

Research



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Community ecology

Social spider webs harbour largely consistent bacterial communities across broad spatial scales

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Social animals that live in domiciles constructed from biomaterials may facilitate microbial growth. Spider webs are one of the most conspicuous biomaterials in nature, yet almost nothing is known about the potential for webs to harbour microbes, even in social spiders that live in dense, long-term aggregations. Here, we tested whether the dominant bacteria present in social spider webs vary across sampling localities and whether the more permanent retreat web harbours compositionally distinct microbes from the more ephemeral capture webs in the desert social spider, *Stegodyphus dumicola*. We also sampled spider cuticles and prey items in a subset of colonies. We found that spider colonies across large spatial scales harboured similar web-associated bacterial communities. We also found substantial overlap in bacterial community composition between spider cuticle, prey and web samples. These data suggest that social spider webs can harbour characteristic microbial communities and potentially facilitate microbial transmission among individuals, and this study serves as the first step towards understanding the microbial ecology of these peculiar animal societies.

1. Introduction

Many animals live in domiciles like nests, hives or retreats that are constructed from biomaterials that may facilitate microbial growth [1,2]. Social arthropods, in particular, often live in dense aggregations inside these structures, where the accumulation of microbes can be amplified due in part to increased conspecific density [3]. To combat this phenomenon, some social animals use antimicrobial secretions (e.g. leaf-cutting ants; [4]), volatile fumigants (e.g. termites; [5]) or hygienic behaviours (e.g. honeybees; [6]) to reduce microbial growth in general or foster the growth of protective microbes within the nest.

Spider webs are one of the most conspicuous animal-produced structures in nature, yet almost nothing is known about the potential for webs to harbour microbes, apart from some studies documenting the antimicrobial activity of isolated silk [7–10]. Social spiders live in dense aggregations and interact with prey, predators and parasites on a shared web [11,12]. These traits make social spiders a useful model for studying host–microbe interactions in taxa with some unique social traits and nest attributes. Though field reports have noted that social spider colonies often succumb to idiopathic epizootics [11], a comprehensive inventory of the microbial communities associated with spider colonies remains missing.

Stegodyphus dumicola is a southwestern African social spider that lives in colonies of up to several hundred individuals that cooperate in collective foraging, web-building and shared care of offspring [13]. These spiders live inside dense, three-dimensional silken ‘retreat webs’ replete with tunnels, with two-dimensional ‘capture webs’ of cribellate silk extending outwards from the

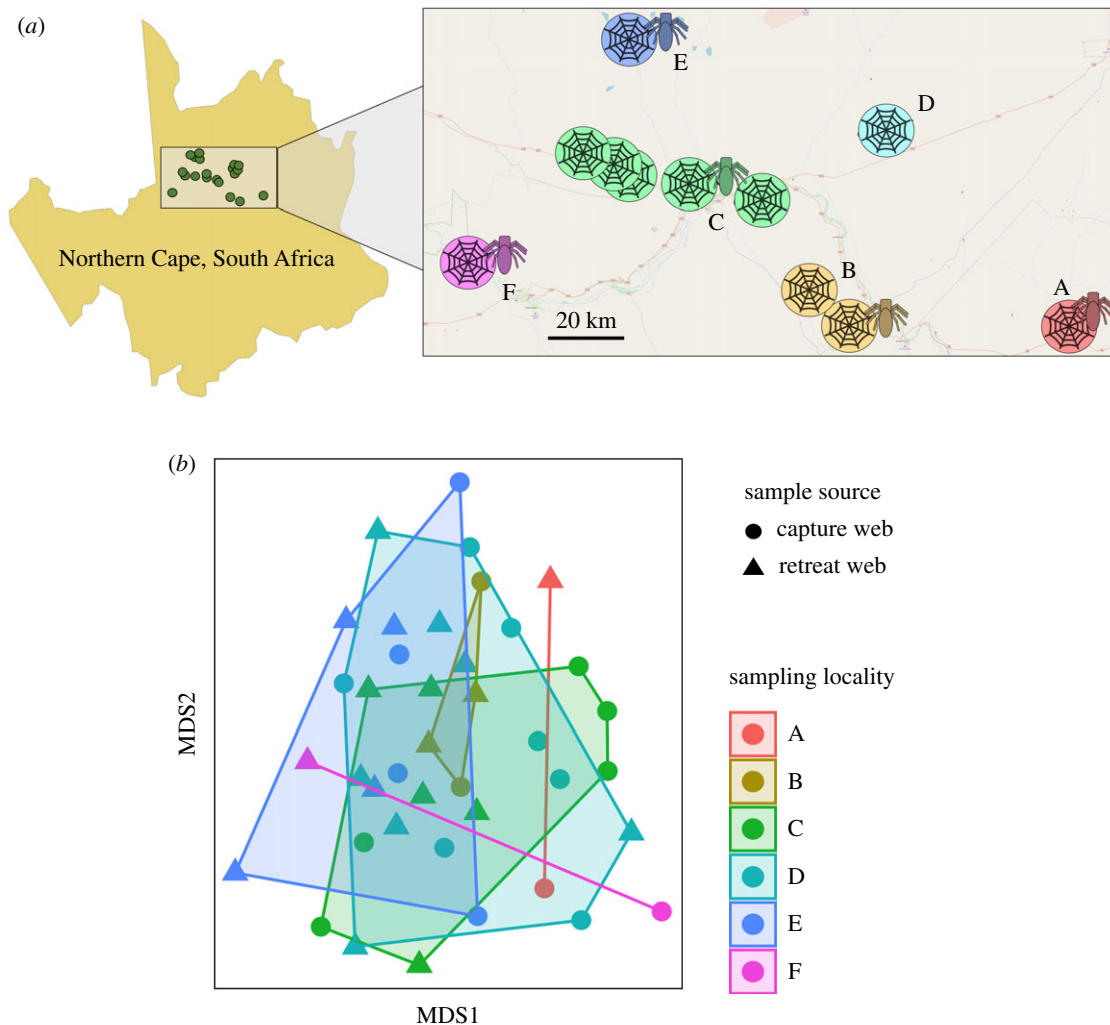


Figure 1. (a) Location of collection sites across the Northern Cape of South Africa. Spider icons represent locations where samples were collected from spider cuticles and prey items in addition to silk samples. (b) Ordination plot of microbiome composition in 40 web samples from six sampling localities. Bacterial community composition was highly consistent among spider colonies across large spatial scales. (Online version in colour.)

retreat, which are regularly repaired and renewed [14,15]. When prey are attacked in the capture web, they are often transported to the retreat for group feeding. As the colony grows, these prey carcasses are often incorporated into the silken walls of the retreat where they remain indefinitely, potentially facilitating microbial growth and microbial transmission to spider hosts. Here, we address two questions: (1) what are the dominant bacteria present in social spider webs, and do they vary across sampling localities? (2) Does the more permanent retreat web harbour microbes compositionally distinct from the more ephemeral capture webs?

2. Material and methods

We sampled the bacterial communities of 20 *S. dumicola* colonies from over an 11 000 km² area in the Northern Cape of South Africa in January 2014 (figure 1a). Colonies were distributed among six sampling sites (labelled A–F) that were separated by 13–217 km (mean distance between sites: approximately 85 km). We collected two silk samples from each of 20 colonies: a 1 cm³ sample removed from the colony retreat using a sterilized razor, and the entire capture web was collected by placing a sterile wooden rod into the centre of the web and spinning the rod to collect silk (see electronic supplementary materials for details). Samples were placed directly into 2 ml of MoBio Lifeguard™ preservation solution (MO BIO Laboratories, Carlsbad, CA 92010).

From five colonies separated by approximately 135 km on average, we also collected samples from the cuticle of an adult female spider in the colony by vortexing the spider in 2 ml of MoBio Lifeguard for 10 s. We also collected a fresh prey item in the capture web which was stored as a whole specimen in MoBio Lifeguard (electronic supplementary material, figure S1). Samples were placed on ice at irregular intervals during initial storage and transport, but samples from each locality experienced this intermittent chilling equally. Upon receipt in the laboratory, they were stored at –80°C.

Bacterial community sequencing was performed by Second Genome (South San Francisco, CA 94080) by using the MoBio PowerSoil® (Carlsbad, CA) kit optimized for high-throughput processing (see electronic supplementary materials for details). DNA concentrations in all extracts were quantified via the Qubit® Quant-iT dsDNA Broad-Range Kit (Invitrogen, Life Technologies, Grand Island, NY). The V4 region of the 16S ribosomal RNA gene was amplified with primers including Illumina (San Diego, CA) adapters and indexing barcodes. Samples were PCR-amplified using two V4 fusion primers, concentrated using a solid-phase reversible immobilization method and quantified via electrophoresis (Agilent 2100 Bioanalyzer®). The pool of 16S V4 enriched samples was loaded into a MiSeq® reagent cartridge for cluster formation, the amplicons were sequenced for 250 cycles with custom primers designed for paired-end sequencing. A negative control was included during the entirety of sample processing but did not yield any sequences. Sequences were quality-filtered, dereplicated and denoised to produce

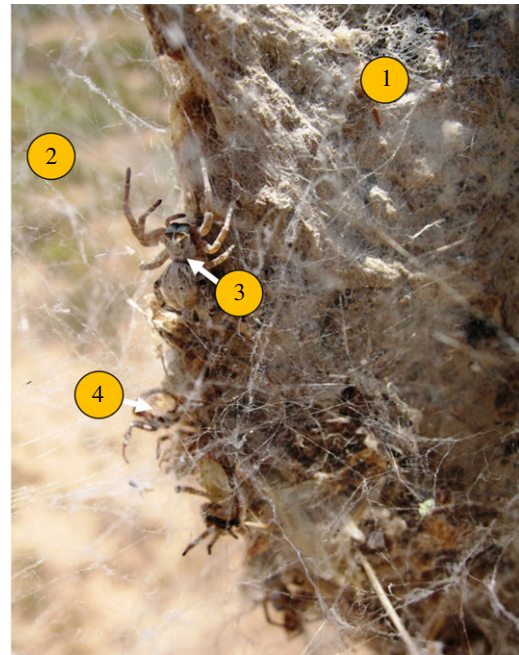
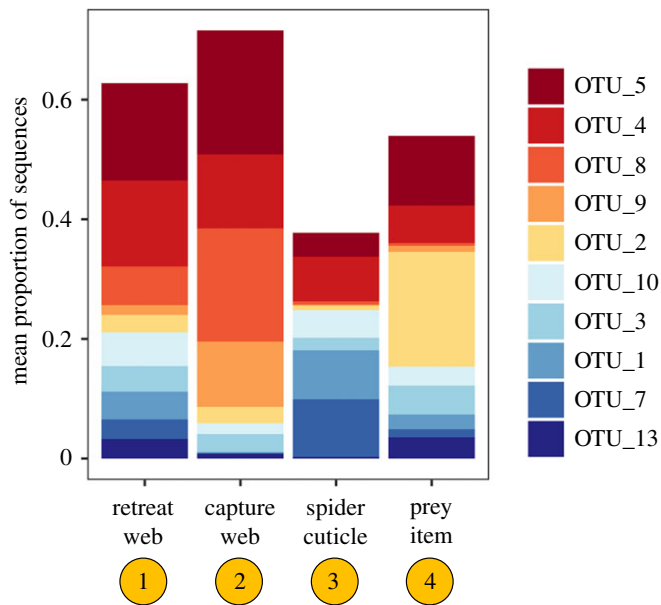


Figure 2. Relative abundances of the top-10 exact sequence variants for each sample type (1–4). There was a minor differentiation in overall bacterial community structure between spider cuticle, prey and contrasting web samples. Overall, these data suggest a high degree of overlap in the composition of dominant bacterial taxa among sample types. (Online version in colour.)

amplicon sequence variants using UNOISE [16] following previous studies (e.g. [17]).

Taxonomy was assigned to amplicon sequence variants (hereafter, OTUs) using the RDP classifier [18] against the GreenGenes database [19]. The resulting OTU table was rarefied to 9413 sequences/sample, and Bray–Curtis dissimilarities were used for permutational MANOVA tests and ordinations. We estimated the coverage of our samples using the bias-corrected Chao estimator, as implemented by the EstimateR function in the vegan package [20]. Across samples, our sequencing libraries are inferred to account for a median 75% of total community diversity. It is possible that rarer bacterial taxa reported from low-biomass samples like ours may originate from contamination via handling or laboratory reagents [21,22], so we also ran identical permanova analyses using only the top 20 most abundant taxa.

In addition to bacterial community sampling, we also collected bacteria using culture-based methods to assess whether OTUs captured in our community sampling were viable bacterial populations. We used sterile cotton swabs to sample the body surfaces of *S. dumicola* spiders, directly after the spider was removed from the colony, at the same time as bacterial community sampling, and plated the samples directly onto separate LB agar plates. We similarly collected bacteria from the cuticles of one haphazardly selected prey item in the same colonies' capture web. LB plates were sealed with parafilm and incubated under ambient temperature (30–35°C, following natural fluctuations where spiders were collected) for 2 days and then stored at 4°C. Bacterial colonies were isolated with a sterile inoculating loop and re-plated four times to obtain monospecific bacterial cultures. Bacterial identification was performed by PCR amplifying a 500 bp region of the prokaryotic 16S ribosomal DNA gene sequencing (SeqWright Genomic Services, Houston, TX 77054). Bacterial identification was verified using FinchTV BLAST software (Geospiza, Inc., Seattle, WA 98119). LB agar is a relatively selective growth media for bacteria, and will only capture a small portion of the bacterial community. However, we were not aiming to culture the entire bacterial community, but rather use culture-based methods to see whether we could recover some of the same bacteria as were identified in our community sequencing.

3. Results and discussion

Spider colonies across sampling sites, even across large spatial scales, harboured similar web-associated bacterial communities (permanova: pseudo- $F_{5,34} = 1.15$, $p = 0.17$; figure 1b). This consistency in microbial communities is notable, given that our sampling efforts covered 20 sites in an 11 000 km² area. Additionally, our negative control did not yield any sequences, and our analyses including only the top 20 most abundant taxa yielded identical results (electronic supplementary material, figure S2). Although we cannot identify the drivers of these results, we propose several non-mutually exclusive explanations which may be tested in future studies: perhaps the environmental microbial communities across this area of arid veld are highly consistent, meaning that social spider webs would be colonized by similar microbial consortia regardless of locality. Spider silk may also act as a habitat filter for certain types of microbes, and thus only a subset of the available microbial communities is able to colonize. Indeed, both the retreat silk and capture web silk of *S. dumicola* have weak antibacterial effects against *Bacillus thuringiensis* [9] and the silk of other spiders has been shown to inhibit the growth of other bacteria [7]. At least a portion of the web-associated microbes could originate from the spiders themselves, which would similarly explain the large degree of overlap between samples collected from silk and from spider cuticles (see below; figure 2). Lastly, *Stegodyphus* spiders consume a diversity of prey types [23,24]. If prey availability is similar across our six sampled sites, then microbes originating from prey could play a role in the consistency of web-associated microbes. While some prey items sampled from different colonies harboured compositionally similar microbiota, others had highly distinct community profiles (electronic supplementary material, figure S1). Future work is necessary to determine which bacteria are resident in webs, spiders and their prey, and which are transiently acquired from the environment. Collecting soil and plant

Table 1. The top 10 most abundant OTUs obtained from our sequencing results. Classifications were made using RDP SeqMatch.

OTU ID ^a	phylum	class	family	genus ^b	mean proportion across samples
OTU_5	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	various	0.165
OTU_4	Firmicutes	Bacilli	Enterococcaceae	<i>Enterococcus</i>	0.121
OTU_8	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	<i>Pantoea</i>	0.102
OTU_9	Firmicutes	Bacilli	Bacillaceae	<i>Bacillus</i>	0.052
OTU_2	Firmicutes	Bacilli	Staphylococcaceae	<i>Staphylococcus</i>	0.043
OTU_10	Firmicutes	Bacilli	Exiguobacteraceae	<i>Exiguobacterium</i>	0.037
OTU_3	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	various	0.036
OTU_1	Firmicutes	Bacilli	Enterococcaceae	<i>Enterococcus</i>	0.029
OTU_7	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	various	0.025
OTU_13	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	various	0.02

^aSee electronic supplementary materials for OTU sequences.

^bVarious refers to multiple equally good matches to different genera within the Enterobacteriaceae.

samples at each collection site would help in this regard and help point towards potential functional underpinnings of these microbial communities [25]. There was a significant, though weak difference in overall bacterial community structure between spider cuticle, prey and contrasting web samples (permanova: pseudo- $F_{3,46} = 2.35$, $R^2 = 0.13$, $p = 0.001$; figure 2). This effect remains with both Bray–Curtis dissimilarities (which account for relative abundance differences) and Jaccard distances (which account for presence/absence only). This pattern is likely driven by differences between prey samples and spider cuticle samples and differences between the arthropod samples and the silks. Despite these community-level differences, there is substantial overlap in microbial composition among sample types even at the level of exact sequence variants (figure 2; see electronic supplementary material, figures S3 and S4 for more detailed plots). Thus, microbes are likely to be readily shared among colony silks and colony constituents and could arrive via prey intercepted in the capture web.

Although we set out to test two specific questions regarding the degree of overlap in microbial communities across sample types and across sampling localities, this research was also partly exploratory, aiming to examine the microbial inventory of this animal society, which is growing as a model system in behavioural and evolutionary ecology [26]. Most notably, we found *Rickettsiella* sp. at appreciable abundances (greater than 10%) in a prey item, the retreat web and from a spider cuticle. *Rickettsiella* sp. are common intracellular pathogens of arthropods [27] that are present in several spider species [28] though other endosymbionts are found in *S. dumicola* [29]. We also found *Snodgrassella alvi*, a highly specific gut symbiont of social bees including honeybees [30], in a retreat web sample at 1.07% relative abundance. These could arise via extracellular DNA from the remnants of partially consumed prey that were in the silk during collection, especially given that spiders undertake extra-oral digestion. Although *Sn. alvi* almost certainly cannot colonize spiders, many of the capture and retreat webs were inundated with honeybees and finding this strain in a retreat web sample suggests a potential route for other prey-associated microbes to colonize silks and potentially spiders.

Five of the seven bacterial OTUs we isolated using culture-based methods were also recovered in our bacterial community sampling (electronic supplementary material, table S1). Two of these bacteria, *Bacillus* and *Pantoea* were the third and fourth most abundant genera (table 1), respectively, and previous experiments have shown that these bacteria can be collected from spider colonies in multiple populations, experimental exposure can have detrimental effects on spider health [31,32] and these spider- and silk-borne isolates can be transmitted among individuals inside colonies [33–35].

These findings suggest that animal domiciles constructed from biomaterials, like silk, can harbour characteristic microbial communities and may facilitate microbial transmission among conspecifics and between prey and predator species. In cases where there is overlap in generations, nests may facilitate the transgenerational transmission of symbiotic microbes without direct vertical transmission. This may be particularly important for arthropod societies which are at risk of experiencing large-scale mortality events (e.g. honeybees; [36]). Although it remains unknown whether this is the case in social spiders, this study serves as the first step towards an understanding of the microbial ecology of these peculiar animal societies.

Ethics. These collections were conducted on invertebrate animals and thus are not under the governance of the US National Research Council, though we adhered to the guidelines of the ASAB/ABS Guidelines for the care and research of animals wherever possible.

Data accessibility. The raw data associated with this manuscript are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.3282cq2> [37].

Authors' contributions. C.N.K. and J.N.P. designed the study; C.N.K. collected the samples; samples were sequenced by SecondGenome and T.J.H. analysed the data. All authors contributed to preparing the manuscript. All authors gave final approval for publication and agree to be accountable for all aspects of the work.

Competing interests. We declare we have no competing interests.

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