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Cuticular bacteria appear detrimental to social spiders in mixed but not monoculture exposure

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Abstract

Much of an animal's health status, life history, and behavior are dictated by interactions with its endogenous and exogenous bacterial communities. Unfortunately, interactions between hosts and members of their resident bacterial community are often ignored in animal behavior and behavioral ecology. Here, we aim to identify the nature of host–microbe interactions in a nonmodel organism, the African social spider *Stegodyphus dumicola*. We collected and identified bacteria from the cuticles of spiders *in situ* and then exposed spiders to bacterial monocultures cultures via topical application or injection. We also topically inoculated spiders with a concomitant “cocktail” of bacteria and measured the behavior of spiders daily for 24 days after inoculation. Lastly, we collected and identified bacteria from the cuticles of prey items in the capture webs of spiders, and then fed spiders domestic crickets which had been injected with these bacteria. We also injected 1 species of prey-borne bacteria into the hemolymph of spiders. Only *Bacillus thuringiensis* caused increased mortality when injected into the hemolymph of spiders, whereas no bacterial monocultures caused increased mortality when applied topically, relative to control solutions. However, a bacterial cocktail of cuticular bacteria caused weight loss and mortality when applied topically, yet did not detectably alter spider behavior. Consuming prey injected with prey-borne bacteria was associated with an elongated lifespan in spiders. Thus, indirect evidence from multiple experiments suggests that the effects of these bacteria on spider survivorship appear contingent on their mode of colonization and whether they are applied in monoculture or within a mixed cocktail. We urge that follow-up studies should test these host–microbe interactions across different social contexts to determine the role that microbes play in colony performance.

Key words: cuticular bacteria, sickness behavior, social spider, *Stegodyphus dumicola*.

Living in groups is one of the most successful and widespread phenomena in the animal kingdom and can be driven by a wide variety of selective pressures. Group living may help individuals subdue large and particularly profitable prey (Nentwig 1985), detect and evade predators with greater precision (Treherne and Foster 1981), or withstand abiotic conditions that would prove lethal to solitary individuals (Jones et al. 2007). Yet, the mode ecological interaction in which a social animal participates is *not* with predators, competitors, or even group mates, but rather internal, epidermal, and

environmental microbes (McFall-Ngai et al. 2013). Given the astounding ubiquity and diversity of bacteria, our understanding of zoology in general is reliant on our understanding of animal–bacterial interactions (McFall-Ngai et al. 2013). Host–microbe interactions encompass all types of biological relationships, from neutralism and commensalism to mutualism and parasitism (Casadevall and Pirofski 2000; Dethlefsen et al. 2007; Newton et al. 2010). Gregarious or group-living animals, however, are at especially high risk of accumulating potentially harmful microbial

pathogens and parasites because of their high conspecific density (Brown and Brown 1986) and the potential for co-feeding on shared prey items (Randolph et al. 1996; Roelke-Parker et al. 1996). Thus, to more completely understand the costs and benefits of sociality for any given social group, we must investigate the relationships between these individuals and their endemic microbiota.

The microbial communities associated with individuals in social groups are a product of individual experiences (e.g., diet) and interactions with the microbiota of group-mates. Animals can consume large amount of bacteria in their diet, either through obligate relationships (Fitt and O'Brien 1985; Hosokawa et al. 2008) or via inadvertent consumption (Del Fiol Federica and Riccardo 2007). The ingestion of bacteria, both pathogenic and benign, can have strong effects on host's health (Freitak et al. 2007), life-history (Ben-Yosef et al. 2008; Freitak et al. 2009), and behavior (Li et al. 2009; Sharon et al. 2010), and individuals who ingest bacteria can remain infected for long periods (Wallace et al. 2010). Additionally, the epidermis, which is essential in protecting the body from invading pathogens, is itself continuously colonized by bacteria (Grice and Segre 2011). This close association with a constantly changing cuticular microbiome can facilitate their passage into the body via body orifices or wounds where previously benign bacteria can become pathogenic (Cogen et al. 2008; Grice and Segre 2011).

Given the inherent complexity of cuticular microbial communities, studying host–bacterial interactions using single-species bacterial cultures might not produce ecologically relevant outcomes (Chandler et al. 2011). Notably, both classic microbiology and modern metagenomic techniques have demonstrated that the interaction between microbes and their hosts must be understood in the context of a microbial community (Hugenholtz and Tyson 2008; Sibley et al. 2008). Here, we test the effects of altering the cuticular bacterial communities of social spiders using both monocultures and concomitant cocktails containing mixtures of liquid bacterial cultures. Although spiders are uncommon subjects for studies of host–bacterial interactions, field observations (Henschel 1998), experimental studies (Gaver-Wainwright et al. 2011; Mascarelli et al., 2013), and metagenomic approaches (Vanthournout and Hendrickx 2015) have highlighted noteworthy interactions between spiders and associated microbes. Some data are observational, for example, colony-wide epizootic mycoses in the African social spider *Stegodyphus dumicola* (Henschel 1998) while others empirically test how intracellular endosymbiotic bacteria (e.g., *Rickettsia* and *Wolbachia*) influence population sex ratios, dispersal, and post-copulatory behavior (Goodacre et al. 2009; Gunnarsson et al., 2009; Vanthournout et al. 2011). In fact, a recent study demonstrated that the bacterial microbiome associated with the dwarf spider *Oedothorax gibbosus* are dominated by bacterial endosymbionts like *Wolbachia*, *Rickettsia*, *Cardinium*, and *Rhabdochlamydia*, prompting Vanthournout and Hendrickx (2015) to question to what degree other spider-associated bacterial communities may be restricted by endosymbionts. However, manipulative studies investigating the relationship between any spider, let alone social spiders, and its associated cuticular bacterial communities are unfortunately absent, given that the arthropod's hard cuticle represents the first physical line of defense against invading microbes (Brey et al. 1993; Vallet-Gely et al. 2008).

Here, we test the hypothesis that increasing the cuticular or internal bacterial load of naturally occurring bacteria will be deleterious to the host in the social spider *S. dumicola*. We exposed *S. dumicola* to bacteria isolated from the cuticles of spiders and prey *in situ*, and also fed them crickets which had been injected with bacteria collected from the cuticles of prey items in their capture web in

the wild. We also injected spiders with spider and prey cuticle-associated bacteria to test whether invasion into the body is a possible means by which resident cuticular microbes can become deleterious. For topical applications, we used both liquid bacterial cultures in monoculture and mixed into “cocktails” containing equal portions of different bacteria (see Table 1 for experimental designs). We then tracked the survivorship, behavior, and body mass of a subset of spiders daily to observe how exposure to these bacteria might shift their behavior and body mass.

Materials and Methods

Study species and behavioral assays

Stegodyphus dumicola is an old-world social spider that lives in age-structured, female-biased colonies of several dozen to a few hundred or more individuals throughout Southwestern Africa (Henschel et al. 1995; Henschel 1998; Avilés et al. 1999). These spiders, primarily adult females, cooperate in web construction, collective foraging, and alloparental care (Bilde et al. 2007). We collected 20 colonies of *S. dumicola* in the Northern Cape of South Africa, near Uptington (S28°27'24.9", E21°24'09.0") and the southern Kalahari Basin (S26°46'24.5", E20°37'56.4") in February 2014. Spiders were transported to the laboratory in their home colonies and then adult females were isolated into 1 ml plastic condiment cups containing a piece of chicken wire as a substrate to promote web-building. All spiders used in this study were adult females, and were fed one 2-week-old domestic cricket weekly.

Twice daily for 2 days, before experimentation, we determined the behavioral type (i.e., “personality”) of individual spiders by determining their individual “boldness,” defined as their latency to resume normal activity after an aversive stimulus (Sloan Wilson et al. 1994). To perform boldness assays, we placed a spider into a plastic container (12.5 cm × 13 cm × 3.5 cm), allowed it a 30 s acclimation period, and then administered 2 rapid puffs of air to the anterior prosoma with an infant nose cleaning bulb. This mimics the approach of a flying predator and represents an antagonistic stimulus (Riechert and Hedrick 1993; Barth and Höller 1999; Uetz et al. 2002). We then measured the latency for the spider to resume normal activity. Spiders that resume movement more rapidly (usually between 1 and 200 s) are deemed more “bold” while those that take longer are deemed “shy” (between 400 and 600 s) (Keiser and Pruitt 2014; Riechert and Hedrick 1993). This is a highly repeatable behavioral metric in this species (repeatability ~ 0.63; Keiser et al. 2014a, 2014b), is indicative of other important behaviors (i.e., collective foraging), and the boldness of group members is even correlated with the success of entire colonies in this and related species (Pruitt et al. 2013; Keiser et al. 2014a, 2014b). Before experimental treatments, we measured the prosoma width and mass of each spider with digital calipers and an analytical balance (Model P-114, Denver instruments, Bohemia, NY 11716), respectively.

Bacterial identification

Bacterial samples were collected from the cuticles of 20 adult female *S. dumicola* each originating from different source colonies by swabbing both the dorsal and ventral body surfaces with a sterile cotton swab *in situ* (i.e., directly after the spider was removed from the colony in the field) and then plating them onto separate LB agar plates. We similarly collected bacteria from the cuticles of 1 haphazardly selected prey item found in the same colonies' capture webs. These plates were sealed with parafilm and incubated under ambient temperature (30–37°C,

Table 1. Identify of bacterial isolates. All bacteria were isolated from the cuticles of live adult female *S. dumicola* and from the cuticles of unidentified Odonata found in the capture web *in situ*. Bacterial phyla are presented in parentheses

Bacterial ID	Source	Characteristics
<i>Bacillus thuringiensis</i> (Firmicutes)	<i>S. dumicola</i> cuticle (2 isolates)	Gram-positive, facultatively anaerobic, spore forming bacteria. Produces insecticidal crystal proteins (exo- and endotoxins) (Gill et al. 1992; Höfte and Whiteley 1989; Raymond et al. 2010).
<i>Pantoea</i> sp. (Proteobacteria)	<i>S. dumicola</i> cuticle	Gram-negative, facultatively anaerobic, some plant endophytes and epiphytes, some opportunistic human pathogens. Diverse environments. (Mandell et al. 2009).
<i>Microbacterium oxydans</i> (Actinobacteria)	<i>S. dumicola</i> cuticle	Yellow-pigmented, Gram-positive rods, aerobic, diverse habitats including clinical specimens. (Schumann et al. 1999; Gneiding et al. 2008).
<i>Planomicrobium</i> sp. (Firmicutes)	Prey: <i>Sparrmannia flava</i> beetle	Gram-positive, aerobic, motile, diverse habitats. (Luo et al. 2014).
<i>Kocuria</i> sp. (Actinobacteria)	Prey: Unidentified Odonata	Gram-positive, obligate aerobic (some facultatively anaerobic). Some opportunistic human pathogens (Savini et al. 2010).
<i>Arthrobacter</i> sp. (Actinobacteria)	Prey: Unidentified Odonata	Gram-positive obligate aerobic soil bacteria, many associated with plants (Jones and Keddie 2006).

Table 2. Experimental design and median time until death, in days, for each set of bacterial applications (injections, topical application, and consumption of bacteria). Treatments found to be significantly different from others (*within* an experiment and trial) via Kaplan–Meier Gehan–Breslow survival analyses are indicated with an asterisk.

Experiment	Trial #	Inoculation style	Bacteria used (source)	Median days until death		
Injections	1	Monocultures	<i>Arthrobacter</i> sp. (prey cuticle)	14		
			<i>Bacillus thuringiensis</i> (spider cuticle)	7*		
			<i>Microbacterium oxydans</i> (spider cuticle)	38.5		
			Phosphate-buffered saline (control)	17.5		
Topical applications	1	Monocultures	<i>Arthrobacter</i> sp. (spider cuticle)	77		
			<i>Bacillus thuringiensis</i> (spider cuticle)	73.5		
			<i>Microbacterium oxydans</i> (spider cuticle)	49		
			Sterile LB broth (control)	73.5		
			2	Monocultures	<i>Arthrobacter</i> sp. (prey cuticle)	28
					<i>Bacillus thuringiensis</i> (spider cuticle)	34
	<i>Kocuria</i> sp. (spider cuticle)	18.75				
	<i>Microbacterium oxydans</i> (spider cuticle)	14				
	<i>Pantoea</i> sp. (spider cuticle)	23.5				
	Sterile LB broth (control)	33.75				
	3	Cocktail	<i>Bacillus thuringiensis</i> (spider cuticle)	16*		
			<i>Microbacterium oxydans</i> (spider cuticle)			
<i>Pantoea</i> sp. (spider cuticle)						
Sterile LB broth (control)			27			
Consumption of prey-borne bacteria			1	Cocktail	<i>Planomicrobium</i> sp. (prey cuticle)	11
					<i>Kocuria</i> sp. (prey cuticle)	
	<i>Arthrobacter</i> sp. (prey cuticle)					
	Sterile LB broth (control)	5*				

following natural fluctuations where spiders were collected) for 2 days and then placed in a cooler at 4°C. Forty different bacterial colonies were isolated with a sterile inoculating loop (Thermo Fisher Scientific Inc., Waltham, MA 02451), re-plated and incubated as before 4 times to obtain monospecific bacterial samples. LB agar is nutrient rich medium, but can be relatively selective, and thus only a subset of the community can be cultured in this way. However, our aim here was to culture cuticular bacteria *in situ* that could then be used for manipulative experiments. Bacterial identification was performed on a subset of these isolated bacteria by PCR amplifying a 500 bp region of the prokaryotic 16S ribosomal DNA gene sequencing and MicroSeq® BLAST Software (SeqWright Genomic Services, Houston, TX 77054). Bacterial identification was verified using FinchTV BLAST software (Geospiza, Inc., Seattle, WA 98119).

We identified 6 species of bacteria; 3 from the cuticles of spiders: *Microbacterium oxydans*, 2 isolates of *Bacillus thuringiensis* isolated

from 2 spiders originating from 2 different localities (> 20 km distance between sites), and *Pantoea* sp.; and 3 from the cuticles of prey items: *Planomicrobium* sp., *Kocuria* sp. and *Arthrobacter* sp. (Table 1). For full BLAST report, see online [Supplementary Material S1 Text](#). Preliminary microbiome sequencing data also suggest that these bacteria are not uncommon in the bacterial communities associated with *S. dumicola* colonies, as they are present on colony silk, spider cuticles, and prey items across multiple populations (Keiser CN, unpublished data). Henceforth, we only use 1 of the 2 *B. thuringiensis* isolates for experimental inoculations. All bacterial strains were stored at –80°C in 25% glycerol stocks, and then revived on LB agar before experimentation.

Preparation of liquid cultures

We produced liquid bacterial cultures by isolating a single bacterial colony on the end of a sterile micropipette tip and placing it in 1 ml

of LB broth in a 14 ml polypropylene round-bottom tube. These liquid monocultures were incubated for 24 h at 30°C, and then vortexed to homogenize the solution. The micropipette tip was removed and the solution was transferred to a clean round-bottom tube. Thus, we produced 6 liquid bacterial monocultures which could then be used to create solutions containing mixtures of equal volumes of different bacterial strains (henceforth referred to as bacterial “cocktails”). Immediately before experimental inoculations, we created 2 bacterial cocktails containing equal portions of 3 different bacteria, 1 containing only exogenous bacteria collected from the spiders (*M. oxydans*, *B. thuringiensis*, and *Pantoea* sp.) and 1 containing bacteria collected from the cuticles of prey items (*Planomicrobium* sp., *Kocuria* sp., and *Arthrobacter* sp.). The average OD₆₀₀ of these bacterial cocktails (OD₆₀₀ = 1.25 ± 0.01) were not significantly different from the average OD₆₀₀ of each of the bacterial solutions therein (Average OD₆₀₀ = 1.31 ± 0.09; $F_{1,50} = 0.14$, $P = 0.71$; online [Supplementary Material S2 Text](#)).

Bacterial exposure

We exposed spiders to bacteria via 3 different techniques, in 3 different experimental blocks, to understand if the location of bacterial colonization is an important factor for host health. Spiders were exposed topically, in the body cavity via injection, and orally by feeding spiders crickets which had been injected with a bacterial cocktail. Throughout the duration of the experiment, individual spiders were maintained in isolation in their home containers (1 oz polystyrene plastic cup with a piece of chicken wire to facilitate web-building). Spiders were maintained at approximately 22°C under a natural 16:8 light:dark cycle.

Injections

To inject bacterial monocultures into spiders' hemolymph, spiders were CO₂ anesthetized for 30 s, secured on their dorsal side with 2-sided tape, and 2 μl of bacterial monoculture solutions was injected into their abdomen with a Hamilton micro-syringe directly posterior to the epigastric furrow. Fifteen spiders per treatment group were injected with monocultures of *M. oxydans*, *B. thuringiensis*, *Arthrobacter* sp., or a procedural control (2 μl of autoclaved phosphate-buffered saline). Since spiders have positive hemolymph pressure (Paul et al. 1994; Foelix 2010), injection techniques are likely to cause high procedural mortality via hemolymph loss. To account for this, spiders whose wounds did not have evidence of clotting and died within 12 h of the injection were removed from further analysis (Final sample sizes: *M. oxydans* $n = 10$, *B. thuringiensis* $n = 15$, *Arthrobacter* sp. $n = 9$, control = 12).

Topical applications

To apply liquid bacterial solutions topically to the spiders' cuticle, we placed each spider in a 14 ml round-bottom tube containing 2 ml of the bacterial solution and vortexed the solution at 1,500 rpm for 3 s using an MS-3 Basic vortex (IKA® Works, Inc., Wilmington, NC). This process disrupts the hydrophobic properties of hairs on the spider cuticle (Suter et al. 2004; Stratton and Suter 2009) and allows the solution to completely coat the subject. Spiders were treated with monocultures of *M. oxydans* ($n = 20$), *B. thuringiensis* ($n = 21$), *Pantoea* sp. ($n = 14$), *Arthrobacter* sp. ($n = 21$), *Kocuria* sp. ($n = 13$), or a control solution of autoclaved LB broth ($n = 19$). Topical applications of bacterial monocultures were carried out across 2 trials. In a third trial, spiders were also treated with a bacterial cocktail containing equal mixtures of the 3 exogenous spider

bacteria: *Microbacterium oxydans*, *B. thuringiensis*, and *Pantoea* sp. ($n = 30$).

Consumption of prey-borne bacteria

Lastly, to test the effects of consuming live bacterial cultures, we prepared a bacterial cocktail as before, but used only bacteria that were collected from prey items found in *S. dumicola* capture webs in the field: *Planomicrobium* sp., *Kocuria* sp., and *Arthrobacter* sp. We then injected 5 μl of the prey-bacteria cocktail into the abdomen of a recently frozen and thawed 2-week old domestic cricket ($n = 25$). Control crickets were injected only with LB broth ($n = 24$). The use of a dead cricket ensures that variation in prey behavior does not influence the likelihood that a spider will capture and consume the prey item. A single injected cricket was placed into the web inside each spider's home container. The spiders were starved for 2 weeks before experimentation to increase their hunger level and the likelihood they would consume the entire cricket. Although there was some variation in the time it took for spiders to begin consuming the crickets, all spiders consumed their cricket within a few hours and thus it is unlikely there would have been significant bacterial replication inside the cricket hemocoel.

For both bacterial cocktail treatments (topical inoculation of spider-cuticle bacteria and consumption of prey-borne bacteria) and their associated LB-control groups, we also measured the boldness and body mass of each spider daily after experimental inoculation. Finally, we checked spiders daily and recorded the date that each spider in every treatment group died after experimental inoculations.

Statistical analyses

Spider mortality was assessed using Kaplan–Meier Gehan–Breslow survival analysis (Mathew et al. 1999). We analyzed survivorship for the injected and topical applications until 50% of the spiders had died (i.e., the LT50). Full survival analyses (until all spiders had died) are available in [Supplementary Figures 1 and 2](#). Changes in individual boldness and body mass were analyzed using general linear mixed models with treatment, days since inoculation, and a treatment × days since inoculation interaction term. Individual spider ID and source colony ID were included as random effects in the model. We only analyzed post-inoculation boldness and body mass until 50% of 1 of the treatments had died, because anything beyond this reduction in sample size would likely violate homogeneity of variances across treatment groups. We performed post hoc q -value correction for false discovery rate to account for the possibility of type I error from multiple testing using the QVALUE software package in R. If the p -value resulting from a statistical test is smaller than its respective q -value, the conclusion is not likely the product of type I error (Storey 2002). All other statistical analyses were performed in JMP version 10 (SAS Institute Inc., Cary, NC, USA).

Results

Host mortality

When injected into the spiders' hemolymph, *B. thuringiensis* was the only bacteria that caused a significant increase in mortality relative to control spiders (Median time to death: 7 days; Gehan–Breslow Test statistic = 11.7, $df = 3$, $P = 0.008$, $Q = 0.07$; [Figure 1](#)). No bacterial monoculture increased spider mortality when applied to their cuticle in either trial (Trial 1: Gehan–Breslow test statistic = 1.5, $df = 3$, $P = 0.70$; Trial 2: Gehan–Breslow test statistic = 5.95, $df = 5$,

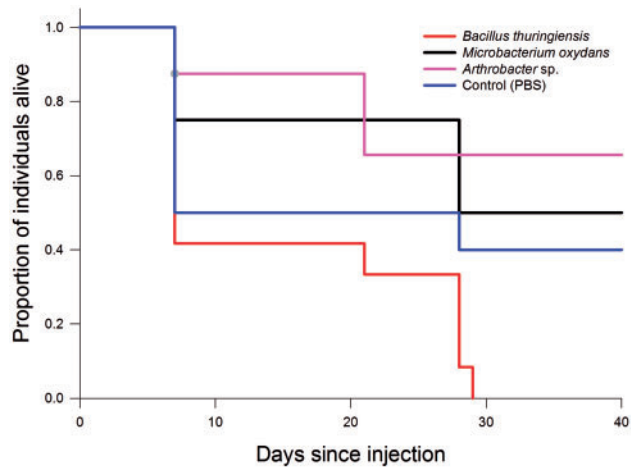


Figure 1. Spiders that were injected with $2\ \mu\text{l}$ of a *B. thuringiensis* liquid culture had increased mortality relative to spiders that were inoculated with any other monoculture or a control injection of phosphate buffered saline. Median time to death for spiders injected with *B. thuringiensis* was 7 days, compared to 38.5 days for *M. oxydans*; 14 days for *Arthrobacter* sp., and 17.5 days for the control spiders.

$P=0.31$). However, in trial 3, when spiders were inoculated topically with a bacterial cocktail containing 3 strains of spider-borne bacteria, the median time until death for was 73% sooner than that of control spiders (Gehan–Breslow test statistic = 9.37, $df=1$, $P=0.002$, $Q=0.08$; Figure 2). Unexpectedly, after spiders were fed crickets which had been injected with a cocktail of bacteria collected from prey cuticles *in situ*, they actually survived twice as long, on average, compared to spiders that ate control crickets (Gehan–Breslow test statistic = 8.3, $df=1$, $P=0.004$, $Q=0.01$; Figure 3).

Post-inoculation behavior and mass

The boldness of individual spiders was not altered by topical treatment with a bacterial cocktail of cuticular bacteria ($F_{19, 401.5}=1.36$, $P=0.14$) or by consuming prey-borne bacteria with a cricket meal ($F_{6, 163}=0.75$, $P=0.61$). However, spiders that were exposed to the bacterial cocktail lost more mass over the next 20 days as compared to LB-control spiders ($F_{19,403.9}=1.77$, $P=0.02$, $Q=0.05$). The change in mass of spiders that were fed prey-borne bacteria was not different from spiders that ate control crickets ($F_{6,79}=0.48$, $P=0.82$).

Discussion

The composition of the internal and epidermal microbiomes associated with an individual animal are fundamental to maintaining its health and modulating its life history and behavior (Zilber-Rosenberg and Rosenberg 2008; Ezenwa et al. 2012; McFall-Ngai et al. 2013). Thus, perturbations to that microbial milieu could potentially have profound, even fatal, consequences. Here, we found that isolating and re-inoculating resident bacteria from the cuticles of social spiders and their prey can have detrimental effects on the host, depending on the bacterial species and application method: relative to their respective control treatments, only *B. thuringiensis* was harmful when injected into the hemolymph and cuticular bacteria were harmful in when applied in concomitant cocktails. Thus, an increased bacterial load, or a potentially altered microbiome, can represent a potentially overlooked biotic stressor for the subjects of

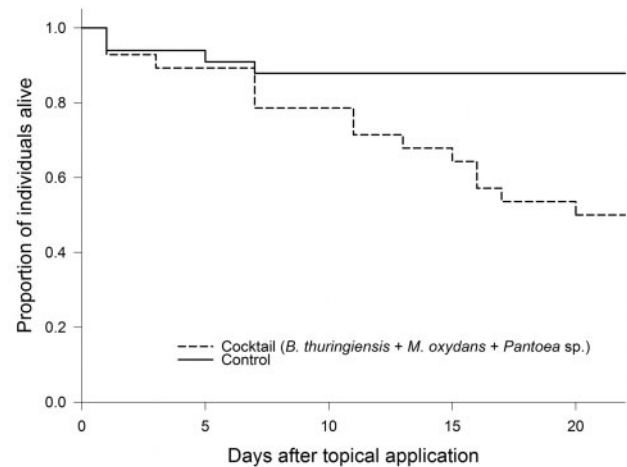


Figure 2. Spiders that were inoculated topically with a cocktail of 3 cuticular bacterial species collected from the cuticles of spiders *in situ* (*B. thuringiensis* + *M. oxydans* + *Pantoea* sp.) had increased mortality relative to spiders that had been inoculated with LB broth alone. The median time until death for spiders inoculated with the bacterial cocktail was 73% sooner than that of control spiders.

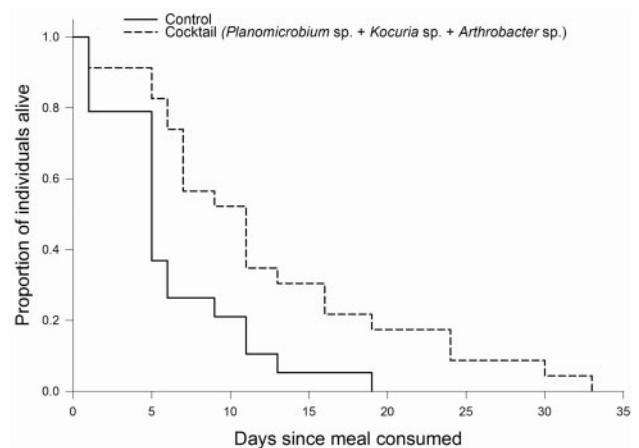


Figure 3. Spiders that ate crickets that had been injected with $5\ \mu\text{l}$ of a cocktail containing 3 bacterial species collected from the cuticles of prey items *in situ* (*Planomicrobium* sp. + *Kocuria* sp. + *Arthrobacter* sp.) lived twice as long, on average, compared to spiders that ate control crickets injected with LB broth. Median time to death for spiders that ate bacteria-treated crickets was 11 days compared to 5 days for the control spiders.

arachnological studies. We used different exposure methods (i.e., monocultures vs. cocktails) independently across experiments, and thus did not compare them statistically, but rather we indirectly infer from each result that mixed-inoculations were more harmful to spiders than monocultures. Further, because we used high concentrations of bacteria, and partially destructive inoculation techniques (injections and topical applications via vortexing), these data should be taken as a starting point for future studies which more closely probe the mechanisms of host–microbe and microbe–microbe interactions in this system.

Bacillus thuringiensis was the only bacterial species that caused significantly quickened mortality in individual spiders when injected into their hemolymph. It should be noted, however, that the mortality rate was relatively slow (~ 7 days for 50% mortality, ~ 30 days for all spiders to die). Further, this treatment regime used PBS as a

control solution instead of LB broth. We are confident that the effects observed in the *B. thuringiensis* treatment are not due to the presence of LB broth, since the other bacterial monocultures (*M. oxydans* and *Arthrobacter* sp.) were also grown and inoculated in LB broth—and these treatments were not significantly different from the control treatment of PBS. *Bacillus thuringiensis* is a common worldwide soil bacterium that is the source of the world's most common microbial insecticide (Lambert and Peferoen 1992) which also exhibits toxicity against some spider mites (Chapman and Hoy 1991). During its vegetative growth phase, *B. thuringiensis* multiplies normally but forms endospores when the environmental conditions become adverse. Concurrent with spore formation, *B. thuringiensis* produces insecticidal crystal proteins, and the ingestion of these proteins causes mortality in host insects (Höfte and Whiteley 1989; Gill et al. 1992; Schnepf et al. 1998). In fact, the virulence of *B. thuringiensis* can be dependent on the presence of resident enteric (gut-associated) bacteria in the host (Broderick et al. 2006, 2009; but see: Raymond et al. 2009).

The mode of action regarding *B. thuringiensis*-induced septicemia remains under debate, though studies have demonstrated that vegetative cells can escape the midgut into the hemolymph (Sutter and Raun 1967), and more recent experiments suggest that the intrahemocoelic route of infection can cause mortality and immune priming (Fedhila et al. 2002; Roth et al. 2009). We have not identified serotype(s) of *B. thuringiensis* associated with *S. duminicola*, which could be informative for both the topical and injected treatments (Hall et al. 1977), and for investigations into the mode of action of specific cry toxins against these spiders (Crickmore 2005).

No cuticular bacteria that we isolated, and re-inoculated topically in monocultures, had an effect on individual survivorship relative to control spiders. This could be due to many nonmutually exclusive mechanisms. For example, although perhaps unlikely here, the resident microbial community inhibited the growth of the new bacteria (Mans et al. 2009), or external immune defenses inhibited colonization, as the cuticle of other arthropods can play an active role in mounting an immune response (Brey et al. 1993). Recent research has identified cuticular antifungal substances in a subsocial crab spider (González-Tokman et al. 2014), suggesting that cuticular immune-related properties could be at play. Since these spiders were maintained in isolation after exposure to the bacteria, the role of allogrooming or social-facilitation of immunity are unlikely (Rosengaus et al. 1998, 1999; Traniello et al. 2002; Pie et al. 2005).

Interestingly, topical application of a cocktail containing equal parts of 3 bacterial species collected from the spiders' cuticles (*B. thuringiensis*, *M. oxydans*, and *Pantoea* sp.) caused reductions in body mass and faster mortality in spiders compared to a control inoculation. Admittedly, whether or not and by what mechanism this cocktail invades the body, establishes an infection, and causes increased mortality in *S. duminicola* is entirely unknown. Although increased bacterial diversity in experimental cultures can increase host invasibility (e.g., Hodgson et al. 2002; Ramsey and Whiteley 2009; Ramsey et al. 2011), the nature of the interactions between the 3 bacterial species in this experiment are currently unknown. In the gypsy moth, fatal septicemia associated with *B. thuringiensis* toxicity can depend on interactions with resident enteric bacteria (Broderick et al. 2006, 2009), though this often occurs after an oral route of infection for *B. thuringiensis*. Bacterial persistence in the body can also occur after passage into the body wall at other locations (Navon and Ascher 2000) and bacteria in this experiment could have entered the body via a number of other orifices (e.g.,

spiracles, Basset et al. 2000; esophagus or gut via grooming, Forster 1977). Further, we are unsure if our topical application technique caused minute dermal abrasions on the spiders, providing another point of entry.

Consumption of recently killed crickets injected with a cocktail of bacteria collected from prey items in *S. duminicola* capture webs (*Planomicrobium* sp., *Kocuria* sp., and *Arthrobacter* sp.) increased spider survivorship/lifespan relative to spiders that ate similarly sized control crickets. This suggests that interactions between spiders and bacteria associated with their diet could have important consequences, which represents a largely unexplored facet of spider foraging studies. One study, however, demonstrated that the consumption of ice-nucleating active bacteria endogenous to their prey can reduce the cold-tolerance of the common house spider *Achaearanea tepidariorum* (Tanaka and Watanabe 2003). Here, consumption of this bacterial cocktail either altered the nutritional resources in the prey in some way or actually had a positive impact on spider physiology. In the larvae of necrophagous flies, the presence of bacteria on their food is beneficial either because they consume the bacteria directly or their presence makes nutrients more available to the larvae (Thompson et al. 2013). Although, others have demonstrated that the presence of nonpathogenic bacteria in the diet can trigger an immune response, slow development time, and reduce body mass in the cabbage looper (Freitag et al. 2007). Given that many of the most prominent insect–pathogen interactions, including *B. thuringiensis* and its diverse hosts, begin with ingestion (Vallet-Gely et al. 2008), further studies should address the consequences of consuming prey-associated bacteria for spiders and their broader foraging ecology (Wise 1995).

These experiments, though exploratory in nature, represent a novel investigation into the relationship between increased bacterial load and/or altered cuticular bacterial communities and host survivorship in spiders. Given that we used exogenous bacteria from spider cuticles and prey items, collected *in situ*, our results could garner real-world insights for the natural history of these social spiders. This species in particular exhibits some fascinating traits that warrant future research on host–bacterial interactions. Extremely high genetic relatedness within colonies via serial inbreeding (Johannesen et al. 2002; Smith et al. 2009), cooperative maternal care via regurgitation of food (Salomon and Lubin 2007), and juvenile consumption of parental spiders (i.e., “gerontophagy”; Seibt and Wickler 1987) all represent practical aspects of microbial transmission among individuals. Follow-up experiments should utilize next-generation sequencing to achieve a more complete view of the cuticular bacterial communities associated with individuals and colonies, especially characterizing and comparing the bacterial communities associated with *S. duminicola* spiders living socially versus solitarily. These studies will be instrumental in investigating the consequences of individual bacterial infections on the performance and success of entire colonies.

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Supplementary Material

Supplementary material can be found at <http://www.cz.oxfordjournals.org/>

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