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Increased bacterial load can reduce or negate the effects of keystone individuals on group collective behaviour

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In many societies certain individuals play a central role in the execution of collective behaviours and group success, termed 'keystone individuals'. To date, most studies on keystone individuals have focused on their mere presence/absence and have failed to consider how their influence changes as a function of their condition or recent experiences. Here we explore how the influence of putative keystone individuals on group collective behaviour changes as a function of recent increases in cuticular bacterial load. In the spider *Stegodyphus dumicola*, individuals that exhibit the greatest 'boldness' are important determinants of colony foraging behaviour and success. We topically exposed individual spiders that varied in their boldness to a combination of naturally occurring cuticular bacteria (*Bacillus thuringiensis*, *Microbacterium oxydans*, *Pantoea* sp.) known to be harmful to *S. dumicola*, and then tracked the effects that this exposure had on their colonies' foraging and web-building behaviour. We found that colonies with unexposed keystones attacked prey more quickly and with more attackers than colonies in which the keystone was exposed to bacteria. Moreover, the relationship between keystone individuals' boldness and colonies' attack speed differed based on whether or not the keystone was recently exposed. The number of spiders that participated in nightly web building was greater in colonies containing unexposed keystones than in colonies lacking a keystone, whereas colonies containing recently exposed keystones deployed an intermediate number of individuals. This trend, however, disappeared after the second night of observation. Together, our results suggest that a group's collective behaviour can be altered based on a single individual's recent experience with microbes.

The collective behaviours of animal societies are more than just a stunning display of biological organization; they are also a key determinant of the success or collapse of societies across the animal kingdom. The transition from solitary living to multilevel sociality has been described as one of the great evolutionary transitions in biological organization, by allowing animal societies to perform feats that are unachievable by solitary individuals (Maynard Smith & Szathmáry, 1997). Although theorists have classically maintained an egalitarian view regarding the organization of collective behaviours for many animal societies, behaviourists are becoming increasingly aware of the role that certain key individuals play in the execution and performance of collective traits. These range from well-established examples like leaders in fish schools (Bumann & Krause, 1993) to lesser-known examples like tutors in bat roosts (Knörnschild, Nagy, Metz, Mayer, & von Helversen, 2010) or knowledgeable matriarchs in elephant herds (McComb, Moss, Durant, Baker, & Sayialel, 2001). These individuals that, in some instances, exert an inordinately large effect on the success of their social group have been termed 'keystone individuals' (henceforth referred to as 'keystones'), akin to the keystone species concept of community ecology (Modlmeier, Keiser, Watters, Sih, & Pruitt, 2014; Paine, 1969).

There are myriad examples demonstrating how the presence of keystones can augment collective behaviours or enhance group productivity and survivorship (reviewed in Modlmeier, Keiser, et al., 2014). However, circumstances also exist where the presence of key individuals can dampen collective behaviours or even incite the demise of the entire group. In fact, the term 'keystone individual' was first coined to describe a particular case of destructive keystones, where the presence of so-called 'hyperaggressive males' can depress the reproductive success of entire groups of water striders (*Aquarius remiges*: Chang & Sih, 2013; Sih & Watters, 2005). A similar phenomenon had also previously been observed in yellow baboons, *Papio cynocephalus* (Alberts, Sapolsky, & Altmann, 1992). Other examples exist where the collective

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exploratory behaviour of feral guppy (*Poecilia reticulate*) schools is restricted by the least active member of the group (Brown & Irving, 2014), and the foraging preferences of dominant pairs of brant geese, *Branta bernicla*, can monopolize preferred food plants for up to 2 years (Prop & Deerenberg, 1991). Even more intriguing, however, are instances where the effects of keystones shift from positive to negative (or vice versa) as a consequence of their condition (e.g. via ageing, injury, or reproductive status; Brent et al., 2015; Horner, Proctor, Bonnie, Whiten, & de Waal, 2010; McComb et al., 2011). These cases are particularly informative because they can help us identify the factors driving the tipping point between when keystones become advantageous, disadvantageous, or entirely impotent. Unfortunately, our present understanding of how keystones' condition alters their social influence remains limited because of a scarcity of experimental evaluation. Many of the case studies mentioned above are correlative and/or unreplicated.

One instance where keystone individuals might lose their influence or where their presence may become disadvantageous is when they suffer microbial infection or an altered resident microbial community, given that infection risk is often associated with functionally important behavioural traits like social dominance (Sapolsky, 2005), aggressiveness (Jin, Su, Tao, Guo, & Yu, 2013) and exploratory behaviour (Boyer, Réale, Marmet, Pisanu, & Chapuis, 2010). Perhaps coincidentally, the most well-publicized examples of disadvantageous keystones come from epidemiology, where 'superspreader' individuals generate a disproportionately large number of secondary infection cases relative to other 'generic' infected individuals (Lloyd-Smith, Schreiber, Kopp, & Getz, 2005). Unfortunately, superspreaders are often only identified a posteriori as the index cases of larger epidemics or local transmission events in human (Gahr et al., 2014; Gibbins, 1998; Shen et al., 2004) and animal populations (Hampson et al., 2009; Kao et al., 2007; Matthews et al., 2006). Furthermore, cases where formerly influential keystone individuals, such as leaders or elites, develop microbial infections and thus potentially become detrimental (or at least impotent) to their group are almost entirely absent (but see Sapolsky & Share, 2004).

In social spiders of the genus *Stegodyphus*, the collective performance of an entire society (which can contain a few dozen to several hundred individuals) can hinge on the behaviour of one or a few key individuals (Pruitt, Grinsted, & Settepani, 2013; Pruitt & Keiser, 2014). The magnitude by which keystones augment collective foraging and group success is positively associated with the keystone's boldness, defined as the latency to resume normal activity after an aversive stimulus (Sloan Wilson, Clark, Coleman, & Dearstyne, 1994). Pruitt and Keiser (2014) recently demonstrated that the presence of just one extremely bold individual can enhance the foraging aggressiveness of an entire colony, increasing the average mass gained by their colony-mates. These keystone individuals initiate more foraging bouts than their less bold colony-mates, although their presence seems to eventually catalyse increased foraging aggressiveness in their previously shy colony-mates. Given our knowledge of the role that these keystone individuals play in their colonies, and the ease by which *Stegodyphus* spp. colonies can be manipulated experimentally in both the laboratory and field (Grinsted, Pruitt, Settepani, & Bilde, 2013; Keiser, Jones, Modlmeier, & Pruitt, 2014; Keiser & Pruitt, 2014; Pruitt et al., 2013; Pruitt & Keiser, 2014), this system represents a superb model to test questions on how interactions between keystone individuals and microbes can change the collective behaviour of their colonies. In *Stegodyphus dumicola*, experimental increases in resident cuticular bacterial load can, depending on the bacteria under consideration, result in depressed weight gain and increased mortality in some individuals, although there is no evidence that cuticular microbes alter host behaviour (Keiser et al., 2016).

Here, we test the collective behaviours of *S. dumicola* colonies containing keystones of varying boldness and bacterial exposure by presenting them with a pair of ecological challenges: prey capture and web construction/repair. We aimed to address the following three questions. (1) To what degree will a putative keystone individual's prior exposure to harmful bacteria alter the collective behaviour of the colony? (2) To what extent will the personality type (boldness) of the keystone alter the association between its bacterial exposure history and its colony's collective behaviour? (3) Does a putative keystone individual's propensity to participate in collective behaviour change based on prior exposure to harmful bacteria? Addressing these questions is important because their answers will help probe the robustness of complex systems that rely on just one or a few highly influential individuals.

METHODS

Collection and Maintenance

Stegodyphus dumicola is an Old World social spider that lives in female-biased, age-structured colonies of a few dozen to several hundred individuals throughout southwestern Africa (Avilés, Varas, & Dyreson, 1999; Henschel, 1998; Henschel, Lubin, & Schneider, 1995). Female *S. dumicola* cooperate with colony-mates in collective foraging, web maintenance and alloparental care (Bilde et al., 2007). *Stegodyphus dumicola* colonies are composed of two discrete functional units: a three-dimensional, dense silken retreat and one or a few two-dimensional capture webs where spiders interact with prey. Spiders repair damage to this capture web nightly (Keiser, Jones, et al., 2014). We collected 19 colonies of *S. dumicola* along roadside *Acacia* trees in the Northern Cape of South Africa in January 2015. Spiders were transported back to our research site near Griekwastad, Northern Cape (28°54′32.0″S, 23°24′33.7″E) where the colonies were maintained indoors in 500 ml plastic cups at ambient temperature and natural light:dark cycle. Prior to experimentation, we isolated each adult female from the colony in 1 ml plastic condiment cups.

Bacteria Collection, Identification and Maintenance

We collected bacteria from the cuticles of adult female spiders in the field following aseptic technique by swabbing the cuticle of a haphazardly chosen spider with a sterile cotton-tipped swab and plating these isolates directly on Luria-Bertani (LB) broth. Bacteria were identified using 300 bp 16S ribosomal DNA sequencing and MicroSeq® BLAST Software (SeqWright Genomic Services Houston, TX, U.S.A.). From the cuticles of three different spiders, we identified *Microbacterium oxydans*, *Bacillus thuringiensis* and *Pantoea* sp., among others not used in the present study. Bacteria were stored in 25% glycerol at −80 °C until the onset of experimentation. Resurrected bacteria were maintained on LB agar, and liquid cultures were prepared by isolating a single colony with a sterile micropipette tip and placing it in 1 ml of LB broth overnight at ambient room temperature. Directly prior to experimental application, equal parts of the three bacterial liquid monocultures were mixed to form a bacterial 'cocktail'. Although we were unable to estimate cell densities for these bacterial solutions, previous experiments have verified that the cell density of this cocktail (estimated via optical density) when grown in this way is not significantly different from that of each bacterial species contained therein when grown in monoculture (Keiser et al., 2016).

Experimental Bacterial Exposure

We exposed putative keystone individuals to bacteria 24 h prior to their introduction into experimental colonies. The spiders were placed individually in 1 ml of bacterial cocktail or sterile LB agar and shaken in the solution at 1500 revolutions/min for 3 s with a vortex to disrupt the hydrophobic barrier of the spider's cuticle and completely coat the spider with the solution. Admittedly, we are unsure of any potential spatial components of colonization by these resident bacteria via this application technique. That is, to what degree, and for how long these bacteria colonize different parts of the cuticle or host body cavity is presently unknown. Thus, we will henceforth refer to spiders that were exposed to bacteria as 'exposed' and those that were exposed only to sterile LB broth as 'unexposed' or 'control' for the sake of brevity. Previous work verified that a concomitant topical application of these three bacteria results in weight loss and higher mortality rates (median time to death = 16 days, versus 27 days for control spiders; Keiser et al., 2016). Prior to bacterial exposure, keystone individuals were given a dot of nontoxic blue acrylic paint atop their cephalothorax so we could track their behaviour within the colonies.

Bacterial Load Assay

To quantify the degree to which our experimental application of bacteria altered the cuticular bacterial communities of our focal spiders, we conducted a bacterial load assay by exposing spiders as before with either the bacterial cocktail ($N = 16$), sterile LB broth ($N = 16$), or no exposure ($N = 16$). We then estimated the bacterial load on the cuticles of four spiders per day for 4 days from each treatment group by vortexing the spiders in 1 ml of LB broth at 2000 revolutions/min for 10 s. We then performed four 10-fold serial dilutions of this solution in LB broth and plated 100 µl of each dilution onto LB agar and spread the solution evenly across the surface of the agar with a sterile polystyrene cell spreader (Sigma-Aldrich, St Louis, MO, U.S.A.). We incubated these plates for 24 h at 30 °C and counted the number of bacterial colonies visible to the naked eye for the dilution amount where colonies were separated from each other and could be reliably counted (between 30 and 300 colonies). The number of colony forming units (CFU) counted was multiplied by the dilution factor of that plate (i.e. multiplied by 100 for a dilution factor of 1:100) to estimate the CFU/ml that had been transferred to the LB broth from the spider's cuticles. Prior to exposure, we also measured the prosoma width and mass of each spider with digital callipers and an analytical balance (Model P-114, Denver instruments, Bohemia, NY, U.S.A.), respectively. We also estimated the body condition of each spider by calculating the residuals of a linear regression of spiders' body mass on body size (Jakob, Marshall, & Uetz, 1996).

Experimental Colony Construction

We collected adult female spiders from five source colonies for the collective foraging experiment and seven source colonies for the web maintenance experiment. We measured each spider's mass and prosoma width, and then subjected them to an anti-predator behavioural assay to determine their 'boldness', defined as the latency to resume movement after receiving an aversive stimulus (Sloan Wilson et al., 1994). Boldness in *S. dumicola* is a highly consistent behavioural metric (repeatability ≈ 0.5–0.7) (Keiser, Jones, et al., 2014; Keiser, Modlmeier, Singh, Jones, & Pruitt, 2014; Pruitt et al., 2013) that is linked with individuals tendencies to perform a variety of tasks (Keiser, Jones, et al., 2014; Wright, Keiser, & Pruitt, 2015).

We used an assay developed by Riechert and Hedrick (1993), where the spider is placed in a black plastic arena (12 cm diameter × 4 cm height) and given a 30 s acclimation period. We then administered two rapid puffs of air to their anterior prosoma using an infant nose-cleaning bulb and measured their latency to resume activity. We allowed spiders 600 s to resume movement, where 'bold' individuals resume activity more quickly and 'shy' individuals have longer latencies to resume activity. We categorically define 'shy' spiders as those that did not resume activity during the assay. Prior to analyses, we inverted the latency of a spider to resume movement (maximum latency of 600 s − spider latency) to make the interpretation of results more intuitive. That is, a higher boldness score represents bolder behaviour (i.e. spiders that resume activity faster after the stimulus).

We then constructed artificial colonies containing nine 'shy' spiders (latency score = 600 s) and later added one bolder spider, the putative keystone individual (latency score range 1–600 in both treatment groups), in 240 ml clear plastic cups. Keystones were assigned to treatment groups (exposed versus unexposed) and experimental colonies haphazardly, and individuals were not mixed from different source colonies to maintain naturally occurring levels of within-colony familiarity (Modlmeier, Laskowski, et al., 2014) and relatedness (Lubin & Bilde, 2007).

Collective Foraging Assay

Experimental colonies ($N = 30$ exposed keystone; $N = 29$ control keystone) containing nine shy spiders were hung in *Acacia mellifera* trees with clothespins at 2000 hours to allow capture web construction/expansion overnight. At 0600 and 1800 hours the following day, we measured their collective foraging by placing a 1.5 cm^2 piece of paper in the centre of the capture web, allowing a 30 s acclimation, and vibrating the paper with a wire attached to a hand-held vibrator (Model: Flamenco Purple no. 4, Golden Triangle). The vibrator was set to a low-frequency 'pulse' setting with a pulse frequency of approximately 3 pulses/s. This stimulus is meant to simulate the flittering behaviour of a prey item captured in the web. We then recorded (1) the latency for the first spider to emerge from the retreat, (2) the latency for the first spider to attack the paper and (3) the total number of individuals that participated in the attack sequence. After these two collective foraging assays, we added a putative keystone individual to each colony at 2000 hours. The keystone individuals had been exposed either to the bacterial cocktail described above or to a control treatment of sterile LB broth. We measured the collective foraging as before twice per day for the next 3 days (six measurements total) and noted whether or not the keystone participated in the attack.

Web Maintenance Assay

We constructed an additional 34 experimental colonies containing nine shy spiders as before, but placed these colonies into one of three treatment groups: (1) the introduction of an exposed keystone individual; (2) the introduction of a control keystone individual exposed only to sterile LB broth; or (3) colonies into which a keystone individual was never introduced. We allowed these experimental colonies 24 h to build a retreat in 240 ml plastic cups, and then placed the colonies in *A. mellifera* trees at 2000 hours as before. At 0500 hours the following two mornings, we assessed the capture web area of each colony by estimating the approximate shape of the capture web (e.g. rectangle, triangle, etc.) and then measuring each of the sides using a tape measurer to calculate the total area (cm^2). We scanned each colony between 1900 and 2000 hours each night for the next five nights to count how many individuals were actively participating in web maintenance. We

also noted whether or not the keystone was participating in this task.

Statistical Analyses

Bacterial load estimates

Bacterial load data were log transformed and analysed with a general linear mixed model (GLMM) with keystone exposure, spider prosoma width, days since exposure and a days since exposure * treatment interaction term as independent variables. Source colony ID was included as a random effect in the model.

Collective foraging

First, to test whether the addition of exposed versus control keystones was associated with a change in the collective behaviour of colonies, we performed two separate GLMMs (control versus exposed colonies separately) with absence versus presence of a keystone as an independent variable predicting (1) the latency for the first spider to emerge, (2) the latency for the first spider to attack the paper and (3) the number of individuals that participated in collective foraging. We excluded instances where the keystone individual initiated the attack in order to test the effect of their presence on the behaviour of their colony-mates. To further analyse the effect of keystone bacterial exposure and boldness on colonies' collective foraging, we performed separate GLMMs predicting latency to emerge, latency to attack and the number of individuals that participated in the attack with keystone exposure (exposed versus control), keystone boldness score and a keystone exposure * boldness interaction term. Again, we removed from the analysis any instance where the keystone individual initiated the attack. This helped us characterize the effect that keystone individual's exposure history had on the behaviour of other colony members, rather than on the keystone individual itself.

Collective web maintenance

To analyse differences in capture web area and the number of individuals actively repairing the web at night, we used separate GLMMs with keystone status (exposed, control, or no keystone), keystone boldness score and a keystone status * boldness interaction term as independent variables. Assay number was also included as a random effect in models predicting collective foraging measurements. For the models predicting the number of individuals that participated in web maintenance, we excluded the keystone individual from the number of participating individuals counted.

Keystone task participation

Participation in both collective foraging and web maintenance were analysed with binomial logistic regressions with keystone boldness, keystone exposure, observation number, keystone exposure * observation number and a keystone exposure * boldness interaction term as independent variables. Response variables were whether or not the individual participated in the task (participated versus did not participate). We removed colonies where a keystone was absent from the analysis predicting keystone participation in web maintenance. For all statistical analyses, source colony ID and experimental colony ID nested within source colony ID were included as random effects in our statistical models. All analyses were performed in JMP version 12.0 (SAS Institute Inc., Cary, NC, U.S.A.).

RESULTS

Bacterial Load

On average, exposure to the bacterial cocktail was associated with an increased cuticular bacterial load two to three orders of magnitude greater than that estimated for the cuticles of control spiders, which did not differ significantly from that of untreated spiders ($F_{2,28.6} = 22.0$, $P < 0.0001$; Fig. 1, Table 1). Across all treatments, estimations of bacterial load decreased over the next 4 days after exposure ($F_{1,34.7} = 9.2$, $P = 0.005$; Fig. 1). Lastly, our estimations of cuticular bacterial load were not influenced by the body size measurements of the spiders ($F_{1,25.8} = 0.004$, $P = 0.95$).

Collective Foraging

Colonies that were assigned keystones of different bacterial exposure statuses did not differ in terms of their collective foraging behaviour before the addition of keystones (all $P \geq 0.37$). For colonies where a control keystone was added, colonies attacked prey stimuli faster ($F_{1,118.1} = 4.19$, $P = 0.05$) and with more individuals ($F_{1,116.3} = 6.30$, $P = 0.01$) after the addition of the keystone individual. However, in colonies where an exposed keystone was added, the latency for the colony to attack a prey stimulus and the number of attackers that participated were not different from those before the keystone was added (all $P > 0.28$). That is, colonies with an exposed keystone appeared no different from colonies completely lacking a keystone individual. Neither keystone boldness, exposure status, nor the exposure * boldness interaction term were significantly associated with the latency for the first individual to emerge from the colony retreat after the onset of the simulated prey stimulus (all $P \geq 0.15$; Table 1).

Colonies containing exposed keystones attacked prey stimuli more slowly than colonies containing control keystones ($F_{1,118.1} = 4.19$, $P = 0.05$; Fig. 2a). An exposure * boldness interaction term was also significant in our model predicting colonies' latency to attack prey ($F_{1,31.8} = 4.93$, $P = 0.03$; Fig. 3). That is, the relationship between the boldness of the keystone individual and the colony's collective foraging was positive ($r^2 = 0.06$) for colonies containing exposed keystones. In contrast, we did not detect a significant relationship between keystone boldness and latency to attack in the control treatment.

On average, nearly twice as many individuals participated in staged foraging events in colonies containing a control keystone

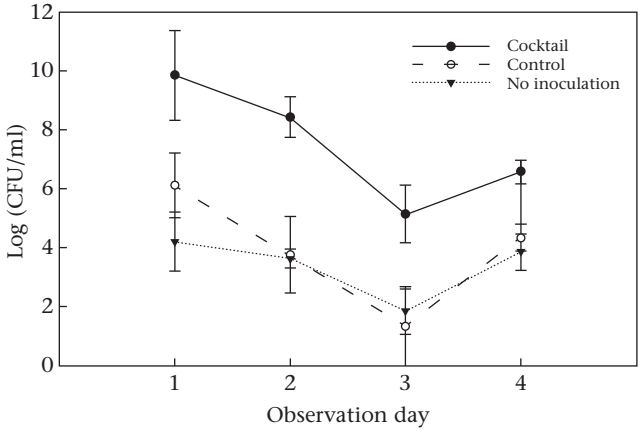

Figure 1. Cuticular bacterial load (colony forming units, CFU) for spiders in each exposure treatment (bacterial cocktail; LB control; no inoculation) across observation days 1–4. Values are means ± SE.

Table 1
Summary of effect tests from three general linear models and a nominal logistic regression predicting different aspects of the collective foraging of colonies containing keystone individuals of varying boldness and bacterial exposure

Source	*df*	*F*	χ^2	*P*
Latency to emerge				
Keystone exposure	1, 31.0	3.44		0.07
Keystone boldness	1, 26.8	0.10		0.76
Keystone exposure*boldness	1, 30.3	2.18		0.15
Latency to attack				
Keystone exposure	1, 32.2	4.25		**0.05**
Keystone boldness	1, 27.8	0.70		0.41
Keystone exposure*boldness	1, 31.8	4.93		**0.03**
Number of attackers				
Keystone exposure	1, 36.3	13.24		**0.001**
Keystone boldness	1, 32.2	0.09		0.76
Keystone exposure*boldness	1, 38.1	2.6		0.12
Participation by keystone				
Keystone exposure	1		0.32	0.57
Keystone boldness	1		0.006	0.94
Keystone exposure*boldness	1		1.30	0.25
Observation number	1		3.06	0.08
Keystone exposure*observation	1		0.009	0.92

Significant *P* values are shown in bold.

compared to colonies containing an exposed keystone ($F_{1,36.3} = 13.24$, $P = 0.0008$; Fig. 2b). Prior to the addition of keystones, the number of individuals observed participating in collective foraging was nearly identical for colonies in both treatments. However, the proportion of trials in which the keystone individual participated in foraging events did not differ based on the keystone's boldness or bacterial exposure (all $P \geq 0.08$; Table 1).

Web Maintenance

Colonies' capture web area (cm^2) did not differ between colonies containing keystones of either exposure status, or colonies where a keystone was absent (all $P \geq 0.23$; Table 2). Colonies containing control keystones deployed nearly twice as many web-builders each night as colonies in which a keystone was absent, while colonies containing an exposed keystone deployed an intermediate number of web-builders. This trend, however, changed over time ($F_{8,95.1} = 2.94$, $P = 0.006$; Fig. 4). That is, the number of spiders maintaining the web at night was only greater in colonies containing keystones of different exposure status compared to those lacking a keystone completely for the first two nights of observations. Finally, the proportion of observations in which the keystone individual participated in web maintenance did not differ based on the keystone's boldness or bacterial exposure (all $P \geq 0.12$; Table 2).

DISCUSSION

The successful execution of a group's collective behaviours can often depend on the actions of just one or a few keystone individuals. Although, how such individuals' conditions or recent experiences affect group behaviours remains little explored. Here, using the social spider *S. dumicola*, we demonstrate that recent

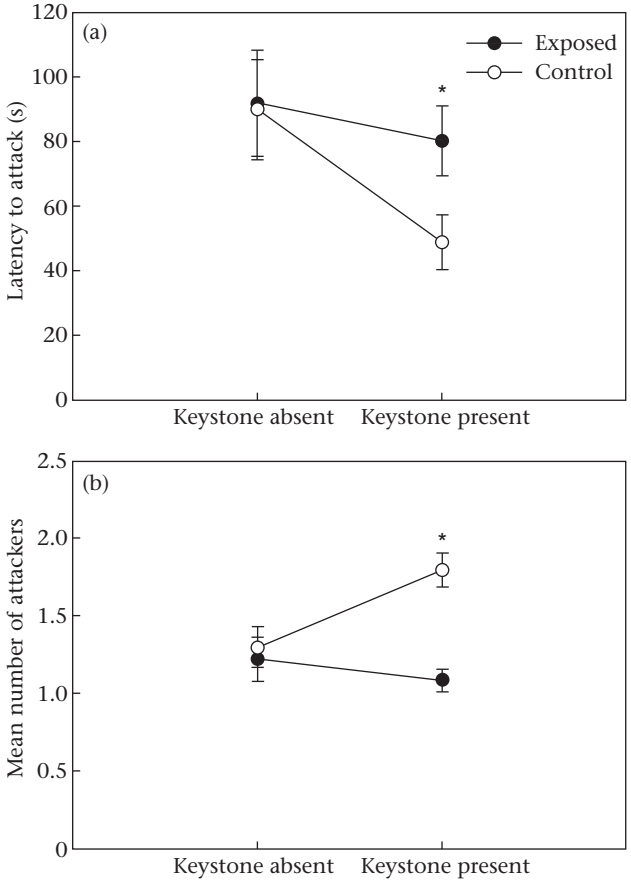

Figure 2. Effects of keystone presence and treatment (bacterial exposure vs LB control) on collective foraging: (a) latency to attack prey; (b) number of attackers. Significant differences ($P < 0.05$) are denoted with an asterisk. Values are means ± SE.

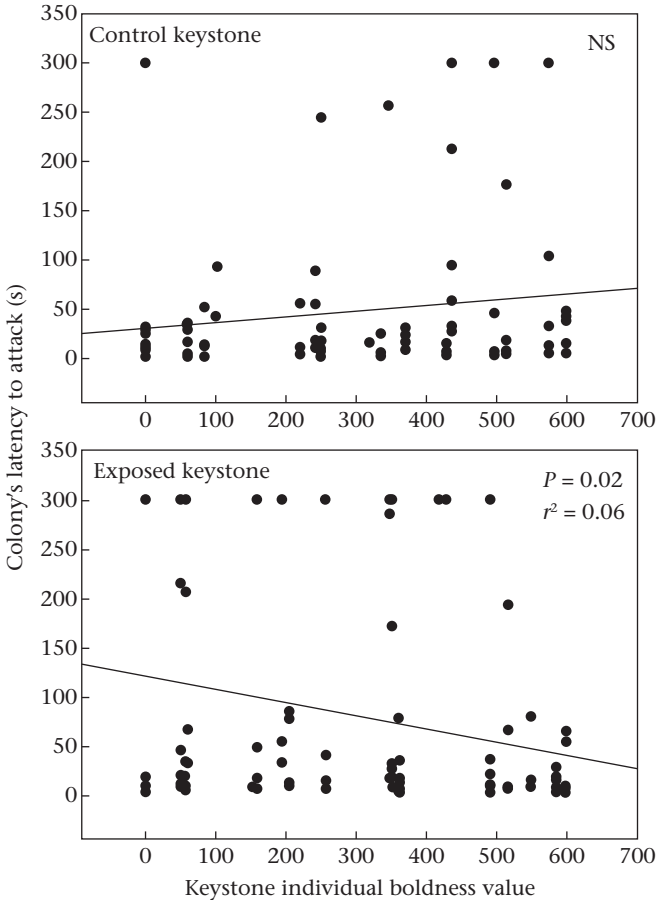

Figure 3. Effects of keystone boldness and treatment (bacterial exposure vs LB control) on each colony's latency to attack a prey item.

Table 2
Summary of effect tests from two general linear models and a nominal logistic regression predicting different aspects of the collective web maintenance of colonies containing keystone individuals of varying boldness and bacterial exposure

Source	*df*	*F*	χ^2	*P*
Capture web area				
Keystone status	2, 28	0.14		0.87
Keystone boldness	1, 17	0.20		0.66
Keystone status∗boldness	1, 17	1.54		0.23
Number of spiders active				
Keystone status	2, 27.4	1.79		0.19
Night of observation (no.)	4, 95.2	6.41		**0.001**
Keystone status∗night no.	8, 95.1	2.94		**0.006**
Keystone boldness	1, 26.2	0.37		0.55
Keystone status∗boldness	1, 21.7	0.39		0.54
Participation by keystone				
Keystone status	1		0.78	0.38
Night of observation (no.)	4		3.97	0.41
Keystone status∗night no.	4		1.19	0.88
Keystone boldness	1		0.02	0.90
Keystone status∗boldness	1		0.05	0.82
Night of observation (no.)	4		3.99	0.41
Keystone status∗night no.	4		0.43	0.98

Significant *P* values are shown in bold.

increases in keystone individuals' cuticular bacterial load can alter the collective behaviour of their colonies. Specifically, colonies containing recently exposed keystone individuals attacked prey items more slowly and with fewer attackers than colonies whose keystone individuals were not exposed. Less intuitively, the relationship between the boldness of keystone individuals' and the collective behaviour of their colony changed based on the keystone's recent bacterial exposure history. That is, colonies containing bolder keystones attacked prey more quickly than colonies with less bold keystones only when the keystones were exposed to bacteria. Lastly, colonies containing unexposed keystones were more active during nocturnal web-building forays than colonies where keystones were absent. In this case, colonies containing recently exposed keystones were initially intermediate in terms of their web-building activity. Taken together, our results suggest that a keystone individual's recent exposure to bacteria can have context-dependent effects on the collective behaviour of its colony.

The success of social spider colonies is often dependent on the spiders' ability to capture and consume large and particularly profitable prey items (Nentwig, 1985; Powers & Avilés, 2007; Rypstra & Tirey, 1991). In the present study, colonies containing keystones that were recently exposed to a cocktail of harmful bacteria attacked prey with half as many attackers, on average. This is potentially problematic for the colony, because collective foraging relies on the ability to subdue large prey and this requires the recruitment of large numbers of attackers (Harwood & Avilés, 2013; Rypstra & Tirey, 1991). Thus, we argue that the differences in collective foraging behaviour displayed by colonies with exposed versus unexposed keystones could result in reduced foraging efficiency (Krafft & Cookson, 2012; Ward & Enders, 1985) and potentially lower colony success. Consistent with this extrapolation, previous laboratory experiments (Pruitt & Keiser, 2014) showed that colonies with bolder keystone individuals attack prey with more attackers and that this is associated with heightened mass gains and survival rates for the whole colony.

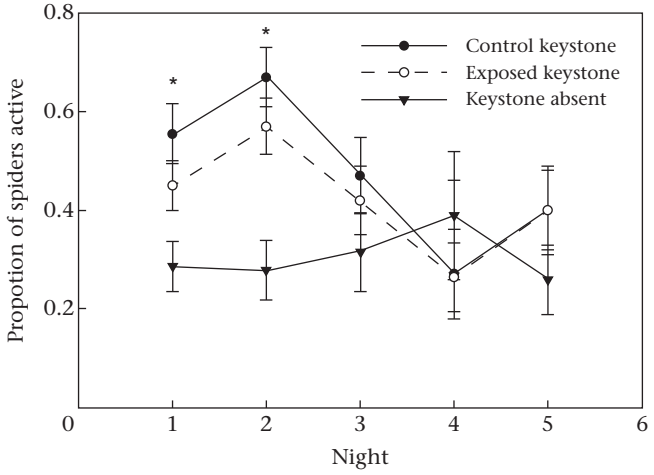

Figure 4. Effects of keystone presence and treatment (bacterial exposure vs LB control) on colony web construction/maintenance. Significant differences ($P < 0.05$) are denoted with an asterisk. Values are means ± SE.

Notably, we detected no relationship between keystone boldness and latency to attack for colonies containing unexposed keystones. This is intriguing, as numerous laboratory experiments on *S. dumicola* (Pruitt & Keiser, 2014; Pruitt & Pinter-Wollman, 2015) and a field experiment with a congener (Pruitt et al., 2013) have suggested that heightened boldness of the boldest individual in the group can increase the speed with which colonies attack prey stimuli. This effect appears to vanish under field conditions. Unfortunately, any number of factors could have differed between former studies and this one. For example, spiders in the field experience abiotic and biotic stressors that are not an issue in laboratory settings (e.g. strong predation risk; Henschel, 1998; Keiser, Wright, & Pruitt, 2015), and colonies in the field have ad libitum space to construct large capture webs, resulting in a greater distance between the colony's retreat and the prey stimulus (maximum capture web size: in our field experiment: 990 cm^2; in the laboratory: ~100 cm^2). This may further obscure our ability to detect the effects of keystones on colonies' latency to attack. Why we did see a trend between keystone boldness and latency to attack in exposed colonies is a more interesting unresolved mystery. In contrast, the catalytic effects of keystone individuals on the number of attackers that respond to prey and the number of individuals that participate in web building are much clearer.

One wonders whether potential 'sickness behaviours' of exposed keystone individuals may be responsible for the observed patterns, since recent studies have demonstrated that early exposure to pathogens can decouple the consistency of individual personalities (DiRienzo, Niemelä, Skog, Vainikka, & Kortet, 2015). Admittedly, it is still too premature to make any grandiose mechanistic statements, especially since (1) previous data suggest that exposure to this cocktail does not alter host boldness (Keiser et al., 2016) and (2) we found no evidence that the propensity for keystones to participate in collective behaviours was altered by bacterial exposure. Thus, indirect evidence from these two conclusions suggests that keystone boldness and task participation is not altered after exposure to this bacterial cocktail. However, evidence from the decreased participation in collective foraging and web maintenance by the colony-mates of exposed keystones makes it difficult to ignore the possibility of sickness behaviour playing a role in their reduced involvement. At present, we still disfavour this interpretation because the individual with the highest bacterial load of all (i.e. the keystone itself) did not alter its involvement in any task (all $P > 0.25$). This interpretation therefore, while still possible, does not stand to reason. Another potential explanation is that keystone individuals more readily transmit these bacteria to their shy colony-mates, thus depressing their task participation. Again, at odds with this interpretation, preliminary evidence suggests that shy *S. dumicola* exhibit stronger immunocompetence than their bolder colony-mates (Keiser, DeMarco, Shearer, Robertson, & Pruitt, 2015), so this remains an unlikely

explanation for the depressed collective behaviour of colonies composed of mostly shy individuals. Unfortunately, the studies described herein were not designed to elucidate underlying mechanisms. Only identifying causal linkages between the physiological symptoms of exposed keystones, exposed nonkeystones, detailed social interactions and putative sickness behaviour will truly resolve these explanations (Klein, 2003; Lopes, Adelman, Wingfield, & Bentley, 2012).

One could argue that the mere addition of one more exposed or unexposed colony member (keystone or otherwise) could be responsible for the increased number of attackers in the unexposed keystone treatment. However, three lines of evidence are at odds with interpretation. First, the number of attackers nearly doubled in colonies where an unexposed keystone was added, despite only an 11% increase in group size, verifying that the addition of an unexposed keystone had an uncharacteristically large effect. Second, keystones were no more or less likely to participate in the staged foraging event based on their boldness or exposure status (all $P \geq 0.31$; Table 1), indicating that their effects on collect behaviour are not merely the result of their direct participation in prey capture. Third, all of our analyses were conducted after removing all instances where the keystone individual had direct involvement with the task. Therefore, the results presented herein reflect the behaviour of all other colony members and not the participatory behaviour of the keystone itself. Thus, as documented in previous studies (Pruitt & Keiser, 2014), the presence of a keystone appears to catalyse more aggressive foraging behaviour in their otherwise shy colony-mates, but this effect was not observed when the keystone had recently been exposed to bacteria.

Bacterial exposure appears to have only a subtle impact on colony web-building behaviour. We first showed that colonies containing exposed keystones, unexposed keystones, or no keystones at all had similarly sized captured webs. This is not an entirely shocking result given that a previous study on *S. dumicola* indicated that even large differences in group composition had little or no effect on capture web size (Keiser & Pruitt, 2014). However, when an unexposed keystone was added to a colony, we observed roughly twice as many spiders engaged in web-building behaviour than in colonies lacking a keystone individual, again suggesting a potential catalytic effect of keystone presence. Colonies containing an exposed keystone were intermediate in web-building activity but more closely resembled the unexposed keystone treatment. Interestingly, even a colony containing an exposed keystone deployed 50–200% more web-builders than colonies in which the keystone was absent. Thus, the effects of the keystone exposure treatment seem modest in this context. We further observed that the effects of keystones on web-building behaviour disappeared with time (i.e. by night 3 or 4). While there are numerous potential explanations for this pattern, we propose that the most reasonable explanation is that colonies had largely finished constructing their webs by night 3 or 4 (Fig. 4).

Recent developments in metagenomic research have determined that the composition, evenness and successional state of an individual's microbiome can have strong effects on health, life history and behaviour in diverse taxa (Cho & Blaser, 2012; Ezenwa, Gerardo, Inouye, Medina, & Xavier, 2012; McFall-Ngai et al., 2013; Newton, Sheehan, Lee, Horton, & Hicks, 2013; Zilber-Rosenberg & Rosenberg, 2008). Furthermore, many empirical and theoretical investigations have made strides towards understanding the role of host–microbiome interactions in the evolution and functioning of animal societies (Cornman et al., 2012; Poulsen & Sapountzis, 2012; Sanders et al., 2014; Scheuring & Yu, 2012). Our manipulative experiments here, although much less complex in nature than microbiome studies, indicate that bacteria could also have a large effect on the functioning of animal societies in the context of keystone individuals and group collective behaviours. The size of these impacts depends, however, on the particulars of the system and the collective traits under consideration. Given that keystone individuals have been identified as significant determinants of collective behaviours in a variety of social groups (e.g. ant colonies, elephant herds, fish schools, etc.; Modlmeier, Keiser, et al., 2014), our findings suggest that a single individual's experiences with microbes could play an important and unappreciated role in determining the collective behaviour of social groups.

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