ANIMAL HOST–MICROBE INTERACTIONS

The primary case is not enough: Variation among individuals, groups and social networks modify bacterial transmission dynamics

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Abstract

1. The traits of the primary case of an infectious disease outbreak, and the circumstances for their aetiology, potentially influence the trajectory of transmission dynamics. However, these dynamics likely also depend on the traits of the individuals with whom the primary case interacts.

2. We used the social spider *Stegodyphus dumicola* to test how the traits of the primary case, group phenotypic composition and group size interact to facilitate the transmission of a GFP-labelled cuticular bacterium. We also compared bacterial transmission across experimentally generated "daisy-chain" vs. "star" networks of social interactions. Finally, we compared social network structure across groups of different sizes.

3. Groups of 10 spiders experienced more bacterial transmission events compared to groups of 30 spiders, regardless of groups' behavioural composition. Groups containing only one bold spider experienced the lowest levels of bacterial transmission regardless of group size. We found no evidence for the traits of the primary case influencing any transmission dynamics. In a second experiment, bacteria were transmitted to more individuals in experimentally induced star networks than in daisy-chains, on which transmission never exceeded three steps. In both experimental network types, transmission success depended jointly on the behavioural traits of the interacting individuals; however, the behavioural traits of the primary case were only important for transmission on star networks.

4. Larger social groups exhibited lower interaction density (i.e. had a low ratio of observed to possible connections) and were more modular, i.e. they had more connections between nodes within a subgroup and fewer connections across subgroups. Thus, larger groups may restrict transmission by forming fewer interactions and by isolating subgroups that interacted with the primary case.

5. These findings suggest that accounting for the traits of single exposed hosts has less power in predicting transmission dynamics compared to the larger scale factors of the social groups in which they reside. Factors like group size and phenotypic composition appear to alter social interaction patterns, which leads to differential transmission of microbes.

1 | INTRODUCTION

Hosts' traits are important determinants of individuals' propensity to acquire and transmit microbes which may be infectious agents of disease (Martin, Burgan, Adelman, & Gervasi, 2016; Salje et al., 2016). Thus, differences among individuals in traits like immunocompetence (Gopinath, Lichtman, Bouley, Elias, & Monack, 2014) and behavioural tendencies (Barron, Gervasi, Pruitt, & Martin, 2015) can have implications for the nature and magnitude of host–microbe interactions. In the most extreme cases, certain individuals can be the tipping point upon which epidemics arise or halt (Lloyd-Smith, Schreiber, Kopp, & Getz, 2005). However, the influence of a single individual on its social group or population will be, at least in part, mediated by the traits of nearby individuals with whom the focal individual interacts. Unfortunately, reliably detecting and tracking transmission among individuals is one of the most difficult problems in disease ecology (Buhnerkempe et al., 2015; Fenton, Fairbairn, Norman, & Hudson, 2002; Haydon et al., 2003; Read, Edmunds, Riley, Lessler, & Cummings, 2012). Furthermore, the traits of pairs or groups of interacting individuals are rarely studied empirically or in unison. Thus, a more complete understanding of how individual traits unite with group traits to explain epidemiological dynamics will require (i) a priori characterization of important host traits; (ii) analyses of social interaction networks; and (iii) information regarding how microbes are transmitted through different structures of social networks.

Many emerging infectious disease outbreaks begin with a single individual contracting an infection, although inferring the origin of an epidemic (i.e. the "primary case" or "patient zero") does not establish their aetiology (Lokhov, Mézard, Ohta, & Zdeborová, 2014). It is tempting to expect that the traits of the primary case will predict the likelihood and magnitude of the ensuing outbreak, and some empirical evidence supports this expectation (Adelman, Moyers, Farine, & Hawley, 2015). However, this is not always the case (Keiser, Howell, Pinter-Wollman, & Pruitt, 2016), and we propose that the effects of the traits of the primary case will depend on factors of the group in which they reside (e.g. group size) and the traits of the individuals with whom it interacts. For instance, the spread of infectious pathogens may be halted if the primary case resides in a small social group, or interacts with a large number of individuals of low infection competence (Barron et al., 2015). In the 2002–2004 SARS outbreak, simulations showed that two identical communities can experience vastly different outbreaks if the primary cases differed in their social contact patterns (Meyers, Pourbohloul, Newman, Skowronski, & Brunham, 2005). Even after a successful transmission event from the primary case to the primary cases of infection, the propensity to transmit infectious agents to secondary cases will depend on the traits of the interacting individuals. Thus, studying individuals' traits in isolation is likely a less powerful predictor of disease dynamics relative to more comprehensive analyses of group traits. We therefore reason that the degree to which infectious microbes travel through a social network will depend on the series of host individuals through which the microbe is transmitted.

There are several non-mutually exclusive paths through which microbes may be transmitted from a single exposed individual to its social partners (Pinter-Wollman, Keiser, Wollman, & Pruitt, 2016). For example, the microbe could follow a daisy-chain or diffusion chain pattern (i.e. a "line network"), where the primary case transmits to a secondary individual, who then transmits to a third individual, and so on. However, models have suggested that transmission along daisy-chains may be altered by the traits of its members (Moussaïd, Brighton, & Gaissmaier, 2015; Moussaïd & Yahosseini, 2016). Alternatively, the primary case alone may transmit to multiple individuals simultaneously or in succession (i.e. via a "star or "radial" network; Perkins, Cagnacci, Stradiotto, Arnoldi, & Hudson, 2009). These two opposing transmission patterns are rarely differentiated experimentally, although evidence for each exists distinctly. For example, the transmission of entomopathogenic fungal spores through interaction chains of cabbage maggot flies and tephritid fruit flies has been shown to extend to at least six and three hosts respectively (Dimbi, Maniania, & Ekesi, 2013; Meadow, Vandenberg, & Shelton, 2000). Alternatively, the superspreader concept in epidemiology represents an extreme case of the star network structure, where a single individual of very high transmission competence can spread pathogens to a large number of susceptible individuals (Lloyd-Smith et al., 2005; Paull et al., 2012).

Here, we use the social spider *Stegodyphus dumicola* as a model to evaluate how individual traits, group phenotypic composition and social network structure influence the degree of group-wide transmission of a GFP-labelled cuticular bacterium (*Pantoea* sp.). Individual *Stegodyphus* vary in a diagnostic behavioural trait, "boldness," that is consistent across long periods and is associated with individuals' propensity to participate in several collective behaviours (Beleyur, Bellur, & Somanathan, 2015; Grinsted, Pruitt, Settepani, & Bilde, 2013; Keiser, Jones, Modlmeier, & Pruitt, 2014; Wright, Keiser, & Pruitt, 2015). Previous experiments demonstrate that transmission of cuticular bacteria between pairs of individuals is biased and directional: more likely to occur from bolder to shyer spiders (Keiser, Pinter-Wollman, et al., 2016), and the degree of group-wide transmission depends on the phenotypic composition of the unexposed individuals in a colony (Keiser, Howell, et al., 2016). Thus, boldness is a measure of individuals' behavioural tendencies that influence their interaction patterns within colonies and likely the transmission of cuticular microbes (Pinter-Wollman et al., 2016). Here we ask (i) whether the traits of the primary case influence bacterial transmission across groups of different sizes and phenotypic compositions; (ii) to what degree transmission dynamics are a product of different social interaction patterns, specifically "daisy-chains" vs. "star networks"; and (iii) whether network attributes that may be important for transmission vary across groups of different sizes.

2 | MATERIALS AND METHODS

2.1 | Animal collection and maintenance

Stegodyphus dumicola is a group-living spider from arid southwestern African that lives in female-biased colonies of two to several hundred age-structured individuals. Adult females in these societies cooperate in several group behaviours like alloparental care, collective foraging and web-building (Avilés, Varas, & Dyreson, 1999; Bilde et al., 2007; Henschel, 1998; Henschel, Lubin, & Schneider, 1995; Keiser et al., 2014). For the bacterial transmission experiments, we collected 19 *S. dumicola* colonies from *Acacia* trees in the Northern Cape of South Africa in March 2015, transported them to the laboratory, and maintained colonies on an ad libitum diet of domestic crickets in 500-ml plastic containers. For the social network observations, colonies were collected from the field in May 2016. We isolated adult females, measured their prosoma width and body mass, and housed them in 30-ml plastic cups during individual behavioural assays. We estimated spiders' body condition (a measure of health or nutritional status) by the residuals of a linear regression of individuals' body mass on body size (prosoma width) (Jakob, Marshall, & Uetz, 1996). Thus, greater values of body condition indicate that individuals weigh more than expected based on their body size, an indication of positive health status.

2.2 | Boldness assays

We characterized individuals' boldness (a behavioural traits associated with individuals' propensity to engage in risky behaviour; Sloan Wilson, Clark, Coleman, & Dearstyne, 1994) by placing each spider in a clear plastic arena (diameter = 12 cm), allowing a 30-s acclimation period, and administering two rapid puffs of air atop the spider using an infant nose-cleaning bulb. Spiders responded by halting movement and huddling their legs close to their body. We then measured their latency to resume activity after this aversive stimulus, and we designated bold individuals as those that resumed movement within 1–200 s and shy individuals required ≥600 s to resume activity (similar to Keiser & Pruitt, 2014).

2.3 | Bacterial exposure, transmission and sampling

We exposed spiders to *Pantoea* + pGLO by submerging them individually in 1 ml of a liquid bacterial solution (*c.* 10^9 CFU/ml in phosphate buffered saline) for 3 s and allowing it to dry for 24 hr before transferring the spider to a colony (see below). Topical application of GFP-labelled *Pantoea* does not appear to be harmful nor alter spiders' boldness (Keiser, Shearer, et al., 2016), thus these transmission patterns may be thought of as a "null model" for transmission in the absence of infection, sickness behaviours and mortality, similar to studying prey behaviour in the absence of predation (e.g. Bastille-Rousseau et al., 2016). To differentiate the primary case from other spiders, we tagged the experimentally exposed individuals with a small green paint dot atop their dorsal abdomen. All susceptible individuals were marked with a blue dot. After allowing the spiders to interact for 24 hr, we sampled the susceptible spiders' cuticles for the presence of *Pantoea* + pGLO by vortexing each spider separately in 1 ml of sterile selective growth media (LB broth with 100 μg/ml ampicillin, 20% arabinose; Data S1) for 10 s, removing and euthanizing the spider, and incubating the solution for 20 hr at 30°C. Then, we checked each solution for green fluorescence under long-wave UV light. Here, we measured transmission solely in the context of a "susceptible-infected model"-type epidemiological framework, where we consider the transition of hosts from unexposed to exposed and disregarded intensity (i.e. bacterial load) (Hethcote, 1976).

2.4 | Bacterial transmission within colonies

To examine bacterial transmission dynamics, we constructed 93 experimental groups of 10 spiders or 30 spiders each containing either all shy spiders (*n* = 23 for groups of 10, *n* = 6 for groups of 30), one bold spider (*n* = 26 for groups of 10, *n* = 5 for groups of 30) or three bold spiders (*n* = 24 for groups of 10, *n* = 9 for groups of 30) with the remaining spiders in the colony being shy. These group sizes and colony compositions are within natural distributions (Keiser & Pruitt, 2014). Colonies were housed in 500-ml plastic containers with a wire substrate for web-building for 48 hr before adding a randomly selected primary case exposed to *Pantoea* + pGLO as described above (latency to resume movement for primary cases ranged from 1 s to 600 s). After interacting with the primary case for 24 hr, we sampled each susceptible spider in the colony for the presence of fluorescing bacteria as described above. A previous study in this system showed that exposure to bacteria via these methods can increase cuticular bacterial load by multiple orders of magnitude and remain elevated for several days (Keiser, Wright, & Pruitt, 2016). The degree of bacterial transmission in each colony was quantified as the number of individuals on which we identified the transformed bacteria, excluding the primary case.

2.5 | Transmission via daisy-chains or star networks

We exposed individuals to *Pantoea* + pGLO, and then 24 hr later, they were split into two experiments for "daisy-chain" or "star networks" social interactions. For the daisy-chain interactions (*n* = 39), primary cases of known boldness and body condition were isolated for 24 hr in their own housing container, and then moved into the housing container of a primary individual. After 24 hr, the primary individual was moved to the housing container of a secondary individual, and the primary case was checked for the presence of *Pantoea*. Twenty-four hours later, the secondary individual was moved into the container of a tertiary individual, and the primary individual was checked for *Pantoea*. This process was continued until the tenth individual was reached (e.g. Figure 3a).

For the star networks (*n* = 20), an primary case was moved into the housing container of a primary individual for 24 hr, and then the primary case was moved into the housing container of a secondary individual and the primary individual was checked for the presence of bacteria (Figure 3b). This process was continued until the tenth individual was reached. For these pairwise interactions, we calculated the

difference in boldness between interacting spiders by subtracting the "latency to move" value of the unexposed individual from the "latency to move" value of the exposed individual, where positive values denote pairs where the exposed spider was bolder (an important predictor of transmission; Keiser, Pinter-Wollman, et al., 2016).

2.6 | Social contact networks and network analysis

To determine the social network structure of intact colonies, we observed resting interactions among spiders in laboratory colonies. Twenty-four colonies each consisting of 10–11 adult female spiders, individually marked with acrylic paint dots atop their dorsal abdomen, were kept in 710-ml round plastic containers with chicken wire that allowed them to build a retreat and a capture web. Individuals in each group came from the same source colony (we used three source colonies which produced 10, 9 and 5 experimental colonies each). We manually noted the resting interaction patterns of all individuals in each colony. We defined interactions between resting group members as a physical contact between any body parts of two spiders. Each group was observed for 6.5 weeks. Resting interactions were observed three times a week with 2–3 days separating each observation. Thus, we obtained 19 resting networks for each group totalling 456 networks. Because groups were tracked for almost 2 months and mortality occurred over time, we obtained interaction networks of groups of different sizes.

To quantify interaction patterns within colonies, we used three network measures calculated using the IGRAPH package in R version 3.1.2 (Csardi & Nepusz, 2006; R Core Team, 2014): (i) network density, the ratio between the number of observed links and all possible links, which quantifies how tightly connected the individuals are in the group; (ii) clustering coefficient, the ratio of observed links connecting a node's neighbours to each other and the maximum possible number of such links, which quantifies the likelihood that an individual's neighbours are also connected to one another; and (iii) Modularity (Q), which quantifies the separation of the network into densely connected subgroups, defined using the "leading eigenvector" clustering algorithm (Newman, 2006). To compare whether the above network measures differed from those expected if individuals interacted randomly, we performed simulations which generated random networks with a given degree sequence, using the degree.sequence.game R

function (Molloy & Reed, 1995), and compared these simulated values to those for our observed networks (Data S1).

2.7 | Statistical analyses

2.7.1 | Bacterial transmission within colonies

We used a generalized linear mixed model with a log-link function for count data (number of spiders exposed) with the following independent variables: the number of bold spiders in the group (0, 1 or 3; categorical variable), group size (10 or 30; categorical variable), the boldness value of the primary case (continuous variable), body condition of the primary case (continuous variable), group mean body condition (continuous variable), and the interaction between the number of bold spiders in the groups and group mean body condition (all non-significant interaction terms were removed for model simplification; Crawley, 2012; see Data S1). Experimental colony ID nested in source colony ID was included as a random intercept in the model (see details in Data S1). For groups that contained both bold and shy spiders, we also performed a univariate nominal logistic regression model predicting the likelihood of an individual acquiring the bacteria based on their own behavioural phenotype (shy vs. bold), where we also included individual ID nested in experimental group ID as a random effect.

2.7.2 | Transmission via daisy-chain and star networks

To analyse whether successful transmission took place at each step, we used nominal logistic regressions with the following independent variables: transmission step, difference in boldness between interacting individuals, primary case body condition, susceptible individual body condition, and the pairwise interaction between these variables and transmission step (Table 1). Then, we removed non-significant interaction terms for model simplification and present the simplified model here (Crawley, 2012).

2.7.3 | Social network analysis

To determine the effect of group size on network density, clustering coefficient and modularity, we ran three linear mixed effects models

TABLE 1 Results from a general linear mixed model predicting the number of individuals that became exposed to the GFP-transformed cuticular bacteria *Pantoea* within 24 hr of interacting with an experimental primary case. Significant *p*-values are denoted with an asterisk

Effect	Parameter estimate (95% CI)	df	χ^2	p-value
Number of individuals exposed to bacteria				
Number of bold spiders in colony	−0.48 (−0.74 to −0.245)	2	17.22	.0002*
Group size	−0.08 (−0.12 to −0.043)	1	27.01	<.0001*
Primary case boldness	0.0002 (−0.0004 to 0.0008)	1	0.55	.46
Primary case body condition	−35.41 (−81.74 to 10.45)	1	2.28	.13
Mean group body condition	162.71 (94.93–232.12)	1	22.81	<.0001*
Group body condition × Number bold spiders in colony	−79.41 (−156.01 to −4.81)	2	7.09	.03*

(LMM) because the distribution of all the response variables was close to normal. Our dependent variables were network density, clustering coefficient or modularity, and number of individuals in the group was the fixed effect in all three models. We further included group identity nested in colony of origin as a random effect and time point as another random effect to control for any variation caused by these variables. LMMs were implemented using the LME4 package (Bates, Mächler, Bolker, & Walker, 2014) in R version 3.1.2 (R Core Team, 2014). To determine the confidence of our estimates, we ran a Wald chi-squared test using the ANOVA R function on the LMM results.

3 | RESULTS

3.1 | Bacterial transmission within colonies

Groups of 10 spiders experienced approximately five times more transmission events compared to groups of 30 spiders (GLMM: χ^2 = 27.01, df = 1, p < .0001; Table 1, Figure 1). Groups containing only one bold susceptible spider experienced over 50% fewer transmission events than groups containing three or zero bold spiders (GLMM: χ^2 = 17.22, df = 2, p = .0002; Table 1, Figure 1). Groups containing spiders in better body condition experienced an increased incidence of bacteria transmission (GLMM: χ^2 = 22.81, df = 1, p < .0001; Table 1, Figure 2). However, this trend differed for groups depending on their phenotypic composition (interaction term: χ^2 = 7.09, df = 2, p = .03; Table 1, Figure 2): this trend was strongest in colonies containing three bold spiders (R^2 = 0.25) compared to those with one or zero bold spiders (R^2 = 0.03 and R^2 = 0.05 respectively). Neither the boldness value (χ^2 = 0.55, df = 1, p = .46) nor the body condition (χ^2 = 2.28, df = 1, p = .13) of the primary case was associated with the degree of bacterial transmission in their social group. Lastly, averaged across all groups containing one or three bold spiders and both group sizes, the per capita likelihood of acquiring bacteria was four times greater for bold spiders (12/87)
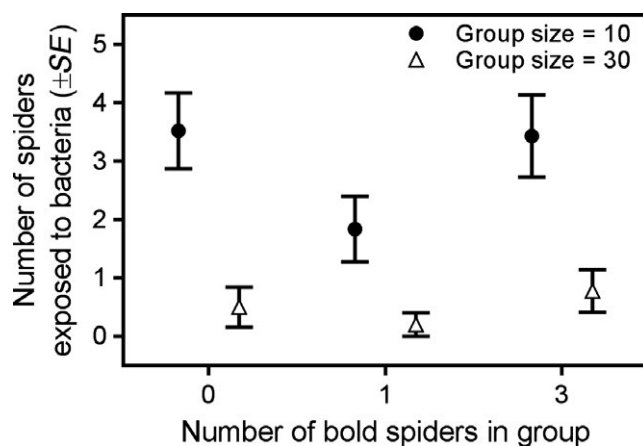

FIGURE 1 Groups of 10 spiders experienced more bacterial transmission compared to groups of 30 spiders, regardless of group composition. However, groups containing only one bold spider experienced the least bacterial transmission in each group size
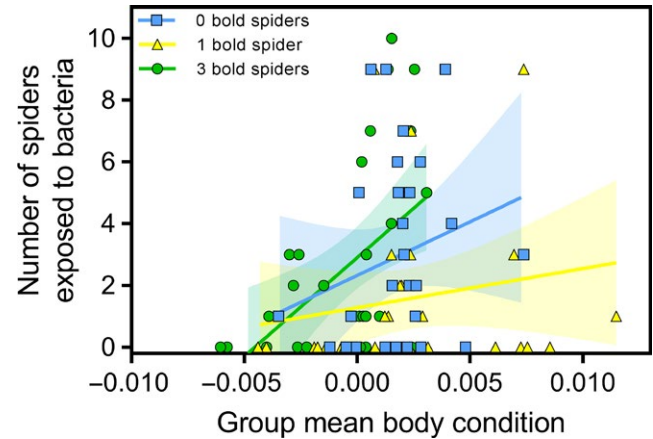

FIGURE 2 Groups containing spiders in better body condition experienced greater bacteria transmission. This trend was strongest in colonies containing three bold spiders (R^2 = 0.25) compared to those with one or zero bold spiders (R^2 = 0.03 and R^2 = 0.05 respectively). [Colour figure can be viewed at wileyonlinelibrary.com]

compared to shy spiders (20/591) (univariate nominal logistic regression: χ^2 = 11.66, df = 1, p = .0006).

3.2 | Transmission via daisy-chain vs. star networks

For each interaction network structure, the likelihood of transmission decreased over each step of transmission (nominal logit: p < .0001; Table 2, Figure 3). For daisy-chain interactions (nominal logit: χ^2 = 6.28, df = 1, p = .01) and star networks (χ^2 = 10.41, df = 1, p = .001), transmission was more likely to take place when the exposed spider was bolder than the susceptible spider with whom it interacted, as previously reported (Keiser, Pinter-Wollman, et al., 2016). For star networks, we found that the rate of transmission across time steps was strongly influenced by the body conditions of both the exposed individual (nominal logit: χ^2 = 15,309.73, df = 6, p < .0001) and the susceptible spiders with which they interacted (nominal logit: χ^2 = 1,302.41, df = 5, p < .0001). That is, bacterial transmission was more likely to occur between spiders in better body condition, although this trend was stronger during earlier steps in the interaction chain.

3.3 | Social network analysis

Interaction density decreased with group size and network modularity increased with group size. Network density was negatively associated with group size (LMM: χ^2 = 192.17, df = 1, p < .0001; Figure 4), while network modularity was positively associated with group size (LMM: χ^2 = 134.47, df = 1, p < .0001; Figure 4). We did not detect a relationship between network clustering coefficient and group size (LMM: χ^2 = 0.21, df = 1, p = .65).

4 | DISCUSSION

The traits of the first individual to become exposed to a novel microbe (e.g. the primary case of an infectious disease outbreak) can impact

Effect	df	χ^2	p-value
Daisy-chain interactions			
Transmission step	3	50.62	<.0001*
Primary case body condition	1	1.96	.16
Susceptible individual's body condition	1	0.41	.52
Difference in boldness	1	6.28	.01*
Star network interactions			
Transmission step	9	9,388.29	<.0001*
Exposed individual's body condition	1	3.33	.0681
Susceptible individual's body condition	1	0.31	.5798
Difference in boldness value	1	10.41	.001*
Transmission step × Exposed body condition	6	15,309.73	<.0001*
Transmission step × Susceptible body condition	5	1,302.41	<.0001*
Transmission step × Difference in boldness	8	901.85	<.0001*

TABLE 2 Results from nominal logistic regressions predicting the number of individuals that became exposed to bacteria via daisy-chain social interactions or iterative social interactions
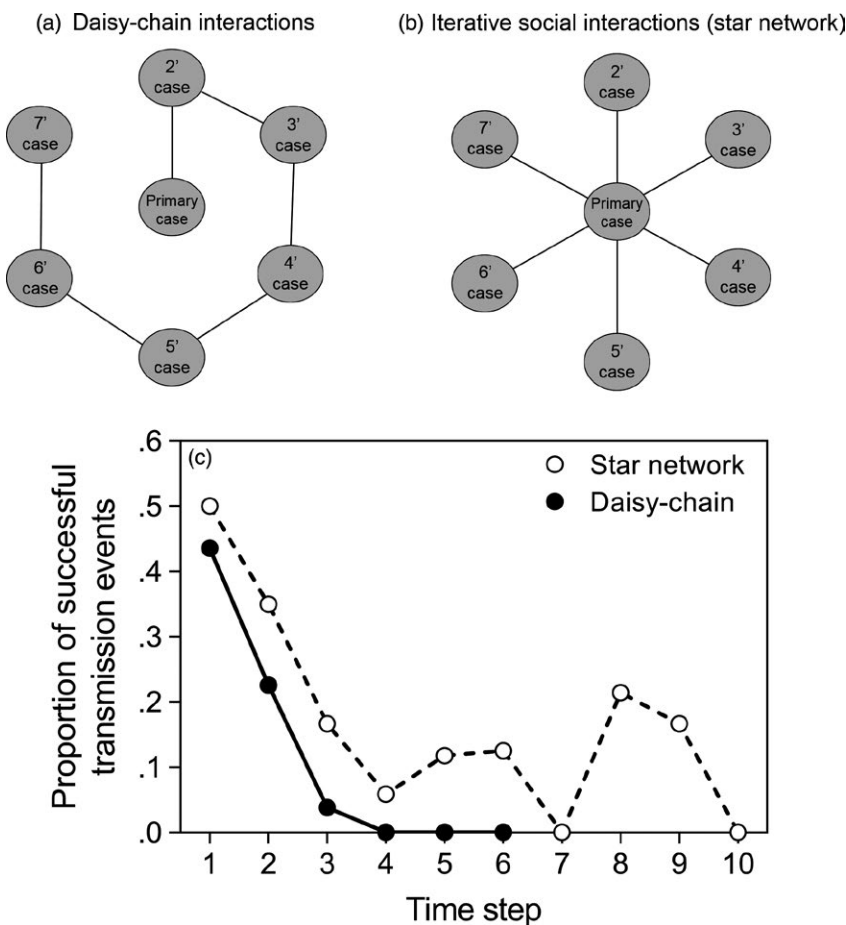
Significant p-values are denoted by an asterisk.

FIGURE 3 Schematic depiction of (a) daisy-chain interactions and (b) star network interactions. Each interaction was separated by 24 hr. (c) The proportion of social interactions which resulted in successful transmission events across 10 days of interactions in each network type. Note that the star networks can reach steps of completely unsuccessful transmission (e.g. time point = 7) and then recover because the same primary case is interacting with new individuals, whereas the daisy-chain transmission cannot recover from an unsuccessful transmission because the bacterial source is lost

the trajectory of transmission dynamics. However, the magnitude of their influence undoubtedly depends jointly on their traits and the individuals with whom they interact (Salje et al., 2016). Here, we found that group-wide transmission of a benign GFP-labelled cuticular bacterium is greater in groups of 10 individuals compared to 30 in colonies of the social spider *S. dumicola*, and that groups containing only one bold individual experienced the least transmission, regardless of the traits of the primary case. Furthermore, we found that bacteria were transmitted to more individuals along a star network of social interactions than along daisy-chains, although transmission depended on the traits of both interacting individuals. Finally, by observing the contact networks of colonies of varying sizes, we infer that the greater incidence of transmission in groups of 10 spiders may be a product of their greater density and lower modularity.
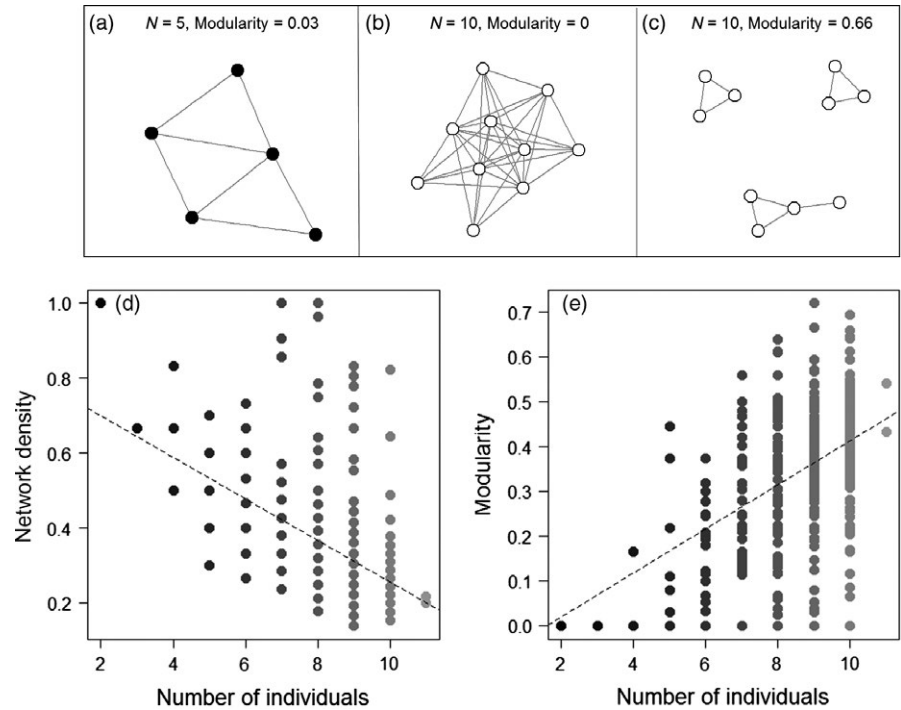
FIGURE 4 Examples of empirical networks differing in groups size and modularity: observed interaction network of (a) 5 spiders with low modularity; (b) 10 spiders with low modularity; and (c) 10 spiders with high modularity. (d) Larger networks were less dense and (e) more modular than their smaller counterparts

Group size and phenotypic composition have both been independently demonstrated as important predictors of groups' susceptibility to disease outbreaks (Gao, Bidochka, & Thompson, 2012). Here, we observed more bacterial transmission in groups of 10 individuals compared to groups of 30. This seems counterintuitive, as spiders could transmit bacteria along star networks to at least six other individuals and up to three steps away along daisy-chains, and both transmission dynamics may have played out simultaneously in social groups. In general, transmission often increases with host density for directly transmitted microbes (i.e. density-dependent transmission: Begon et al., 2002; Côté & Poulin, 1995). However, this trend is not universal (Nunn, Jordán, McCabe, Verdolin, & Fewell, 2015), and may be due to the more modular network structure of larger groups (Sah, Leu, Cross, Hudson, & Bansal, 2017). We also found that groups containing only one bold spider experienced fewer transmission events, regardless of the traits of the primary case, recapitulating the findings of an earlier study on this species (Keiser, Howell, et al., 2016). It may be that the role of a single bold spider plays in structuring the colony has important consequences for group-wide transmission (Pinter-Wollman et al., 2016). Although bold spiders were more likely to become exposed than shy spiders overall, the difference in per capita exposure was minute in groups with only one bold spider. Thus, the reduction in exposure rate in these groups is unlikely to be a product of phenotype-biased exposure. Theory incorporating both group phenotypic composition and size to predict transmission patterns is currently absent from the literature, possibly because the dynamics differ based on the focal phenotype and system under consideration (e.g. social insect caste ratio vs. the ratio of demonstrators to observers in a fish school). Nevertheless, the patterns by which individuals within a group interact (i.e. social network structure) will undoubtedly determine transmission dynamics (Hock & Fefferman, 2012; Stroeymeyt,

Casillas-Pérez, & Cremer, 2014), especially if interaction patterns change across different group sizes or compositions.

We found that network modularity increased with group size, a trend commonly reported and often suggested to reduce parasite transmission in larger groups (Nunn et al., 2015; Sah et al., 2017). High network modularity suggests that because social interactions within larger groups are broken into several smaller subgroups with few connections between subgroups, transmission in large groups (e.g. of 30 spiders) may have been restricted to individuals within a subgroup. However, more transmission events may have eventually taken place had there been time for individuals to move between subgroups (Eubank et al., 2004). In Belding's ground squirrels, for example, juvenile males that move between groups increase the overall incidence of intestinal parasite infections by connecting individuals from different subgroups (VanderWaal, Atwill, Hooper, Buckle, & McCowan, 2013). Furthermore, smaller groups were denser, meaning their interactions networks were closer to interaction saturation compared to larger groups, thus providing more opportunities for transmission among all group members. Similarly, a positive relationship between network density and parasite transmission has been described in bumblebee colonies (Otterstatter & Thomson, 2007). Taken together, group size appears to play a large role in dictating transmission dynamics because it influences network structure, which is the organizing force that determines how many and which individuals will interact.

Our experimental manipulations of social interaction patterns further suggest that network structure may be the most important force in shaping transmission dynamics in this system. More transmission occurred in star networks than along daisy-chains. That is, auxiliary cases of bacterial exposure are more likely to arise from a series of individuals interacting directly with the primary case, rather than transmission occurring along a chain of interacting individuals. This suggests that

TABLE 3 Observed and predicted proportions of successful transmission events across four time steps in a daisy-chain network. The predicted values are calculated based on the proportion of successful transmission events from daisy-chain and star networks in the previous time step (i.e. $Pdc_n = Pdc_{n-1}...Ps_{n-1}...Pdc_{n-2} \cdot Ps_{n-2}...$)

Time step	Star network *observed*	Daisy-chain *observed*	Daisy-chain *predicted*
1 day	0.50	0.44	—
2 days	0.35	0.23	0.22
3 days	0.17	0.04	0.02
4 days	0.06	0.00	0.0001

transmission may be dose-dependent, where recipients of cuticular bacteria harbour smaller and smaller bacterial loads at each step of transmission, as has been observed in some systems (Pulkkinen, 2007). Yet, we observed that the primary case harbours a bacterial load large enough to transmit bacteria to many social partners, even many days after exposure (Figure 3). Thus, transmission along star networks appears to mimic that of "stuttering chains" that cannot initiate self-sustaining epidemics, but rather follows stochastic transmission chains that stutter towards extinction (Blumberg & Lloyd-Smith, 2013). The transmission data arising from our star networks and daisy-chains suggest that one can predict the proportion of successful transmission events for a given step in the daisy-chain via the proportion of successes in the previous time from both networks (Pdc_t and Ps_t, respectively, where t is the time step under consideration). At any given time step, one can calculate Pdc_t as:

$$Pdc_t = Pdc_{t-1} \ldots Psc_{t-2} \ldots Psc_{t-2} \ldots$$

Using this equation, we can predict the probability of transmission on a daisy-chain with high accuracy (Table 3). We hope that future studies will experimentally manipulate interaction patterns to compare transmission along these types of networks in regard to infectious diseases with variable transmission modes (Read et al., 2012).

The transmission dynamics we observed are of a benign cuticular bacteria lacking infection-induced sickness behaviours and mortality. Transmission dynamics could differ when studying coevolved host–pathogen systems or emerging disease with which hosts have no experience. Still, our study can inform the modes by which microbes are shared among hosts of social species and shape resident microbiome communities (Ezenwa, Gerardo, Inouye, Medina, & Xavier, 2012; Song et al., 2013). Our methods represent a step forward for experimental studies tracking transmission across social contacts because of our ability to connect individual traits and group properties (e.g. group size, composition) with network structure and transmission rates, and further experimentally manipulate network structure itself. We therefore hope that these data will prompt other researchers to use similar methods for evaluating the transmission of disease-causing infectious agents and/or beneficial microbes that defend against infection (e.g. chytridiomycosis; Harris et al., 2009; Rebollar, Simonetti, Shoemaker, & Harris, 2016). In conclusion, our data show that reliable and vigilant tracking of social contacts in the initial stages of an outbreak may be vital for controlling disease outbreaks (Meyers et al., 2005), and that

such interactions are likely to change based on the social context of the primary case rather than its own traits, potentially due to consequences on social network structure.

AUTHORS' CONTRIBUTIONS

C.N.K. designed the experiments; C.N.K., M.J.Z. and K.S.K. performed the experiments; N.P.W. performed social network observations and analyses; C.N.K. performed statistical analyses and prepared the manuscript with assistance from J.N.P.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.47p7c (Keiser, Pinter-Wollman, Ziemba, Kothamasu, & Pruitt, 2017).

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