increasingly enlightening test system for studying social dynamics (Pruitt & Avilés, 2018) and host–pathogen dynamics (e.g., Gilbert et al., 2016; Gilbert & Uetz, 2016), and their interaction (e.g., Keiser, Pinter-Wollman, et al., 2016). Particularly exciting future avenues of research will incorporate spider–microbe interactions into broader levels of biological organization, from physiology to community ecology (Eberhard, 2004; Mammola, Michalik, Hebets, & Isaia, 2017; Nentwig, 2013; Wise, 1995). We forecast that animal behaviour could play a role in unifying these diverse fields of study, and spiders may be useful test systems towards this end.

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