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Research Article

Temporal and spatial microbiome dynamics across natural populations of the social spider *Stegodyphus dumicola*

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One sentence summary: High temporal stability of the social spider microbiome, but nest death is often preceded by a massive increase in bacterial numbers.
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Abstract

Host–symbiont interactions may form obligatory or facultative associations that are context dependent. Long-term studies on microbiome composition from wild populations should assess the temporal and spatial dynamics of host–microbe associations. We characterized the temporal and spatial variation in the bacterial microbiome composition in six populations of the social spider *Stegodyphus dumicola* for 2.5 years, using 16S rRNA gene amplicon sequencing of whole spiders. Individuals within a nest exhibit highly similar microbiomes, which remain stable over several generations and are not predictably affected by seasonal variation in temperature or humidity. This stability in nest microbiome is likely due to social transmission, whereas drift-like processes during new nest foundations explain variation in host microbiomes between nests. This is supported by the lack of obligate symbionts (i.e. no symbionts are present in all spider individuals). Quantitative PCR analyses showed that the bacterial load of individual spiders is stable in healthy nests but can increase dramatically in perishing nests. These increases are not driven by specific bacterial taxa but likely caused by loss of host immune control under deteriorating conditions. Spider nests show an annual survival rate of approximately 45%, but nest death is not correlated to microbiome composition, and the bacteria found in *S. dumicola* are not considered to be high virulence pathogens.

Keywords: 16S amplicon, bacterial load, Kaplan–Meier, nest survival, symbiont, time series

Introduction

All animals live in close association with microbes, and the impact of these microbial communities on host fitness is becoming increasingly apparent (Douglas 2009, Feldhaar 2011, Ferrari and Vavre 2011, Engel and Moran 2013, McFall-Ngai et al. 2013). Host–microbe interactions range from obligate mutualistic symbioses to facultative associations (Aanen et al. 2002, Oliver et al. 2003, Jousselin et al. 2009, Hammer et al. 2017, Duplouy and Hornett 2018, Su et al. 2021). To understand the different types of host–microbe relationships, we need to understand the specificity and functional significance of host–symbiont interactions, and the roles of the host vs. the environment in shaping host microbiomes. Gut microbiomes are, e.g. often shaped by host diet, which can lead to substantial variation in microbiota among host individuals (Oliver et al. 2003, Shoemaker and Moisander 2017, Vandeputte et al. 2021). Host microbiomes can also be shaped by other environmental parameters causing spatial variation in microbiome composition (Smith et al. 2015, Epstein et al. 2019, Haydon et al. 2021). Temporal or spatial variation in the host microbiome provides opportunity for development of specific host–symbiont interactions, and may be indicative of beneficial functional associations in certain contexts or local conditions. If local host–symbiont combinations mediate improved host performance, they may have potential to facilitate rapid host responses to local conditions (Smith et al. 2015, Kolodny and Schulenburg 2020, Moeller and Sanders 2020, Houwenhuyse et al. 2021). However, our ability to assess host–microbiome relationships, functional outcomes, and evolutionary trajectories is limited by scarce knowledge on how microbiomes fluctuate temporally and spatially in wild populations. Temporal studies of octocoral (Octocorallia) microbiomes revealed broad microbial diversity with seasonal differences and identified 'core microbiomes' that are independent of the environment and potentially convey important functions to their hosts (Haydon et al. 2021). In contrast, reef location played a larger role than seasonality in driving microbial community composition in *Acropora* coral species (Epstein et al. 2019), a pattern that would be expected if local conditions favor certain host–symbiont associations. The skin microbiome in the frog *Rana sylvatica* is important in the immune defence against pathogens, and an analysis of temporal variation revealed differences across seasons (Douglas et al. 2021), such a pattern could possibly suggest that the microbiome responds to changes in pathogens (Smith et al. 2015). These

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examples illustrate the importance of investigating temporal and spatial variation in host–microbiome associations for developing and substantiating hypotheses on the type of benefits bacterial symbionts might provide in particular environments.

In this paper, we investigate temporal and spatial variation in host–microbiome associations in the African social spider *Stegodyphus dumicola* Pocock, 1898 (Eresidae), which occupies a wide geographical range across Central and Southern Africa (Majer et al. 2013). Groups of closely related spiders live in communal nests containing tens to hundreds of individuals (Avilés 1997), who collaborate on reproduction, brood care, and foraging (Lubin and Bilde 2007). The combination of close proximity and intimate social interactions, which include mixing and exchange of digestive fluids under extra-oral communal ingestion of prey and regurgitation feeding of offspring (Lubin and Bilde 2007), provides ample opportunity for microbiome transmission among nest mates. Indeed, individuals that occupy the same nest exhibit highly similar microbiomes (Busck et al. 2020). Populations are founded by dispersing single mated females that initiate a new nest together with her offspring, and because existing nests can undergo fission, and thereby split, nests occur in aggregations that often descend from a single colonization. The turnover rate of nests is high due to low survival rate of individual nests and frequent extinction–colonization events (Crouch and Lubin 2001, Bilde et al. 2007). Notably, there is no premating dispersal, and reproduction, therefore, occurs among highly related offspring, which leads to a strictly inbreeding mating system (Settepani et al. 2017). The combination of a strict inbreeding mating system, female-biased sex ratio, and frequent extinction–colonization events has severe consequences for population genetic diversity and structure: genetic diversity is extremely low within populations, and despite restricted gene flow between populations, population extinction–recolonization causes remarkably low genetic divergence among populations (Lubin and Bilde 2007, Settepani et al. 2014, 2017, Vanthournout et al. 2018, Bechsgaard et al. 2019). This type of host metapopulation dynamics may have implications for host–microbiome dynamics (Mihaljevic 2012, Miller et al. 2018), but potentially also for the spatial distribution of the microbiome composition.

The population genetic structure in *S. dumicola* is not mirrored in the spider's bacterial microbiome. *Stegodyphus dumicola* have low diversity microbiomes, dominated by one or two bacterial symbionts, on average making up 93% of the microbiome (McNaughton's dominance index of 0.93±0.13, Busck et al. 2020). Individual spiders occupying the same nest have almost identical microbiome compositions, but this similarity does not extend to the population level, where nests from the same population can have different dominating symbionts (Busck et al. 2020). This pattern raises the intriguing question of what causes and maintains substantial variation in the microbiomes of different nests within a population given the lack of genetic divergence of the spider host? Several scenarios are compatible with the observed pattern of different dominating symbionts within nests in close proximity: First, temporal and/or spatial differences in environmental conditions (seasonality or microhabitats; Smith et al. 2015, Epstein et al. 2019, Haydon et al. 2021), could cause shifts in microbiome composition and possibly in the services that microbial symbionts provide to the host. A second possibility is that different microbiome compositions result from a series of transient infections (Chrostek et al. 2017, Hammer et al. 2017), possibly by pathogens of relatively low virulence, which dominate a nest for a period of time until suppressed by host immune response, bacteriophages, or new infections by competing pathogens. Given the lack of migration of spiders between nests (Lubin and Bilde 2007, Settepani et al. 2017), the recurrent acquisition of these symbionts (pathogens) would have to rely on environmental vectors (Busck et al. 2020). A third explanation could be incompatibilities among host genetics and microbiome composition (Duplouy and Hornett 2018, Tabrett and Horton 2020), which requires a genetic polymorphism most probably maintained by balancing selection. This is unlikely, given the extremely low nuclear genetic diversity within and across *S. dumicola* populations (Settepani et al. 2017). However, previous studies have demonstrated polymorphisms in mitochondrial haplotypes among nests (Johannesen et al. 2002, 2009, Smith et al. 2016), which could lead to interactions (compatibilities) between mitochondrial haplotypes and microbiome composition, a phenomenon observed in mice (Hirose et al. 2017, Yardini et al. 2019). A fourth option proposed in Busck et al. (2020) is that a set of obligate symbionts exist but their relative abundance in host individuals fluctuates randomly by a drift-like process; either within the lifetime of a spider, or perhaps more likely, during female dispersal and nest fission, when one or a few individuals form a new nest. A first step towards assessing these different explanations is to determine temporal and spatial variation in the spiders' microbiome together with its total size (bacterial load) within nests and populations, as the current knowledge of the *S. dumicola* microbiome is limited to relative abundances of symbionts measured in spiders sampled at single time points.

In this study, we asked: what is the temporal variation of the microbiome of nests (family groups) within and between generations; what is the spatial variation in nest microbiomes within and between populations; and, which of the four scenarios lined out above can best explain the observed microbiome patterns?

To assess the temporal and spatial microbiome dynamics we conducted an *in situ* time-series analysis by continuous sampling of six natural populations for a period of 2.5 years. Spiders occupying the same nest have a similar microbiome composition (Busck et al. 2020), which provides a unique opportunity to assess temporal variation in the microbiome by sampling individuals from the same nest within and across generations. We analyzed temporal variation by implementing 3 months sampling intervals and determining microbiome composition of three individuals sampled from each nest by whole-body 16S rRNA gene amplicon sequencing. These data enabled analyses of whether microbiome composition within a nest changed over host life span or with time of year (seasonality). We also investigated the survival probability of nests over the sampling period, as nest extinction patterns provide insights into potential transmission dynamics. Finally, we used quantitative PCR (qPCR) to analyze the bacterial load in nests that were perishing, to assess whether changes in the abundance of total bacteria or of specific symbionts were associated with nest mortality. This was done by analyzing individuals collected from nests that were found dead on the subsequent sampling event, enabling us to identify whether changes in bacterial load precede nest mortality.

Materials and methods

Sampling sites

We identified six sampling sites, representing six *S. dumicola* populations, along a North–South and East–West gradient in Namibia (Otavi, Windhoek, Stampriet, Betta, and Karasburg sites) and Botswana (Palapye site; Fig. 1A; Table S1, Supporting Information). The sites were selected to cover a range of temperature and humidity gradients (Fig. 1B) and different vegetation types (Table S1

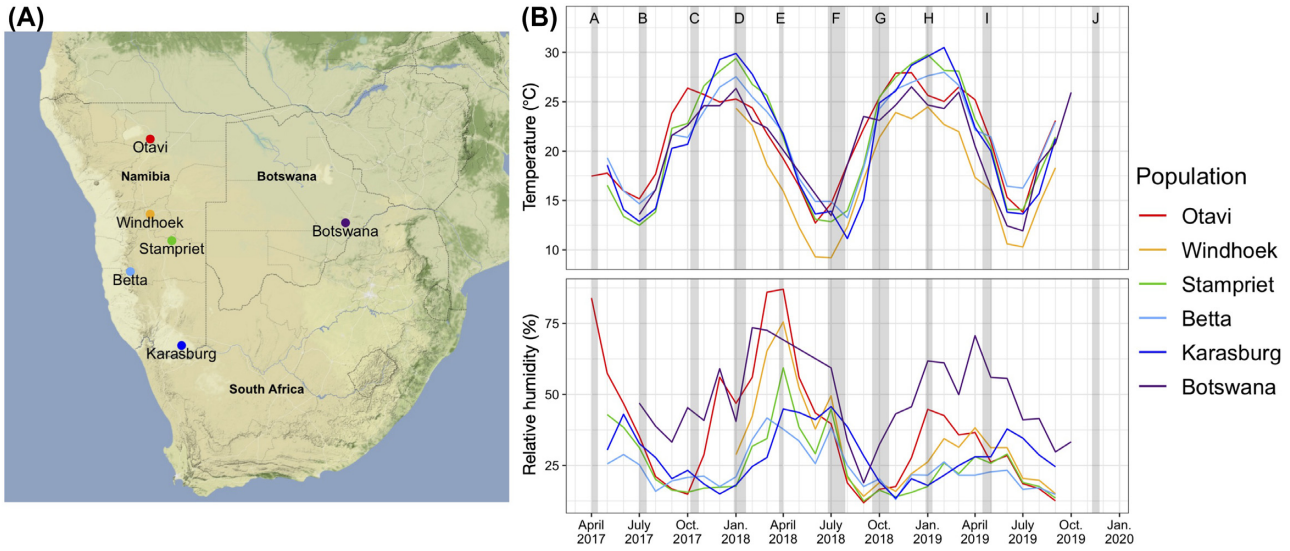

Figure 1. (A) Sampling locations of *S. dumicola* populations **(B)** Monthly averages of temperature and relative humidity for each population. Data collected by several iButtons in each population placed directly under the spider nests.

and Figure S1, Supporting Information). The nests of all except the Windhoek and Otavi sites were next to the road in natural livestock grazing areas. The Windhoek site was on a housing estate in a seminature reserve, about 20 km east of Windhoek. The Otavi site was on farmland.

Sample collection

Stegodyphus dumicola spiders were collected approximately every 3 months in the period from April 2017 to November 2019. In each population, the same 5–10 nests were sampled at each time point, while a replacement nest was included if a nest died or disappeared during the study period (Table S2, Supporting Information). Three spiders were sampled from each nest at each time point with as little damage as possible to the nest, by vibrating the capture web, or by gently squeezing the nest, until spiders came out. Total body length (in mm from front of prosoma to the tip of abdomen) was measured before individuals were placed in ATL buffer (Qiagen, Hilden, Germany) in microcentrifuge tubes, and placed in a portable freezer. For exact sampling times and other sample data see Table S3 (Supporting Information).

Environmental data collection

iButton™ temperature/humidity loggers (Maxim Integrated Products) were placed directly below several nests in each population, logging nest temperature and relative humidity every hour. During each sampling trip, the data were extracted from the iButtons using a dual Blue Dot™ Receptor and OneWireViewer software (Maxim Integrated Products).

DNA extraction and 16S rRNA gene amplicon sequencing

DNA was extracted from 722 whole spiders of 66 nests using DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol (Purification of total DNA from animal tissue—spin-column protocol). The spiders extracted in Denmark (Table S4, Supporting Information) were first homogenized using liquid nitrogen and a sterile pestle, whereas spiders extracted in Botswana were homogenized in the ATL buffer without the use of liquid nitrogen. Fully grown female spiders (larger than 1 cm) were extracted using double reagent volumes and two spin-columns per spider. A blank, i.e. tubes with no sample added, were included throughout the DNA extraction procedure. DNA extracts were used for both 16S rRNA gene amplicon sequencing and qPCR.

16S rRNA gene amplicon libraries were prepared according to Illumina's 16S Metagenomic Sequencing Library Preparation guide, with slight modifications (see Table S4 (Supporting Information) and Supplementary methods for PCR details), using Bac 341F and Bac 805R primers to amplify the variable regions V3 and V4 (Herlemann et al. 2011). The DNA extraction blanks as well as PCR negatives were included for sequencing. For each run, a pooled library containing amplicons from all samples was sequenced on a MiSeq desktop sequencer (Illumina) according to the provided preparation guide.

Determination of bacterial load using qPCR

We defined the bacterial load as the number of 16S rRNA gene copies normalized to the number of copies of a specific spider gene, i.e. two separate qPCR assays were run per sample to quantify bacterial and spider gene copy numbers (see details below). qPCR was only run on a subset of the samples: six long-lived nests were chosen for qPCR of all time points, plus 15 nests that died during the course of our study were analyzed at the last two time points.

The ratio between bacterial 16S rRNA gene copies and spider gene copies was measured by amplifying a 167 bp fragment of bacterial 16S rRNA gene using the universal primer pair Bac908F/Bac1075R (Mateos-Rivera et al. 2018) and a 147 bp fragment of a conserved *Stegodyphus* gene using the primer pair 5F/5R (Settepani et al. 2016). A single plasmid containing target DNA sequences for both assays was synthesized (Eurofins) and used as a qPCR standard. Any given sample was analyzed with both qPCR assays on the same day using the same dilution series (10^1–10^7 copies/μl) of the standard curve, thereby ensuring comparable standard curves for the two assays. All PCR reactions were run in triplicate in 20 μl reaction volumes containing 10 μl LightCycler 480 SYBR Green I Master (Roche), 2 μl Bovine Serum Albumin (10 μg/ml), 1 μl of forward primer (10 pmol/μl), 1 μl of reverse primer (10 pmol/μl), 4 μl dH_2O, and 2 μl template DNA. qPCR as-

says were run on the Stratagene Mx3005P qPCR system with the following conditions: 5 min initial denaturation at 95°C, 45 cycles of (30 s denaturation at 95°C, 30 s annealing at 59°C, 20 s elongation at 72°C, 15 s acquisition at 80°C for the spider fragment, and 78°C for the bacterial fragment) followed by a final melting curve with 95°C for 1 min and ramping temperatures 59–95°C, 30 s at each degree.

16S rRNA gene amplicon analysis

All analyses were done in R v 3.6.1 (R Core Team 2019). Sequences were trimmed to remove barcodes and primers using cutadapt (Martin 2011). Each sequencing run was processed separately for quality filtering, denoizing, and paired-end merging using the R package 'DADA2' v. 1.12.1 (Callahan et al. 2016) with filter settings maxEE = (2, 2), truncLen = 230, and truncQ = 2 to identify Amplicon Sequence Variants (ASVs). Data from the separate sequencing runs were then merged for chimera finding and classification using DADA2 and Silva SSU reference database number 132 (Quast et al. 2013). ASVs were filtered to a minimum length of 400 and to exclude chloroplasts, mitochondria and nonbacteria. The sequencing data from the DNA extraction blanks and PCR negatives were used to decontaminate the sample data. ASVs had to be five times more abundant in the samples than in the controls, otherwise they were considered contaminants and removed from the data. Samples with fewer than 7000 remaining reads were excluded from further analysis.

For any analysis involving diversity measures, amplicon reads were subsampled to a common depth of 7000 reads (with seed = 42). For everything else, amplicon reads are reported as fractions of all reads per sample. If subsampling was used, it will be clear from the figure caption. All remaining analyses and visualization of community data were done with custom R scripts, using published packages such as 'ggplot2' v. 3.3.0 (Wickham 2009) and 'vegan' v. 2.5.6 (Oksanen et al. 2019).

Survival analysis

Kaplan–Meier analysis was run on a dataset of 77 nests. Not all nests included in the survival analysis were used for 16S rRNA gene amplicon sequencing, and several nests used for sequencing were excluded from the survival analysis because sampling of these nests had stopped without a sure observation of natural death (e.g. nests gone because of road-side maintenance; Figure S2, Supporting Information). The analysis was run in R using the package 'survival' v. 2.44.1.1 (Therneau 2015) and plotted using 'survminer' v. 0.4.6 (Kassambara et al. 2019). Nests still alive at the end of the study were right censored. Nest sampling times were corrected to days since sampling start, meaning the first sampling of each nest was set to T_0, even though sampling starts were staggered in real time (Figure S2, Supporting Information).

Data availability

All sequences are available from Genbank SRA under the BioProject accession number PRJNA656699. The supplementary material contains ASV (Tables S6 and S7, Supporting Information), taxonomy (Table S5, Supporting Information), qPCR (Table S8, Supporting Information), and metadata (Table S2 and S3, Supporting Information) tables. Voucher specimens of *S. dumicola* collected at sporadic intervals from the five Namibian populations and from Botswana were deposited at the National Museum of Namibia, Windhoek, and at the Ditsong National Museum of Natural History, Pretoria, respectively.

Results and discussion

Stegodyphus dumicola have low diversity microbiomes dominated by six bacterial groups

We identified 16 324 bacterial ASVs in the 722 *S. dumicola* spiders originating from 66 nests. Detailed sample information and read statistics, as well as ASV sequences and classifications are available in the supporting information (Tables S2, S3, and S5–S7, Supporting Information). Only 1074 ASVs appeared in five or more spiders, and no ASV appeared in all spiders sampled, confirming our previous conclusion that no obligate symbiosis with any specific bacterium exists in *S. dumicola* (Busck et al. 2020).

The individual spider microbiomes had an average Shannon diversity index of 0.66 ± 0.71 (mean ± SD), which is low compared to other spiders (Zhang et al. 2018) and to eusocial arthropods (Raymann et al. 2017, Benjamino et al. 2018, Chua et al. 2018). Note that two factors in our study have likely increased the estimated alpha diversity reported here: first, the denoizing algorithm we used to group reads into ASVs allows for a higher taxonomic resolution than the clustering algorithms used in many comparable studies (Callahan et al. 2017). This is exemplified in the two main *Mycoplasma* ASVs in this study (ASV_1 and ASV_3), which differ by only one out of 426 base pairs, and would have been grouped together in the same operational taxonomic unit by a clustering algorithm. Second, many arthropod microbiome studies are based on surface sterilized animals (Zhang et al. 2018), or even dissected guts (Raymann et al. 2017), while we have sampled whole spiders that were not surface sterilized. Therefore, direct comparisons to other studies should be done with caution.

Corroborating our previous finding (Busck et al. 2020), spiders within a nest had highly similar microbiome composition with the same dominant ASV (Figure S3, Supporting Information). A total of 10 ASVs belonging to six taxonomic groups (Spirochaetaceae, *Mycoplasma*, *Spiroplasma*, *Diplorickettsia*, Rickettsiaceae, and Weeksellaceae) made up more than 90% of all 16S rRNA gene amplicons sequenced in this study. The ASVs classified as either Spirochaetaceae or *Borrelia* have more than 99.5% BLAST identity with the *Stegodyphus*-specific endosymbiont *Candidatus* Arachnospira stegodyphi (Busck et al. 2020). The *Mycoplasma* have more than 99.5% BLAST identity to sequences from Busck et al. (2020) and they form a sister group to *Mycoplasma* found in scorpions (Bolaños et al. 2019). Scorpions also have *Spiroplasma* with 100% sequence identity to the sequences found in *S. dumicola* (Elmnasri et al. 2018, Bolaños et al. 2019). The *Diplorickettsia* and Rickettsiaceae have more than 99% sequence identity to intracellular symbionts of other spiders, ticks, and insects (Mediannikov et al. 2010, Yu and Walker 2015, White et al. 2020). Finally, the Weeksellaceae had 96% identity to sequences from a shrimp gut. In summary, all of the most abundant taxonomic groups detected in *S. dumicola* are host-associated endosymbionts, and many of them are related to arachnid or insect associated bacteria; their function in *S. dumicola* is unknown.

A high McNaughton's dominance index (DMN), which is the sum of the relative abundance of the two most abundant ASVs in a sample, of 0.93 ± 0.12 (Table S2, Supporting Information) confirms the previous finding that individual spiders are dominated by one or two ASVs (Busck et al. 2020). Some of the dominant taxonomic groups were represented by 2–5 ASVs but all shared > 99% sequence identity within the group. There were other ASVs (classified as *Acaricomes* and *Rickettsiella*) dominating individual microbiomes, but they were not as prevalent as the six dominating ASVs mentioned above (Fig. 2). The six main dominant taxonomic groups were previously found in South African *S. dumicola*

Figure 2. Heatmap of relative abundances of ASVs. The 20 most abundant ASVs in populations and nests of *S. dumicola* across all sampling times. Only nests sampled three or more times are included. Each column represents the nest average at a single sampling event (1–3 individual spiders). Bottom panel plots average size (in mm) of sampled spiders. White cells signify relative abundances < 0.1%.

populations (Busck et al. 2020), several even identical at the ASV (~400 bp) level, which confirms the presence of the same symbionts across large geographical distances. This tight association could arise if these ASV's provide essential functions to the host (Aanen et al. 2002, Jousselin et al. 2009), or if the spiders are repeatedly infected with the same low-virulent ASV's though some sort of vector, as proposed for social spiders by Busck et al. (2020). Transmission could be mediated by vectors such as infected prey, parasites, or host plants (Bright and Bulgheresi 2010, Chrostek et al. 2017, Hammer et al. 2017). Another possibility is that the observed pattern is explained by metapopulation dynamics through repeated colonizations by hosts and their associated microbiota, implying that ASV composition and turnover is influenced by spatial and host community components (Mihaljevic 2012, Miller et al. 2018).

The spider microbiome exhibits a clear nest structure with high temporal stability

We found that microbiome composition of a substantial proportion of nests showed high temporal stability (Figs 2 and 3). The dominant ASVs in a nest rarely changed with time, and there were no clear cyclical changes indicative of seasonal effects (Fig. 2). Using Bray–Curtis (BC) dissimilarity to compare individual spider microbiomes (Fig. 3), we found high similarity between nest-mates sampled at the same time point (median BC dissimilarity of 0.087), supporting the previous findings of highly similar microbiome composition within nests (Busck et al. 2020). The BC dissimilarity index was slightly but significantly higher when comparing nest-mates sampled at different time points (median of 0.132). However, neither within-nest BC dissimilarities nor the dominating taxa showed clear generational shifts (Figs 2 and 3; Figure S3, Supporting Information) and were only weakly correlated to the time between sampling points (Spearman $R = -0.047$, $P = .0026$; Figure S4, Supporting Information), indicating that the microbiome composition was undergoing random fluctuations rather than directed change. BC dissimilarities were much higher (median of 0.947) between nests of the same population sampled at the same time point, and almost as high as BC dissimilarities observed between populations (median of 0.994). These data show that nest identity is a better predictor of microbiome composition than either sampling time, generation, or population (Fig. 3); this rejects the hypothesis that differences in environmental conditions (e.g. seasonality, microhabitats, or temperature variation) cause shifts in the overall microbiome composition, however, individual ASVs

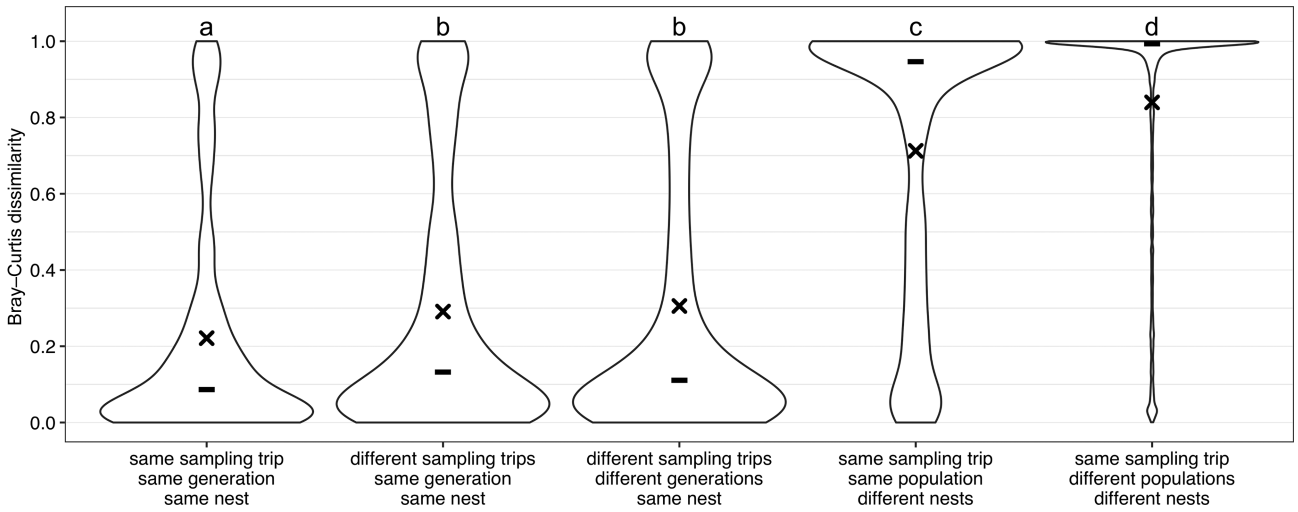

Figure 3. Violin plot of Bray–Curtis dissimilarity between microbiomes of individual spiders grouped at different levels of comparison. Data were subsampled to 7000 reads per sample and filtered to only include ASVs that were present in at least five samples and have a relative abundance above 0.1%. Significant differences between groups at $P < .01$ were determined using pairwise Wilcoxon rank sum test (BH adjusted) and are indicated by letters above the violins. '-' marks the median and 'X' marks the mean.

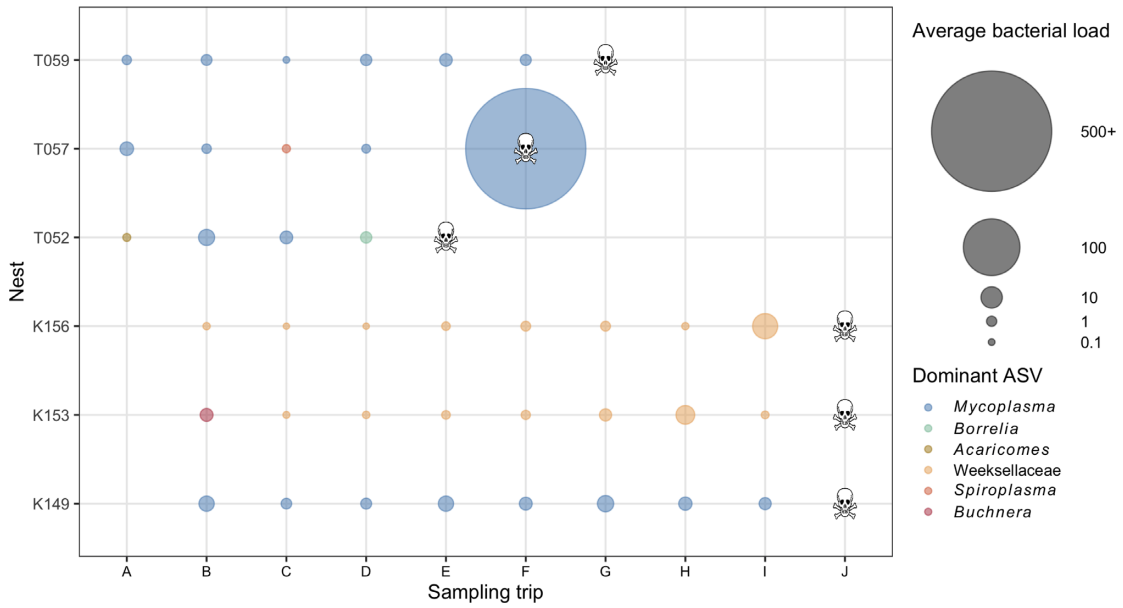

Figure 4. Bacterial load (16S rRNA gene copies/spider gene copy) in six selected nests across sampling trips. Total $n = 99$. Each circle represents the average bacterial load in one to three spiders sampled from a given nest at a given sampling trip. Circle area is scaled to indicate bacterial load and circle color indicates taxonomic group of dominant ASV. Skull and crossbones symbols indicate the nests being found dead (or actively dying in the case of T057). White spaces indicate no sampling. T052, T057, and T059 belong to the Otavi population. K149, K153, and K156 belong to the Karasburg population.

could still correlate with these parameters. Likewise, there is no indication that microbiome composition is driven by a series of transient infections with bacterial pathogens.

The transmission routes of the *S. dumicola* endosymbionts are currently unknown, but the spiders have an annual life cycle with several months overlap between generations and extensive brood care, including regurgitation feeding of juveniles and matriphagy (Salomon and Lubin 2007). This likely facilitates high-fidelity social transmission (Bright and Bulgheresi 2010, Koch and Schmid-Hempel 2011, Wang and Rozen 2017, Drew et al. 2021), as exemplified by social transmission of the cuticular bacterium *Pantoea* sp. in *S. dumicola* (Keiser et al. 2016a,b). In addition, communal feeding (Schneider and Bilde 2008) makes it likely that symbionts are not only passed from mother to offspring, but also continuously passed between nest-mates both within and between generations. While social transmission remains to be proven for the dominating endosymbionts in *S. dumicola*, it would, together with the fact that the spiders are highly philopatric and rarely immigrate into other nests (Lubin and Bilde 2007), provide an explanation for how the microbiome can remain stable within a nest for generations (years), even in the absence of obligate symbiotic relationships. Additional studies are required to assess evidence for vertical transmission, as seen e.g. in honey bees, termites, and aphids (Aanen et al. 2002, Jousselin et al. 2009, Drew et al. 2021), or for mixed transmission routes, e.g. in the red firebug (Kaltenpoth et al. 2009) or in earthworms (Paz et al. 2017).

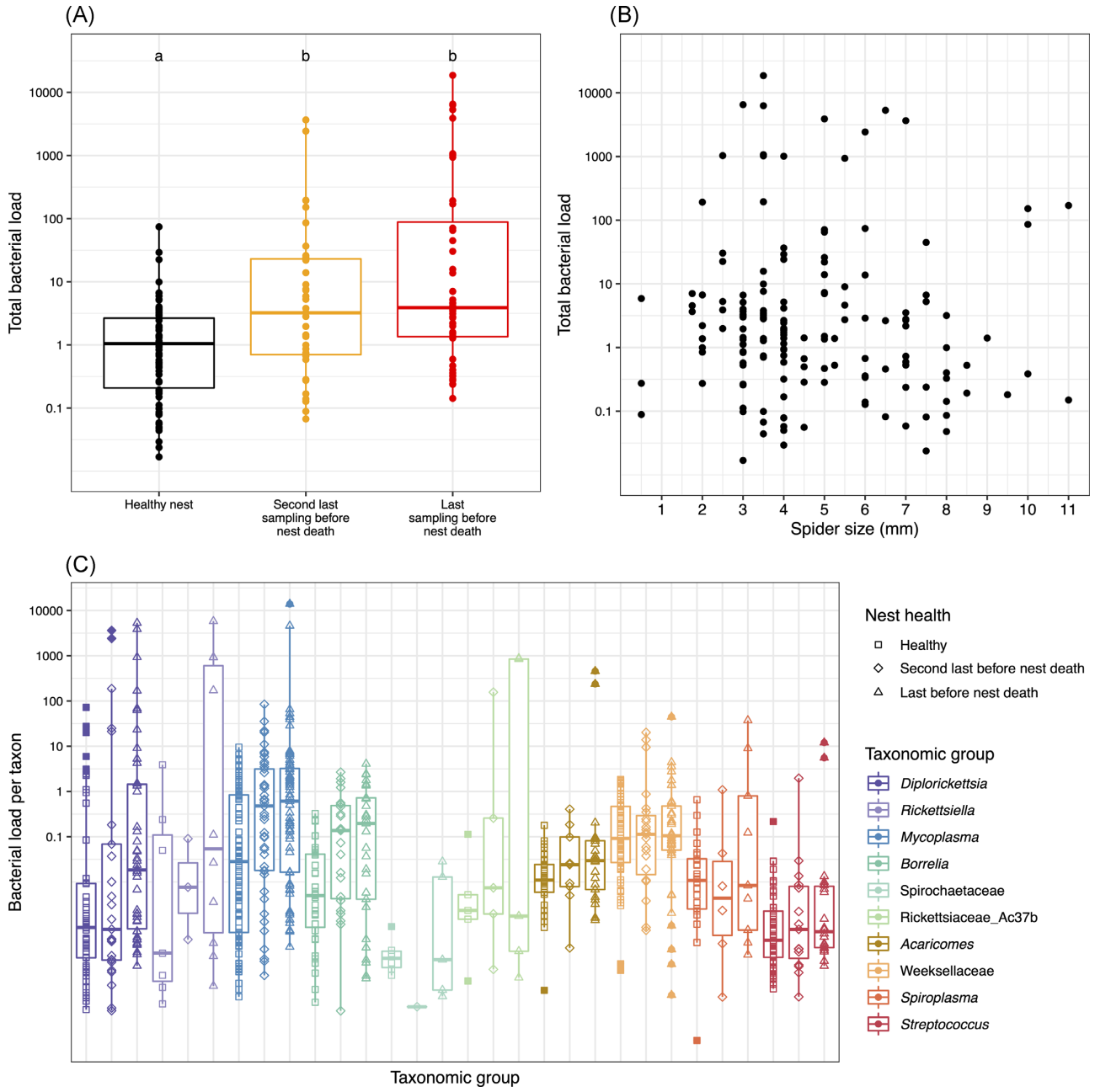

Figure 5. Correlations with total and taxon-specific bacterial load (16S rRNA gene copies/spider gene copy, $n = 151$). **(A)** Total bacterial load in individual spiders sampled from nests at different timepoints before natural nest death. A healthy nest is defined by being found alive at the following two sampling trips. Significant differences between groups at $P < .01$ were determined using pairwise Wilcoxon rank sum test (BH adjusted) and are indicated by different letters above boxes. **(B)** Total bacterial load versus spider body size. There is no correlation between load and size (Pearson's product moment correlation, $P = .658$). **(C)** Taxon-specific absolute abundance of the 10 most abundant taxa. Symbols show if the nests were healthy, or if they would die within the next two sampling events.

Small variation in individual microbiome composition within nests may lead to high variation between nests

In contrast to the finding of clear temporal stability in nest microbiome composition, we document substantially higher dissimilarity between nests found in close proximity (within populations, Fig. 3). This is interesting, given the spiders' high genetic similarity between nests (Lubin and Bilde 2007, Settepani et al. 2017) and the similar environmental and climatic conditions within a population. We suggest that the founding of new nests offers opportunities for shifts in microbiome composition. New nests founded by individual dispersing mated females (Lubin and Bilde 2007) that carry a set of symbionts, may enable minor random variation in individual microbiome composition within a nest to lead to different relative representation or fixation of symbionts in newly founded nests by drift-like processes. The founding female spider will propagate her individual microbiome to her offspring, giving rise to a new nest microbiome that can be slightly different compared to her natal nest-microbiome. Although members of the same nest generally harbor the same microbiome, we did find exceptions. In a small fraction of nests, the microbiome composition differed between individuals, either collected at the same

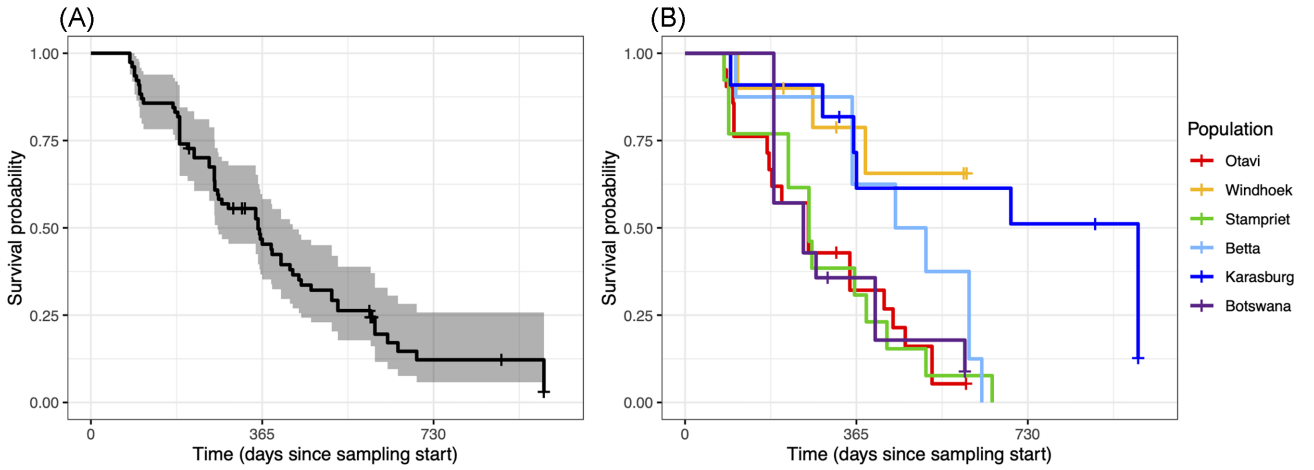

Figure 6. Kaplan–Meier survival analysis of nests (t_0 = sampling start). Nests that were still alive at the end of the study period are right censored (indicated as vertical tick marks on the graphs). **(A)** all nests in study ($n = 77$) **(B)** nests separated into populations.

time or during different sampling trips (Fig. 3, seen as high BC dissimilarity; Figure S4, Supporting Information). If individuals from these heterogeneous nests disperse by nest fission, it will likely result in neighboring nests with different microbiomes. Likewise, founding of new nests through long-distance ballooning dispersal of single mated females would also lead to different microbiome compositions of different nests within the population. This explanation of variation in ASVs among nests propagated by symbiont-carrying females, thereby differs from obligate associations and co-founding events by hosts and their associated obligate symbionts (Aanen et al. 2002, Jousselin et al. 2009).

There is a small possibility for individually dispersing females to join existing nests, although genetic data suggest this is rare (Johannesen et al. 2002). This could lead to fusion of individuals with different microbiomes within the nest and thus give rise to the variation in microbiome composition observed in a small subset of nests (Fig. 3). However, because of social transmission of symbionts, this process is more likely to homogenize the spiders' microbiome composition within and between nests rather than causing differences between nests. It is noteworthy that the variation in microbiome composition between nests belonging to the same population is almost as high as the differences found among populations (Fig. 3), thus suggesting that migration of spiders between nests is very rare.

Bacterial load increases prior to nest death

The bacterial load (number of bacterial 16S rRNA gene copies normalized to a conserved spider gene) in samples from six selected nests from two populations showed only minor temporal changes (Fig. 4), with a median load of 1.2 and maximum and minimum loads of 15.1 and 0.06, respectively (omitting the outlier T057). Bacterial load did not fluctuate predictably with season (Fig. 4), in contrast to what has been shown in bees (Kešnerová et al. 2019), nor was it correlated to body size (Fig. 5B). One extreme outlier (nest T057, trip F) had a bacterial load of 12 444 ± 8717 (Fig. 4; Table S8, Supporting Information). Interestingly, this nest was in the process of perishing, with spiders in bad condition and only few individuals present, and the nest was visually deteriorating. To investigate this connection further, we determined the bacterial load in spiders from 17 additional nests, which perished during our study, focusing on the last two sampling times for a given nest. In seven nests, spiders displayed high bacterial loads (above 50) at the last sampling, and in four of these nests, the increased bacterial load was already apparent, although less pronounced, at the penultimate sampling (Fig. 5A; Figure S5, Supporting Information). As our sampling interval was approximately 3 months, we do not know how soon after the last sampling a given nest perished, or whether nest death is always preceded by a major increase in bacterial load. Nevertheless, our data suggest an increased bacterial load was connected to subsequent nest death.

While it is tempting to interpret this increased bacterial load as if pathogenic bacteria cause nests to perish, the increase in bacterial load was not tied to any specific ASV (Fig. 5C; Figure S5, Supporting Information), and occurred regardless of the microbiome composition of nests. Furthermore, the relative abundances of symbionts were overall not affected, compared to previous sampling times (Figure S6, Supporting Information). In the absence of specific bacterial pathogens, the dramatic increase in bacterial load might be caused by loss of host immune control (Ayres and Schneider 2008). In such cases, bacteria which under some circumstances are beneficial or commensal can become opportunistic pathogens, possibly by invading parts of their host, which were previously protected by the immune system. It is also possible that the immune system gets hampered by an external pathogen, challenging the ability to control bacterial load (Hillyer 2016, Dustin and Ioannis 2017). We cannot be certain what could cause a putative impairment of immune function in *S. dumicola*, as indicated by the increase in bacterial load, but we suspect that fungal infections in the nest or in the spiders themselves may play a role (Henschel 1998). Fungi are known pathogens of arthropods (Lovett and St Leger 2017), and their ability to spread between nests as spores would allow them to be much more virulent than the nonspore forming obligately host-associated bacterial symbionts we have identified (Hughes et al. 2008).

Nest survival rate is low and not correlated to microbiome composition

Of the 42 nests sampled at the initiation of the study (sampling trips A and B), only 2 (5%) were still active and healthy at the end of the 2.5-year sampling period (Figure S2, Supporting Information). In order to keep all populations represented, new nests were added to the study when existing nests perished. Such high turnover of social spider nests has also been reported in different populations of *S. dumicola* and the other African social spider

species *S. mimosarum* Pavesi, 1883 (Crouch and Lubin 2001, Bilde et al. 2007). We were unable to estimate the full lifespan of nests, since we do not know the age of the nests at the initiation of sampling. However, Kaplan–Meier survival analysis showed a median nest survival time of 356 days from sampling start, and an average survival rate of 45% in the first year sampled (Fig. 6A). Social spider nests from Karasburg, Betta, and Windhoek showed higher survival rates compared with nests from Otavi, Botswana, and Stampriet (Fig. 6B). Otavi and Botswana experienced higher relative humidity (Fig. 1B) than the other populations, but this was not the case for Stampriet. Therefore, we cannot determine if relative humidity affects survival rates. It has, however, been proposed that fungal infections are more severe under higher humidity (Henschel 1998) and specifically prominent at Otavi (Nazipi et al. 2021), generating the testable prediction that elevated mortality in areas with higher humidity is driven by fungal infections.

Conclusions

Individuals of the African social spider, *S. dumicola* that occupy the same nest have highly similar microbiomes with a high temporal stability. However, when comparing microbiomes in spiders from neighboring nests, they are almost as different as in spiders from nests of different populations. Temporal and spatial analyses suggest that microbiome variation is not predictably affected by seasonal variation in temperature or humidity. Our data suggest that a high degree of social transmission within a nest leads to these stable nest microbiomes, whereas drift-like processes during the foundation of new nests explain variation in host microbiomes between nests. Nest survival rate was low and not linked to microbiome composition; however, an increase in overall bacterial load in spiders prior to nest death was indicative of a loss of host immune control preceding nest collapse.

Supplementary data

Supplementary data are available at *FEMSEC* online.

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