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Exploring the effects of horizontal pathogen transmission on mortality and behaviour in a cooperatively breeding spider

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Social interactions are a driving force behind disease outbreaks in animal societies. As groups become more complex and build permanent nests, they run the risk of introducing more routes of potential pathogen exposure. Eusocial societies have well-established 'superorganism immunity' that protects against disease. However, fewer studies have investigated social immunity in cooperative breeders, which may provide insight into the evolution of immunity as a form of collective behaviour during major evolutionary transitions in sociality. We exposed groups of social spiders (*Stegodyphus dumicola*) to a generalist entomopathogenic fungus (*Metarhizium robertsii*) using three modes of exposure: directly onto a groupmate, mechanically vectored by prey and nest exposure. We compared spider mortality between exposed groups and pathogen-free controls. We measured space use and colony fragmentation to test whether pathogen exposure route and disease severity affected spiders' aggregation and polydomy behaviour. We found that route of exposure greatly affected spider mortality, with direct exposure resulting in the most rapid mortality. Individuals from groups with a directly exposed spider were also more likely to be observed outside their nest compared to all other treatments. Exposure did not result in differences in colony fragmentation. These data demonstrate that the route of pathogen introduction not only affects the severity of disease outbreaks but can also alter behaviours relevant for social immunity. This study enhances our understanding of the transitional steps in social immunity among cooperative breeders that may lead to the evolution of superorganism immunity.

Group living, with higher frequencies of social interactions often between related individuals, is a major driver of horizontal pathogen transmission. Social interactions in a shared living space increase the number of potential routes of pathogen exposure and transmission. Pathogens can be acquired or transmitted from shared food (Naug, 2008; Rudolf & Antonovics, 2007), overlapping space use (Gorochowski & Richardson, 2017), via shared domiciles (Brown & Brown, 2004) and via direct transmission between hosts. A recent review by Nadler et al. (2024) hypothesized that a pathogen's transmission mode can alter the trade-off between disease-associated benefits and costs of social living, with consequences on disease avoidance and infection-induced changes to host behaviour. Many species have evolved increasingly complex behaviours to respond to this escalating risk, termed social immunity (Cotter & Kilner, 2010; Cremer et al., 2007). Despite the complex interrelatedness between social behaviour and disease, experimental studies rarely test whether different modes of pathogen introduction into a social group (e.g. direct transmission, trophic or environmental) alter host behaviour as well as disease outbreak severity.

Many social animals build nests, burrows or retreats to provide space to raise offspring, protect against the abiotic environment and defend against predators. Although an increase in host density and social interaction frequency within the nest is expected to increase pathogen transmission, the built environment also has buffering effects against disease (Pinter-Wollman et al., 2018). For example, nests can incorporate antiparasitic building materials (Chapuisat et al., 2007; Clark & Mason, 1985) and complex physical structures such as division of nests into discrete chambers can confer 'organizational immunity' in some eusocial insects (Stroeymeyt et al., 2014). Physical separation within a colony could theoretically delay pathogen spread or even potentially stifle disease outbreaks all together (Pie et al., 2004). This may be especially

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true in polydomous colonies, which have a fragmented nest structure such that discrete nests are spatially separated but remain socially connected (Robinson, 2014). Patterns of social interactions within the built environment can also play an important role in 'behavioural immunity', or the ways in which behavioural traits limit the spread of disease. For example, exposure to the fungal pathogen *Metarhizium brunneum* can alter the social network structure in nests of the black garden ant, *Lasius niger*, by increasing network modularity, decreasing intracolony density, increasing colony diameter and decreasing network centrality (Stroeymeyt et al., 2018). Although animal domiciles can be built in ways to limit pathogen spread, infection can also alter space use and social interactions within nests. Therefore, experimental studies on nest building, space use and pathogen spread using different modes of exposure are needed.

Eusocial societies have evolved such complex disease mitigation behaviours that some researchers have designated them as 'superorganism immunity', which can only evolve following a major evolutionary transition to eusociality (Boomsma & Gawne, 2018; Pull & McMahon, 2020). This distinguishes these behaviours from more general social immunity (Cremer et al., 2007). Superorganism immunity is more coordinated and complex compared to basic social immunity behaviours like the grooming and contagion avoidance often found in noneusocial groups (Cotter & Kilner, 2010). However, for cooperative/communal breeders, such as social beetles (Nuotclà et al., 2019) or social spiders (Straus & Avilés, 2018; Tietjen, 1981), the complexity of their social immune systems, how it evolves and under what conditions it is expressed are less clear compared to eusocial insects. Selection for social immunity in cooperative breeders may depend on the virulence of pathogens they are exposed to, their ability to detect pathogens, the likelihood of disease outbreaks and the degree of kin relation within the group. Understanding the individual and collective behavioural defences against disease in cooperative breeding systems is important to understand how social immunity evolves in general.

The African social spider *Stegodyphus dumicola* is a cooperatively breeding spider whose social behaviours and nest architecture have been hypothesized to incur selective pressures from horizontally transmissible pathogens (Henschel, 1998; Nazipi et al., 2021). *Stegodyphus dumicola* lives in large colonies, from several dozen to hundreds of closely related individuals. Colonies are founded by single matrilines that serially inbreed across generations (Johannesen et al., 2002), and a combination of immunological and genomic data suggest that serial inbreeding has resulted in reduced efficacy of selection on immune genes (Bechsgaard et al., 2022).

One way to cope with lower immunocompetence in social *Stegodyphus* spiders could be increased expression of social immunity, which has been suggested in ant colonies (Cassidy et al., 2021). Transmission of microbes within colonies can be driven by social contact and indirect transmission via shared silk (Keiser et al., 2016), co-feeding and regurgitating food to offspring (Rose et al., 2023), and potentially from spiderlings consuming their parental generation before maturing (Junghanns et al., 2019). Evidence from field surveys suggests that nearly 6% of colonies collapse from outbreaks of a fungal pathogen (potentially *Purpureocillium* or *Lecanicillium* sp.; Nazipi et al., 2021), compared to only 1.3% of solitary *S. dumicola* experiencing fungal mortality (Henschel, 1998), although the identity of the apparent fungal parasite that actually kills the colonies has never been confirmed. Despite a combination of compelling evidence for host–pathogen interactions from sequencing and field observations, no experimental study has yet tested the potential routes of pathogen introduction and transmission in a social spider. Investigating disease outbreaks in social spiders may provide insights into the evolution of social immunity in communal breeders.

Here, we record space use behaviour and mortality in cooperative spiders after different modes of pathogen exposure using *S. dumicola* and a generalist fungal pathogen *Metarhizium robertsii* as a model. It has historically been thought that there is little taxonomic overlap between spider and insect fungal pathogens/parasites in nature (Durkin et al., 2021; Evans, 2013; Shrestha et al., 2019). However, there are several published reports of generalist pathogens infecting spiders in the laboratory (e.g. *Metarhizium* and *Beauveria*; Beys-da-Silva et al., 2013; Portilla et al., 2017) and in field studies, where environmental applications of *Metarhizium* resulted in a decrease in spider populations (e.g. Fischhoff et al., 2018). Given, there are no documented pathogens of known taxonomic identity that infect *Stegodyphus* hosts, we attempt to establish *Metarhizium* as a model to investigate *Stegodyphus* disease dynamics. More broadly, the use of generalist arthropod fungal pathogens could be a powerful tool in arachnology, where model pathogen systems are currently nonexistent. *Metarhizium* infect insect hosts by conidiospores attaching to the cuticle where spores are transported around the environment, potentially being shed onto the substrate or being transmitted to other hosts via social contact. Then, spores germinate, penetrate the host cuticle and replicate in a yeast-like stage inside the body, which quickly kills the host via toxins and then saprophytically consumes the cadaver. Finally, *Metarhizium* sporulates, growing back through the host cuticle where it produces conidiospores for further transmission (St Leger & Wang, 2020). While no studies have investigated how this life cycle operates in infected spiders, we expect similar mechanisms of transmission-, infection- and disease-related mortality.

In this study, we ask the following questions. (1) Does the mode of *Metarhizium* exposure affect *Stegodyphus* mortality? (2) Does pathogen exposure affect the proportion of time individuals spend inside versus outside their nest? (3) Does pathogen exposure mode influence colony fragmentation (i.e. polydomy)? (4) Does pathogen exposure drive individuals outside the nest prior to death (i.e. self-removal)? Because these social spiders spend much of their time in body contact with nestmates (Hunt et al., 2019), and we know microbes can be transmitted from direct contact (Keiser et al., 2016), we hypothesized that social transmission is a driving factor of disease outbreaks relative to environmental transmission or trophic transmission. Since disease seems to be an important evolutionary pressure (Henschel, 1998), we predicted that spiders would spend more time outside the nest after pathogen exposure and show an increase in polydomous behaviour to increase modularity in social interactions in an attempt to suppress an outbreak.

METHODS

Animal Maintenance

Stegodyphus dumicola colonies were collected north of Outjo, Namibia in April 2023 (Supplementary Table S1). Spiders were maintained in Percival environmental chambers with conditions mirroring the June averages for the locality of origin (data pulled from Weather Atlas, https://wanderlog.com/weather/80672/6/outjo-weather-in-june): 11:13 h light:dark cycle, 25:12 °C day:-night temperature and ~45% average relative humidity. Three weeks before the experiment started, spiders were separated into groups of five and fed banded crickets, *Gryllodes sigillatus*, every 2 days for 2 weeks to reduce body condition differences between individuals. At the start of the experiment, we measured individuals' mass (mg) and prosoma width (mm) to calculate scaled mass index (SMI), which is a proxy for body condition previously

used in *Stegodyphus* (Parthasarathy & Somanathan, 2018). During the experiment, spiders were fed a maintenance diet of one 2-week-old cricket and misted with 3 ml of water weekly. At the end of the experiment, voucher specimens were accessioned into the Spider Parasite Digital Research Collection (Ecdysis Portal Spider Parasite Digital Research Collection, https://ecdysis.org/collections/misc/collprofiles.php?collid=93) at the University of Florida, in addition to voucher specimens deposited to the National Museum of Namibia.

Ethical Note

Animals were exported from Namibia under research permit number 202302008 issued by the Namibia National Commission on Research, Science, and Technology. These experiments were conducted on invertebrate animals and thus are not under the governance of the U.S. National Research Council, although we adhered to the guidelines of the ASAB/ABS Guidelines for the care and research of animals whenever possible.

Experimental Overview

Colonies collected during this field season had an average group size of 46 spiders per nest, with 11% of nests (26/184) containing five or fewer spiders and 7% of polydomous colonies (3/44) containing a nest with only a single spider. We removed 140 female spiders from four nests we collected from the wild and divided them into 28 groups, each containing five spiders that originated from the same wild colony. While this colony size is smaller than the average wild colony, many studies have been published on this species using similar experimental group sizes (e.g. Najm et al., 2020; Rose et al., 2023). We used only female spiders in this experiment because *Stegodyphus* colonies have a highly female-biased sex ratio in nature (~90% female; Lubin & Bilde, 2007), so the disease dynamics between females are likely to drive disease outbreaks in wild colonies. We then split the 28 groups of spiders into seven blocks in a growth chamber ($N = 4$ groups per block, one per treatment). We measured body mass and size for each spider and gave them one of five coloured identity (ID) markings using markers to track individuals over the experiment. Three weeks after experimental colony formation we exposed colonies to conidiospores from the generalist fungal pathogen *M. robertsii*. Within each block, we implemented four routes of pathogen exposure: (1) procedural control (Control); (2) exposed filter paper in the web to mimic pathogen introduction from the environment via an inanimate substrate (Paper); (3) cricket exposed and fed to spiders (Cricket); (4) direct exposure to one spider in the group (Spider).

Daily, we recorded individual mortality, location of each individual (inside/outside nest) and number of nests (>1 nest indicates a polydomus colony) within the experimental colony. In the wild, a single spider making an additional nest may induce a group dispersal event where other individuals in the group follow a silken bridge created by an individual to the new nest site (Parthasarathy & Somanathan, 2018, 2020). Combined with the data we presented above, showing that ~11% of all nests found in nature contain group sizes of five or less, we think that relatively small group sizes may provide relevant insights in the response of individuals and groups to pathogens because this is likely to be a critical stage in colony development at a time when survival is very low. We conducted the experiment for 35 days (28 days after *M. robertsii* exposure), afterwards all remaining spiders were euthanized at -4 °C and kept as voucher specimens. We monitored spiders that died during the experiment for an additional 16 days to track fungal sporulation from corpses.

Pathogen Exposure

We cultured fungi from a *S. dumicola* corpse previously exposed to *M. robertsii* in a pilot study (Supplementary Fig. S1) but inadvertently exposed our experimental spiders to an unidentified nonpathogenic fungus from this culture that appeared similar to *M. robertsii* but produced no conidiospores. We observed colonies for 9 days and found no mortality following contaminant exposure, which would not have been expected if we had exposed colonies to pathogenic fungi based on the pilot study (LD50 = 9 days for *M. robertsii* exposure; Supplementary Fig. S1). Attempts to extract DNA to identify the contaminant fungus were unsuccessful. We do not believe exposure to this unknown fungus had any effect on our experimental colonies because all spiders remained alive for >1 week following contaminant exposure.

We exposed colonies to *M. robertsii* spores via three exposure methods: (1) Paper, (2) Cricket or (3) Spider, and compared them to (4) Controls that were disturbed but not exposed to spores. Our justification for these treatments is based on the hypothesized routes of exposure to potential fungal pathogens in the wild (Nazipi et al., 2021): exposure from the environment/nest (Paper), from prey items (Cricket) or from a single nestmate (Spider). We grew *M. robertsii* (ARSEF number 2576) cultures by removing ~10 µl of a -80 °C frozen glycerol stock and plating on potato dextrose agar. We allowed the culture to grow for 3 weeks and collected conidiospores using a sterile inoculating loop. We collected spores from four plates of *M. robertsii* culture, homogenized them in a single tube and then equally distributed 1 ml aliquots into 21 separate sterile 15 ml tubes. We exposed either a 1.2 cm^2 piece of filter paper, a live 4-week-old banded cricket or a randomly selected spider to spores by placing them individually in a 15 ml tube with 1 ml of spores and gently spun (i.e. vortex set to 3/10) for 3 s. No spider or cricket vortexed appeared to die prematurely due to vortexing and all seven spiders vortexed with spores lived for at least 1 week after vortexing. After pathogen exposure, we used forceps to place each exposed item in the colony containers ~2 cm from the entrance to each nest. To minimize any effect of treatment application, we performed the same procedural disturbance on all colonies but without spore exposure, where we vortexed one spider without spores, vortexed each cricket without spores before feeding and placed a piece of filter paper 1 cm from the nest entrance of every colony. In other words, all colonies had a spider disturbed by vortexing, were fed a vortexed cricket and had a piece of filter paper added to the colony, but only some colonies had fungal spores exposed to them.

Data Collection

All daily observations on spiders occurred around midday to ensure consistency in behaviour (i.e. location of individuals in/out of nests). To remove observer bias, colonies were labelled with only random IDs so we would be blind to all colony treatments when making daily observations. Colonies were maintained in the environmental chamber for 28 days following *M. robertsii* exposure and checked daily for individual mortality, number of new nests built and the location of each individual (inside/outside of nest). Once all spiders died, all dead spiders were removed and placed in sterile petri dishes atop moistened filter paper in environmental chambers at 30 °C to monitor for sporulation. To determine the spiders' locations of inside/outside the nests, we observed where they were relative to the nest's tunnels. If they were at least one body length outside the entrance to a tunnel (~1–2 cm), then we recorded them as 'outside'; otherwise, they were marked as being 'inside' the nest. These are not definite categories, since it requires an observer's visual estimation of one body length of each spider, but it is likely to

be limited in its subjectivity. Additionally, if we were ever unsure about spiders being inside or outside, we categorized them as inside to conservatively estimate any social distancing. We defined a nest in this experiment by visually identifying opaque silken tunnel structures characterized by their yellowish colour and lack of sticky cribellate silk as compared to the lighter coloration, much finer capture silk. We measured the number of nests by counting spatially distinct nests (i.e. tunnels that were at least 5 cm apart) that contained at least one individual at the time of any given observation in a container, where the furthest possible distance was roughly 15 cm. We scored any colony with more than one nest as a polydomous colony.

Statistical Analyses

We analysed time to death after fungal exposure using a Cox proportional hazards model from the 'coxme' package (Therneau, 2020) with treatment and SMI as independent variables and colony ID, block ID and colony of origin (i.e. source retreat) as random effects. We quantified space use by counting the number observations each spider was observed inside the nests and divided it by the total number of days that individual was alive, because the number of days alive varied across many individuals. The denominator was set to 28 days for individuals still alive at the end of the experiment. We analysed space use using a generalized linear mixed model from the 'glmmTMB' package in R (Brooks et al., 2017), specifying a Gaussian error distribution, with treatment as the independent variable and colony ID, block ID and source retreat as random effects. We tested for differences among fixed effects with Wald chi-square tests using 'Anova' in the 'car' package (Fox & Weisberg, 2018) with type III sums of squares. We compared observed to simulated model residuals in the 'DHARMa' package (Hartig, 2018) to test for overdispersion and to confirm our model fits conformed to the distributional assumptions. We calculated least-squares means and performed post hoc Tukey tests in the 'emmeans' package (Lenth et al., 2019). Finally, we analysed formation of polydomous colonies across our treatments using a Pearson's chi-square test to determine whether each treatment resulted in the creation of a multinest colony. We performed all analyses in R version 4.3.1 (R Core Team, 2018).

RESULTS

To verify that our observed mortality was the product of infection rather than background mortality or handling, 90% (66/73) of spiders from exposed treatments that died showed *M. robertsii* fungal growth after their death (Fig. 1 insert). All pathogen-exposed groups experienced more rapid mortality than control groups, in which only one control spider died with no indication of sporulation (Fig. 1). Mortality from all treatment groups significantly differed from each other: groups in which a spider was directly exposed to spores experienced the most rapid mortality, followed by groups in which crickets were exposed and finally those in which a piece of filter paper was exposed (integrated log link: $\chi^2_7 = 100.7$, $P < 0.001$; Fig. 1, Supplementary Table S2). This suggests that direct horizontal transmission between hosts can be a driving force in social spider disease outbreaks not only because the spider-exposed groups experienced the most rapid mortality, but also because this pattern held true when we assessed mortality excluding the experimentally exposed spiders (Supplementary Fig. S2, Table S3). Spider body condition (SMI) had no effect on survival time ($P = 0.7$; regression coefficient = - 0.0020).

To test whether moribund spiders leave the nest before death (i.e. self-removal), we measured whether spiders were inside or outside their nest the day before their death. Only 10.7% of spiders that died (8/75) were found outside the nest the day prior to death, suggesting that moribund infected spiders did not leave their nest 24 h prior to death. Pathogen-exposed groups were also no more or less likely to build polydomous colonies (i.e. more than one nest) during our observation period compared to control groups ($\chi^2_3 = 3.111$, $P = 0.374$; Fig. 2). However, every treatment contained at least one polydomous colony with ~25% of all experimental colonies constructing at least one additional nest (Fig. 2).

To test whether pathogen exposure mode affected spider space use, we tested whether spiders spent different proportions of time inside or outside their nest across each treatment group. Colonies that had a spider directly exposed to the fungal pathogen spent less time in the nest compared to all other treatments ($\chi^2_3 = 38.904$, $P < 0.001$; Fig. 3). This pattern held true even when we excluded all spiders that did not die during the experiment (triangles in Fig. 3, Supplementary Fig. S3). In control groups, 76.5% of individuals were

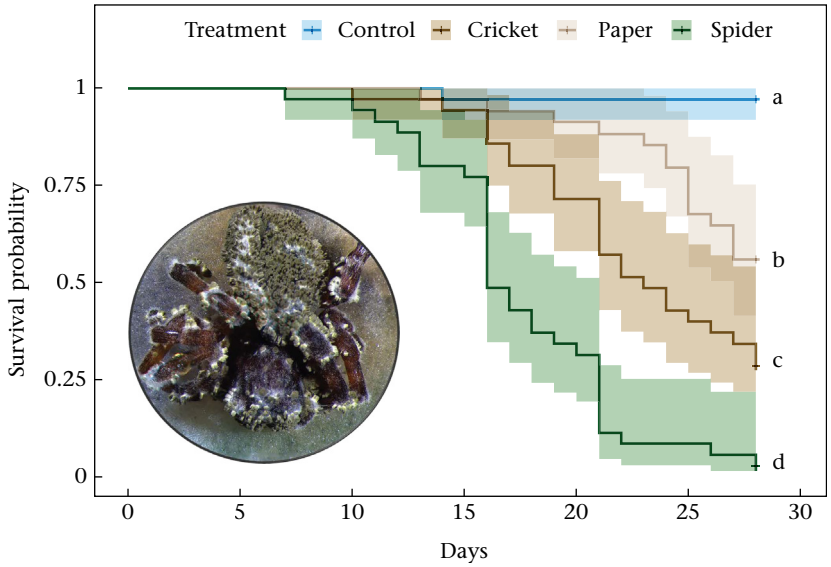

Figure 1. Survival curve representing the three pathogen exposure groups compared to a pathogen-free control. Letters on the right side of the mortality curves represent statistically significant differences. (insert) *Stegodyphus dumicola* cadaver after *Metarhizium robertsii* has begun to sporulate.

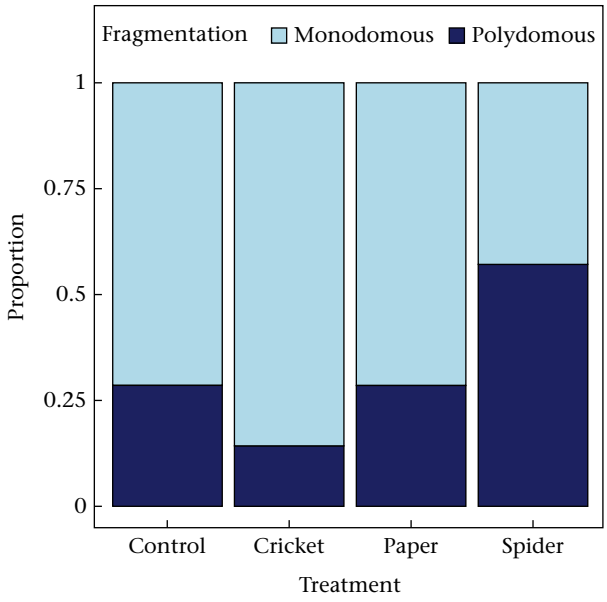

Figure 2. The proportion of colonies that built one nest (monodomous) or multiple nests (polydomous) for each experimental treatment.

recorded inside the nest at every observation, compared to 37.1% for paper-exposed groups and 31.4% for cricket-exposed groups, while no individuals in the spider-exposed groups spent 100% of their time in the nest (Fig. 3). Interestingly, only 4.2% (3/72) of spiders that died over the course of experiment across all treatments were observed to be inside the nest for 100% of observations, all of which were in the cricket treatment (Supplementary Table S4). No other treatment differed from each other in their time spent inside the nest (Supplementary Table S4).

DISCUSSION

One cost of social living is the increased potential for horizontal transmission of pathogens, although experimental studies usually focus on a single mode of exposure. Using groups of social spiders, we found that colonies experienced the most rapid mortality when a single spider was directly exposed to a generalist fungal pathogen. *Stegodyphus dumicola* spend much of their time in contact with each other inside the nest and during feeding events (Hunt et al., 2019), introducing many opportunities for horizontal transmission (Keiser et al., 2016). Environmental exposure to spores, via a contaminated piece of paper, produced the weakest mortality of pathogen exposed groups, likely because this stationary source of spores only contacts spider hosts haphazardly as they move directly over it. Spore-exposed feeder crickets produced an intermediate level of mortality because multiple spiders can co-feed on a single prey (although prey is not always shared evenly; Whitehouse & Lubin, 1999). Although we controlled for the approximate volume of spores present in the application vials (~1 ml), we are unable to account for differences in the number of spores that adhered to different exposed items (i.e. spider, cricket, or paper). It may be that the cuticular hairs on *S. dumicola* increased the pathogen load acquired during exposure and therefore increased the dose that those treatments received. Unfortunately, we were not able to quantify the spore load acquired by spiders, crickets or the paper substrate. Nevertheless, exposing groups to an infectious cricket or piece of paper reduced survival of all nestmates, highlighting the importance of social interactions and space use in transmission.

Changes to space use in and around the built environment after pathogen exposure can be the product of one or more phenomena, including pathology, social immunity, adaptive sickness behaviours or parasite manipulation (Vale et al., 2018). We found that individuals in colonies from the Spider treatment, which endured the most severe outbreaks, spent significantly less time inside their nest compared to spiders from colonies from all other treatments. Reducing or altering the patterns of social interactions in the nest is considered part of the social immune systems (Cotter & Kilner, 2010; Cremer et al., 2018). Spending less time in the nest may be an attempt for uninfected individuals to socially distance themselves from sick groupmates ('contagion avoidance'). Self-removal from nests by infected individuals may also have evolved in highly related groups of social spiders via kin selection. Reducing time spent in the nest could also have been an attempt to mitigate the symptoms of infection ('adaptive sickness behaviour'), but given

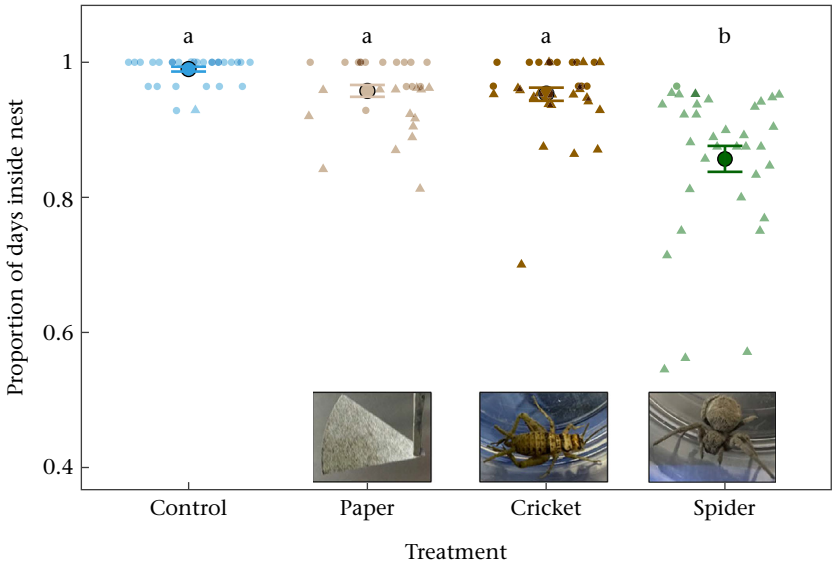

Figure 3. Proportion of days that each individual spider spent inside the nest versus outside the nest on the capture web before they died or the experiment ended. Raw data are smaller points and the means are larger points outlined in black. Triangles indicate individuals that died from disease and circles represent spiders that lived for the entire experiment. Bars indicate standard error and letters indicate significance. Pictures show the treatment application of spores to each of the three exposed treatments.

that all but one individual from the Spider treatment died in less than a month, any attempt at mitigating the outbreak at the individual or collective level appeared ineffective. This could either be because the spiders still spent ~86% of their time in the nest, or be a product of the confined experimental set-up, meaning we were not able to observe the benefits of self-removal that could be present in natural colonies. Alternatively, this behaviour could be the product of parasite manipulation, where leaving the nest may increase the transmission potential or fitness of the fungal parasite. Parasite manipulation is an unlikely explanation given that *M. robertsii* is a generalist pathogen and therefore likely lacks the tight coevolutionary relationship between hosts and specialist parasites that leads to reliable behavioural changes (de Bekker et al., 2021). At present, we interpret the observed changes to space use after pathogen exposure to be pathological side effects of the infection, although future studies could identify additional evidence in support of the social immunity hypothesis.

One goal of this experiment was to assess whether spiders leave their nest immediately prior to death (i.e. self-removal), as has been observed in moribund workers in eusocial insect colonies (Giehr & Heinze, 2018; Heinze & Walter, 2010). Our observations suggest that moribund infected spiders do not leave their nest 24 h prior to death. In ants and termites, workers exiting the nest before dying is considered an important component of social immunity (Cremer et al., 2018), although social immunity has not been thoroughly investigated in social spiders beyond defence against kleptoparasites (Straus & Avilés, 2018) and sanitary behaviour (Tietjen, 1981). In this study, we unfortunately did not track where spiders were the day of death; we only marked them as dead. Spiders might leave the nest only shortly before death or may even be removed by other nestmates, which means we could have missed self-removal or corpse removal behaviours. Future studies should measure these data as it could be very interesting to see if spiders perform corpse removal or if individuals self-remove in the hours prior to death. Social spiders do not possess the highly developed superorganism immunity possessed by their eusocial counterparts (Pull & McMahon, 2020), but they may have a less intricate form of social immunity that is conditional and dependent on kin relation to nestmates. Future research should test the extent to which social spiders collectively implement protective behaviours in the face of disease, the presence of different social immune behaviours (including self-removal and corpse removal) and how that relates to the degree of sociality (i.e. social versus subsocial).

Multinest ('polydomous') *Stegodyphus* colonies are commonly found in nature (~20% of *S. dumicola* colonies; Cassidy, 2025), which has been hypothesized to be a product of group emigration due to food inequality (Parthasarathy & Somanathan, 2018, 2020), attacks by predators such as ants (Keiser et al., 2015) and potentially birds (S. T. Cassidy, personal observation). We hypothesized that pathogen exposure may induce polydomy but found no evidence supporting this hypothesis, possibly due to our small group sizes and confined experimental set-up. Group fragmentation by polydomy may alleviate some risk associated with disease outbreaks in many eusocial species (Debout et al., 2007; Ellis & Robinson, 2014; Robinson, 2014), but fragmentation may not be a viable defence for smaller groups. Colonies experiencing the worst disease outbreak (i.e. the Spider treatment) did build at least one additional nest in 57% of colonies (4/7 colonies; Fig. 2) and spent 10–13% less time on average inside their nest. These observations may indicate an attempt to disperse as the outbreak proliferated. This dispersal could be a sign that the costs of cooperation, in the form of disease transmission, may have outweighed the benefits provided by group living, leading to a breakdown in group cohesion. Ultimately, their attempted dispersal may have failed due to the confined nature of their small enclosures and the few colonymates that were available to assist in building more nests. Solitary nests are unlikely to survive in the wild (~90% mortality; Bilde et al., 2007) and colony fragmentation tends to happen when groups of spiders disperse together (Parthasarathy & Somanathan, 2020). The likely benefits of dispersal, despite the high mortality, is increased body condition via food competition release, reduced pathogen pressure via social isolation and fresh nesting material that is unlikely to harbour disease. Disease-induced colony collapse, as we may have observed in our Spider treatment, could push individuals to leave their nest once the benefit of escaping from the outbreak outweighs the high cost of single-female dispersal. Future studies should test the effect pathogen/parasite exposure has on dispersal on larger colonies in the field to determine whether individuals disperse (in groups or alone) to escape severe outbreaks, leading to colony collapse.

In cooperative breeders, it is currently unclear to what extent social immunity evolves and is expressed. Studies on cooperatively breeding species have proven very important to understand the evolution of eusocial 'superorganisms' (e.g. Nuotclà et al., 2019). Within noneusocial cooperative breeders, there exists a gradient of social complexity from kin-dependent reproductive division of labour (Downing et al., 2020) to facultative eusociality (Boomsma & Gawne, 2018; Kirkendall et al., 2015). Social spiders represent a cooperatively breeding group that live in highly related family groups (Avilés & Guevara, 2017; Lubin & Bilde, 2007), seemingly have reproductive division of labour (Salomon et al., 2008) and produce groups for at least a few generations by retaining offspring (Crouch & Lubin, 2001), and yet they remain unexplored systems for social immunity behaviour outside kleptoparasite defences (Straus & Avilés, 2018). To our knowledge, this is the first study to test differences in mortality between modes of exposure in a spider host and the first to test transmission dynamics in a social spider species (Beys-da-Silva et al., 2013; Fischhoff et al., 2018). Our work corroborates previous demonstrations of *Metarhizium*'s ability to readily infect spider hosts, and we are the first to show that *Metarhizium* can alter spider host behaviour. We think this study and others demonstrate the importance of using investigating behavioural responses to disease outbreaks in cooperatively breeding species to gain insights into the evolution of social and superorganism immunity.

Author Contributions

Steven T. Cassidy: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Seth J. Eiseb:** Writing – review & editing, Resources, Project administration, Investigation. **Martha Haufiku:** Writing – review & editing, Methodology, Investigation. **Tresia Kavili:** Writing – review & editing, Methodology, Investigation. **Carl N. Keiser:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Nolan Missigman:** Writing – review & editing, Methodology, Investigation. **Abigail Pope:** Writing – review & editing, Supervision, Methodology, Investigation. **Kara J.M. Taylor:** Writing – review & editing, Methodology, Investigation.

Data Availability

The data, associated metadata and R code required to reproduce all the analyses are available on Figshare (https://figshare.com/s/899373c40e6b67775187).

Declaration of Interest

The authors declare that we have no competing interests.

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Supplementary Material

Supplementary material associated with this article is available at https://doi.org/10.1016/j.anbehav.2025.123113.

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