

**GROUP COMPOSITION IN SOCIAL SPIDERS: COLLECTIVE BEHAVIOR,
KEYSTONE INDIVIDUALS, AND BACTERIAL TRANSMISSION DYNAMICS**

by

Carl Nicolas Keiser

Bachelor of Arts, Arcadia University, 2009

Master of Science, Georgia Southern University, 2012

Submitted to the Graduate Faculty of the
Dietrich School of Arts and Science in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

University of Pittsburgh

2016

UNIVERSITY OF PITTSBURGH
Dietrich School of Arts and Science

This dissertation was presented

by

Carl Nicolas Keiser

It was defended on

April 18, 2016

and approved by

Corinne L. Richards-Zawacki, Associate Professor, Department of Biological Sciences

Jon P. Boyle, Associate Professor, Department of Biological Sciences

Wayne M. Getz, Professor of Wildlife Ecology, University of California Berkeley

Jonathan N. Pruitt, Assistant Professor, University of California Santa Barbara

Dissertation Advisor: Walter P. Carson, Associate Professor, Department of Biological
Sciences

Copyright © by Carl Nicolas Keiser

2016

GROUP COMPOSITION IN SOCIAL SPIDERS: COLLECTIVE BEHAVIOR, KEYSTONE INDIVIDUALS, AND BACTERIAL TRANSMISSION DYNAMICS

Carl Nicolas Keiser, M.S., PhD

University of Pittsburgh, 2016

The global success of animal societies is due, in part, on the ability of groups of animals to perform collective behaviors that would be unachievable by a single individual. One major determinant of collective behavior is the composition of different types of individuals within the group. For example, individuals often differ consistently in their behavioral traits and the tasks in which they participate, and a group's composition of individuals expressing different behavioral phenotypes (i.e., "personalities") can be a fundamental driver of collective behaviors. Though, the same compositions that excel in one aspect of collective behavior may also incur costs in other, opposing patterns of social interactions. Here, I use the social spider *Stegodyphus dumicola* to test how group personality composition explains patterns of collective behaviors, social interactions, and bacterial transmission. *Stegodyphus dumicola* is an African social spider that lives in colonies of several dozen to many hundreds of individuals whose collective behaviors are determined by the composition of "bold" and "shy" spiders present in the colony. I found that group personality composition is a more important predictor of the execution of collective behaviors than more conspicuous colony traits like group size. Then, using social network analyses I found that colony contact networks are disassortive, where individuals preferentially interact with others of opposing personality types. Using experimental exposures to a fluorescence-transformed cuticular bacterium (*Pantoea* sp.), I found that horizontal bacterial transmission is predominantly directional, occurring more so from "bolder" to "shyer" spiders.

Thus, it could be reasoned that animal groups containing diverse personality types may experience augmented success during collective tasks, but may also incur costs in the context of horizontal bacterial transmission. Taken together, it appears that personality compositions may impose constraints on the social organization of animal societies.

TABLE OF CONTENTS

PREFACE.....	XV
1.0 INTRODUCTION.....	1
2.0 EXPLORING THE EFFECTS OF INDIVIDUAL TRAITS AND WITHIN- COLONY VARIATION ON TASK DIFFERENTIATION AND COLLECTIVE BEHAVIOR IN A DESERT SOCIAL SPIDER	4
2.1 INTRODUCTION	4
2.2 METHODS.....	7
2.2.1 Study system.....	7
2.2.2 Individual level personality assays.....	9
2.2.3 Colony-level personality assays	10
2.2.4 Statistical analyses	12
2.3 RESULTS.....	14
2.3.1 Individual personality assays.....	14
2.3.2 Task performance.....	15
2.3.3 Colony level personality assays	15
2.4 DISCUSSION.....	16
2.4.1 Task differentiation	17
2.4.2 Colony-level personality.....	18

2.4.3	Synthesis of social spider literature	20
2.4.4	Conclusions.....	22
3.0	PERSONALITY COMPOSITION IS MORE IMPORTANT THAN GROUP SIZE IN DETERMINING COLLECTIVE BEHAVIOR IN THE WILD	34
3.1	INTRODUCTION	34
3.2	METHODS.....	37
3.2.1	Study species and field site.....	37
3.2.2	Individual personality assays.....	37
3.2.3	Collective behavior assays.....	38
3.2.4	Statistical analyses	40
3.3	RESULTS	40
3.3.1	Web architecture and dispersal.....	40
3.3.2	Prey capture	41
3.3.3	Web evacuation.....	42
3.4	DISCUSSION.....	43
3.4.1	Web architecture and dispersal.....	43
3.4.2	Prey capture	44
3.4.3	Web evacuation.....	46
3.4.4	Conclusions.....	47
4.0	INCREASED BACTERIAL LOAD ON KEYSTONE INDIVIDUALS ALTERS GROUP COLLECTIVE BEHAVIOUR.....	53
4.1	INTRODUCTION	53
4.2	METHODS.....	57

4.2.1	Collection and maintenance.....	57
4.2.2	Bacterial collection, identification, and maintenance.....	57
4.2.3	Experimental bacterial exposure	58
4.2.4	Bacterial load assay	59
4.2.5	Experimental colony construction	60
4.2.6	Collective foraging assay.....	61
4.2.7	Web maintenance assay	62
4.2.8	Statistical analyses	62
4.3	RESULTS	64
4.3.1	Bacterial load	64
4.3.2	Collective foraging.....	64
4.3.3	Web maintenance	66
4.4	DISCUSSION.....	66
5.0	INDIVIDUAL DIFFERENCES IN BOLDNESS INFLUENCE PATTERNS OF SOCIAL INTERACTIONS AND THE TRANSMISSION OF CUTICULAR BACTERIA AMONG GROUP-MATES.....	78
5.1	INTRODUCTION	78
5.2	METHODS.....	82
5.2.1	Animal collection and maintenance	82
5.2.2	Behavioral assays.....	82
5.2.3	Social interactions.....	83
5.2.4	Bacterial exposure	84
5.2.5	Transmission experiments	85

5.2.6	Statistical analyses	86
5.3	RESULTS	88
5.3.1	Social network analyses.....	88
5.3.2	Bacterial transmission.....	88
5.4	DISCUSSION.....	90
5.4.1	Network patterns and bacterial transmission.....	91
5.4.2	Transmission through contact vs. shared silk.....	92
5.4.3	Future directions.....	93
	BIBLIOGRAPHY	100

LIST OF TABLES

Table 2-1 (a) Tests for repeatability in individual and colony-level behavioral assays. (b) Associations between individual-level personality and individuals' body size or condition.	23
Table 2-2. Results of three ANOVAs predicting the relationship between individuals' traits (behavioral tendencies, body size, body condition) and their participation in various colony tasks.	24
Table 2-3. Model comparison of those obtained via independent model selection procedures predicting latency of emergence, latency of attack, and number of individuals participating in ambient web-building in experimental colonies.	25
Table 2-4. Comparisons in the relationship between individual traits and colony social organization and success across nine species of social spider, representing eight independent derivations of sociality.	26
Table 3-1. Summary of effect tests from three general linear models predicting three aspects of colonies' collective foraging behavior in the wild. An asterisk denotes significant values.	48
Table 4-1. 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.	72

Table 4-2. 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. 73

Table 5-1. (A,B) Two nominal logistic regressions predicting the presence or absence of CNK02 on the cuticles of susceptible individuals after interacting with exposed individuals directly or indirectly. (C) A GLMM predicting the estimated bacterial load collected 95

LIST OF FIGURES

Figure 2-1. Frequency histograms depicting the average personality traits exhibited by individual female <i>S. dumicola</i>	29
Figure 2-2. Individuals with larger than average mass for their body size, an indication of body condition, were less likely to participate in foraging-related tasks.....	30
Figure 2-3. Larger individuals were less likely to participate in ambient web construction after during colony expansion into a larger environment.	31
Figure 2-4. The latency of emergence from the nest after prey stimulus increased along with within-colony variance in individuals' prosoma width.	32
Figure 2-5. Colonies' latency to attack a prey item increased along with within-colony variance in aggression.	33
Figure 3-1. Without a capture web, the latency for the first individual to emerge from the retreat after prey stimulus was a product of the colony's personality composition. Values significantly different from each other are represented by different letters within each panel	49
Figure 3-2. Without a capture web, colonies composed of all shy individuals were 1.3 times faster in attacking the prey stimulus when their colony size was thirty as opposed to ten. Values significantly different from each other are represented by different letters within each panel. ...	50

Figure 3-3. The number of individuals which participated in the attack was influenced both by the composition of the group and the group size. Values significantly different from each other are represented by different letters within each panel. 51

Figure 3-4. A speculative depiction of the predicted relationship between colony size and participation in collective prey capture for shy/average colonies (solid line). 52

Figure 4-1. Bacterial exposure increases cuticular bacterial load..... 74

Figure 4-2. Bacterial exposure alters colony collective foraging. 75

Figure 4-3. Keystone boldness, bacterial exposure, and collective foraging..... 76

Figure 4-4. Bacterial exposure and web construction/maintenance. 77

Figure 5-1. Interaction patterns among 19 spiders in an experimental colony of marked individuals..... 96

Figure 5-2. Average assortativity of all observed networks (red vertical line) is significantly smaller than the average assortativity of each of the 10,000 sets of randomized networks (histogram)..... 97

Figure 5-3. Spiders under long-wave UV light: (a) exposed to with sterile PBS (control). (b) 4h after exposure to CNK02. (c) 48h after exposure to CNK02. (d) An unexposed spider that has interacted with an exposed spider for 24h. Areas of green fluorescence suggest the presence of *Pantoea* +pGLO. 98

Figure 5-4. Direct transmission of cuticular bacteria is influenced by the phenotypes of both exposed and susceptible individuals. (a) a greater proportion of pairs in which the exposed individual was bolder resulted in successful transmission and (b) transmission was more likely when exposed individuals were bolder than their susceptible colony-mates (positive values denote a bolder exposed individual, while negative values denote a bolder susceptible). (c)

Transmission was also more likely when susceptible individuals had a positive body condition, *i.e.* weighed more than predicted based on their body size. 99

PREFACE

I extend a distinct appreciation to my advisor, Jonathan N. Pruitt, for his unending support for my development as a scientist and mentor. I would like to acknowledge the South Africa Department of Tourism, Environment, and Conservation for providing permits for animal collection and research. Lastly, I would like to dedicate this dissertation to my mother and father, Catherine A. Keiser and Christopher S. Keiser.

1.0 INTRODUCTION

The execution of collective behaviors, and the factors that shape their performance, are vital for the success of animal societies (Gordon, 2013; Wilson, 1987). One major determinant of collective behavior is the composition of different types of individuals within the group, as individuals often differ consistently in their behavioral traits and the tasks in which they participate (i.e., “animal personality”; Kralj-Fišer & Schuett, 2014; Sih, Bell, & Johnson, 2004). Yet, the same group compositions which promote success in one context (e.g., a larger proportion of socially connected individuals) may also produce costs in another context, such as an increased transmission of infectious agents of disease (Naug & Camazine, 2002).

The composition of different personality types in a social group can be a major factor driving the execution of collective behaviors like cooperative foraging (Keiser, Jones, Modlmeier, & Pruitt, 2014) and group exploratory behavior (Brown & Irving, 2014). Further, evidence from social arthropods shows that societies containing the right mixture of personality types can even be more productive (Modlmeier, Liebmann, & Foitzik, 2012) and stave off colony collapse (Pruitt & Goodnight, 2014). Because individuals of different personality types vary in their behavioral tendencies and task participation (Wright, Keiser, & Pruitt, 2015), they also vary in the frequency of social interactions they have with group mates (Pike, Samanta, Lindström, & Royle, 2008). What’s more, this heterogeneity in contact rate and interaction

preferences can influence the transmission of potentially harmful microbes among individuals (White, Forester, & Craft, 2015).

In fact, heterogeneity among hosts in their propensity to transmit pathogens is becoming increasingly emphasized in disease ecology (Galvani & May, 2005). Especially in the context of social groups, social network analysis has become instrumental in characterizing transmission dynamics among group-mates (Adelman, Moyers, Farine, & Hawley, 2015). The structure of social interaction networks and the mechanisms of network formation can impact transmission patterns (Bansal, Grenfell, & Meyers; Hock & Fefferman), especially network assortativity, where individuals tend to interact with others of similar phenotypes (Croft et al., 2009). Disassortative networks, alternatively, where individuals preferentially interact with individuals of opposing phenotypes (Newman, 2002, 2003), have been shown to shorten the duration of disease outbreaks (Kiss, Green, & Kao, 2006). Taken together, group composition can influence patterns of social interaction networks and the transmission dynamics they produce, which may or may not act in congruence with the execution of important collective behaviors.

Here, using both field and laboratory experiments, I examine how group personality composition influences collective foraging, contact network formation, and bacterial transmission in *Stegodyphus dumicola* (Araneae, Eresidae). *Stegodyphus dumicola* is an African social spider that lives in colonies of up to several hundred individuals that exhibit cooperative foraging, the execution of which can be based on the composition of “bold” and “shy” spiders present in the colony (Bilde et al., 2007; Keiser, Jones, et al., 2014). I found that group personality composition is a more important predictor of the execution of collective behaviors than more conspicuous traits like group size (Keiser & Pruitt, 2014). Then, using social network analyses and experimental inoculations using a genetically transformed cuticular bacterium, I

found that colony contact networks are disassortive and that bacterial transmission is biased and directional, predominantly from “bolder” to “shyer” spiders (Keiser et al., 2016). Thus, it appears that personality compositions may impose constraints on the social organization of animal societies.

2.0 EXPLORING THE EFFECTS OF INDIVIDUAL TRAITS AND WITHIN-COLONY VARIATION ON TASK DIFFERENTIATION AND COLLECTIVE BEHAVIOR IN A DESERT SOCIAL SPIDER

Keiser CN, Jones DK, Modlmeier AP, and Pruitt JN. 2014. Exploring the effects of individual traits and within-colony variation on task differentiation and collective behavior in a desert social spider. *Behavioral Ecology and Sociobiology*. 68: 839-850.

2.1 INTRODUCTION

Social taxa make up some of the most diverse and abundant groups of animal life on the planet. From the most speciose eusocial Hymenoptera to complex primate societies, elucidating the drivers behind the success of social animals has been a pervasive goal of evolutionary and behavioral ecology. Task partitioning, task allocation, division of labor, and behavioral specialization, in particular, have been identified as key mechanisms underlying the collective behavior and success of complex societies (Ratnieks and Anderson 1999; Gordon 2002; Bergmüller and Taborsky 2010). Despite some organizational costs, both the efficiency of collective behaviors and colony-wide performance generally increase as task-sharing and task-switching decrease and individual specialization increases (Ratnieks and Anderson 1999; Gordon 2002). In many cases, behavioral specialization is accompanied by morphological, size, or age

class polymorphisms (Ebert 1998; Beshers and Fewell 2001). In others, however, morphologically similar individuals differ consistently in their predispositions or propensities to carry out certain tasks. For example, *Stegodyphus* social spiders live in age-structured colonies, and individuals differ consistently in their propensity to perform foraging tasks (Grinsted et al. 2013).

In *Stegodyphus* and *Anelosimus* social spiders, the propensity to initiate and participate in foraging tasks can be predicted by individual behavioral tendencies, body size indices, or both (Grinsted et al. 2013; Pruitt et al. 2013; Settepani et al. 2013). Social spiders, therefore, represent a fascinating system in which to study the relative importance of morphology and individual behavioral tendencies in task allocation and division of labor, both from the perspective of a cooperative breeder and from spiders (Shear 1970; Kullmann 1972; Riechert 1985).

One of the fundamental characteristics of task allocation and division of labor is the differential tendency of individuals to perform certain tasks over others and, necessarily, behave differently over time (Beshers and Fewell, 2001; Gordon 2002). Consistent individual differences in behavioral traits (i.e., “animal personality”, “behavioral types” or “behavioral syndromes”) among groups members have proven to be an informative predictor of task participation, collective behaviors, and group productivity across diverse taxa (Le Vin et al. 2011; Pruitt and Riechert 2011b; Pruitt and Riechert 2011a; Modlmeier et al. 2012). Thus, the relative abundance of different behavioral types within groups could be a particularly important driver of group performance. This prediction is difficult to assess in the majority of animal social groups (e.g., primate societies, bird flocks, eusocial Hymenoptera, etc.). Yet, in a select number of tractable model systems, experimentally manipulating the personality composition of social groups has had enormous predictive power for understanding collective behavior and group

success (e.g., acorn ants, Modlmeier et al. 2012; guppies, Brown and Irving 2013; and social spiders, Pruitt and Riechert 2011a; Pruitt and Riechert 2011b).

Social spiders in particular have emerged as a front-running model for understanding how individual personalities shape social group functioning and performance. From these systems, we have established that maintaining behavioral heterogeneity in groups is important for prey capture success (Pruitt and Riechert 2011a), that keystone individuals can differentially shape the collective behavior of groups (Pruitt et al. 2013), and that personality traits have individual-level fitness consequences (Pruitt et al. 2008). Regrettably, at present, the majority of these studies have focused on a very limited number of highly related and recently-evolved species of *Anelosimus*, which draws into question the generality of the observed patterns (Agnarsson et al. 2006; Agnarsson et al. 2007). Thus, we reason that the most exciting new discoveries in this field will come by comparing the role of personalities in the social organization of phylogenetically disparate test systems.

In this study, we test how individual behavioral tendencies and body size determine task participation in the social spider *Stegodyphus dumicola* (Araneae, Eresidae). *Stegodyphus dumicola* lives in foraging societies of up to 2000 individuals in arid Southwestern Africa and is one of three independently derived social species in the *Stegodyphus* genus (Johannesen et al. 2007). In *S. sarasinorum*, individuals differ consistently in their task participation, and this is determined by a combination of behavioral traits and body size (Grinsted et al. 2013; Pruitt et al. 2013; Settepani et al. 2013). In *S. mimosarum*, however, individuals appear to lack task differentiation during group foraging (Ainsworth et al. 2002). Although several studies have investigated the collective foraging behavior of *S. dumicola* (e.g., (Whitehouse and Lubin 1999; Amir et al. 2000), we have yet to investigate the role of individual personality traits in task

participation in this species. Conducting this research is particularly interesting, as comparative data from all three social species in the *Stegodyphus* genus will now be available. This, in turn, will allow us to compare and contrast the relationship between individual personalities and social organization across numerous species (i.e., both within and between the *Anelosimus* and *Stegodyphus* genera). Moreover, we include here a series of non-foraging tasks (web repair and web construction) which have, to date, been ignored in this literature. We argue that this will further augment our understanding of how different tasks are correspondingly related to individual behavioral tendencies.

Specifically, we predict that (I) aggressiveness and boldness will be positively linked together in a behavioral syndrome; (II) foraging participation and web repair will be positively associated with boldness, aggression, and body size; (III) an individual's propensity to participate in one activity will be negatively associated with its propensity to participate in another (i.e. consistent with division of labor); (IV) colonies will exhibit characteristic differences in their collective behavior (i.e., "colony-level personality", Jandt et al. 2013); and (V) differences in colony-level personality will be explained by their degree of within-colony behavioral variation.

2.2 METHODS

2.2.1 Study system

Stegodyphus dumicola builds large webs consisting of a dense communal retreat and a two-dimensional capture web. The spiders used for this experiment were collected along

roadway fences and on *Acacia* shrubs in the southern Kalahari Desert in the Northern Cape, South Africa in January 2013. Colonies were transported to the University of Pittsburgh (Pittsburgh, PA, USA) where colony size was assessed (range: 1-700 individuals). Fifteen female spiders per colony were isolated into 30 ml opaque plastic cups containing a 1×1 cm square piece of poultry wire for substrate. Body mass (range: 0.04-0.21 g) and prosoma width (range: 2.13-3.61 mm) were measured after the fifteen spiders were isolated. Spiders were subject to natural light cycles and fed a maintenance diet of one 7mm cricket weekly.

Spiders remained isolated in the 30 ml opaque plastic cups for the duration of their initial individual-level personality assays (described below). After this time, individuals were given a unique three-color identification pattern atop their abdomen using non-toxic, acrylic paint (Palmer Paint Products, Prism™ Acrylic). Ten individuals were then chosen randomly from each pool of 15 spiders and placed in a 1-L clear plastic container with a 10×10 cm folded piece of wire for substrate. Fifteen artificial colonies were established in this way and these groups were maintained for the remainder of the experiment (32 days). These experimental colonies were provided four days to build a retreat and capture web before being subjected to a series of colony-level behavioral assays. These colony-level behavioral assays were used to (I) test for consistent inter-colony differences in their collective behavior (i.e., prey capture, web repair, web construction), (II) to test for associations between individuals' traits (morphology, behavior) their participation in different tasks, and (III) to test for associations between colonies' phenotypic composition and their colony-level personality.

2.2.2 Individual level personality assays

Boldness assays: Individual spiders were assayed for boldness by assessing their response to an aversive stimulus. Spiders were placed in an opaque, plastic arena (13cm × 12.5cm × 3.5cm) and allowed to acclimate for 60s. To simulate the approach of a predator, two rapid bursts of air were administered to the spider's anterior prosoma using an infant ear-cleaning bulb (see Riechert and Hedrick 1990; Lohrey et al. 2009; Pruitt et al. 2013). Web-building spiders generally have poor eyesight, and thus, rely heavily on air currents, seismic cues, and/or large shadows to detect the approach of predators via (Foelix 1996). This rapid burst of air elicits a huddle response, where the spider draws its legs tightly against its body in a death feign. A stopwatch was used to record the time taken by the spider to resume movement and traverse one full body length. Spiders that failed to move within 10 minutes were assigned the maximum value of 600 seconds. To obtain more intuitive values for "boldness", individuals' latencies to resume movement in seconds were subtracted from the maximum value. Thus, a greater latency to resume movement corresponds with a smaller boldness value. Following each boldness assay, the plastic arena was cleaned with isopropanol. Each spider (n = 182) was assayed four times, once daily for four consecutive days between the hours 9:00-14:00. Spiders were fed individually one day prior to the onset of personality assays. Multiple tests per individual were used to confirm the repeatability of this assay for *S. dumicola* and to obtain a more precise estimate of each individual's behavioral type.

Aggression Assays: Spiders were fed again directly after completion of the four consecutive boldness assays, and aggression assays were initiated on the following day. Aggression trials were initiated by placing the spider in a plastic arena (13cm × 12.5cm × 3.5cm) and providing it 60s to acclimate. Individuals were then prodded with a blunt probe on their

foremost left leg. The immediate response of the individual was then scored and categorized nominally in terms its aggressiveness. Our nominal scoring system resembles the ordinal ranking of Grinsted et al. (2013) but also resembles the ranking systems used in a variety of other spiders (Riechert and Johns 2003; Pruitt et al. 2008). “Non-aggressive” behaviors included: huddle responses, walking away from stimulus, and lunging away from the stimulus. “Aggressive” behaviors included: turning toward the stimulus, raising their anterior legs, shifting their abdomen in place, and walking towards the stimulus with raised front legs. Once the individual’s behavior was scored, it was returned to its 30 ml plastic cup and the arena was cleaned with isopropanol. As with boldness assays, each spider (n = 182) was assayed four times, once daily for four consecutive days between the hours 9:00-14:00.

2.2.3 Colony-level personality assays

Colonies were tested multiply for each assay in order to (I) to test for consistent differences in the collective behavior of colonies (i.e., “colony-level personality), (II) test for associations between individuals traits (body size, personality) and their propensity to participate in each colony maintenance task (Pinter-Wollman 2012; Jandt et al. 2013), and (III) to test for associations between colonies’ phenotypic composition and their colony-level personality type.

Prey capture response: The prey-capture behavior of each colony was assessed daily for ten consecutive days. Collective prey-capture trials were initiated by placing a small (1×1.5cm) piece of white printer paper in the capture web of each colony. We then provided 60s acclimation time before a battery-powered handheld vibratory device (GoVibe) was used to vibrate the piece of paper to simulate a prey item caught in the capture web. To prevent the colony container from moving, it was placed within an identical empty 1-L container which was

stabilized in 7cm of dry sand substrate. A stopwatch was then used to record the time taken for the first spider to emerge from the retreat (“time to emergence”) and the subsequent latency for the first spider to attack the paper (“latency of attack”). The trial ended after the first spider attacked the prey item or until the maximum time was reached (600s). In addition to measuring colonies’ latency to emerge and latency to respond during simulated prey capture events, any individuals that had emerged from their retreat by the time of the first attack were identified by their three-color identification pattern. Such individuals were considered to have “joined” in the collective foraging event (Grinsted et al. 2013; Pruitt et al. 2013).

Web repair: Web repair trials were initiated on the day following the completion of colonies’ ten prey capture assays. To assess the web repair behavior of each colony, we cut all anchor points of the capture web within the 1L colony container using a clean utility knife blade. Then, over a period of five evenings, web repair activity was observed during dusk using a red headlamp every 30 minutes between the hours 19:00-23:00. Web-building in *Stegodyphus* spiders occurs primarily at night, so nocturnal observation using a red headlamp is a valuable method to observe natural web-building behavior (Pasquet et al. 1999). Any individuals engaged in web repair during these checks were identified using their unique three-color identification patterns. In addition, a flashlight with a red filter was used to confirm the silk production of active individuals.

Ambient web building: To assess the ambient web building behavior of each colony, we allowed colonies to expand into a larger environment. Colonies’ 1L colony containers were placed in the corner of a square mesocosm (30.4cm × 30.4cm × 30.4cm) consisting of three chiffon sides for ventilation, two clear plastic sides for observation, and an aluminum bottom. A branch containing a ~90° bend was then inserted into the colony and glued to the adjacent corner

of the mesocosm. Individuals were allowed to explore the mesocosm, and colonies were observed at dusk during the following five nights using a red headlamp. Colonies were checked every 30 minutes between the hours 19:00-23:00. Individuals moving outside their webs and engaging in web construction were identified visually using their unique three-color identification pattern. A flashlight was again used to confirm the production of silk.

2.2.4 Statistical analyses

To test for repeatable differences in individual and colony-level personality, we used ANOVA to partition variance into within- versus among-individual components, where repeatability is estimated as the proportion of total variation attributable to between individual differences (Boake 1989; Falconer and Mackay 1996). Colony ID was included as a random effect in these analyses. We used Spearman's rank correlations to identify a syndrome between boldness and aggressiveness. To determine if personality traits were associated with body size and body condition, we used ANOVAs with aggression and boldness as dependent variables and body size (prosoma width) and body condition as dependent variables. Body condition was estimated by the residuals of a linear regression of spiders' body mass on body size (Jakob et al. 1996). To determine whether individuals' traits were associated with their propensity to participate in various tasks, we first performed z-score normalized Principle Component Analysis on our five observed tasks: number of times each individual joined the attack, number of times first attacker, number of times first to emerge, number of times producing silk during web repair following experimental web destruction, and number of times producing web during standard web-production (cribellate or otherwise). The first principle component (PC1)

explained 55.7% of the variation and was composed of three foraging variables (Factor loadings: number of times first to emerge 0.90, number of times first attacker 0.92, number of times joined in attack 0.93), PC2 explained 21.7% of the variation (Factor loadings: number of times producing silk 0.87, number of times repairing the web 0.51), and PC3 explained 15% of the variation (Factor loadings: number of times producing silk 0.46, number of times engaged in standard web-building 0.70). Thus, values on the PC1 axis denote an individual's propensity to participate in quantify prey capture, values on the PC2 axis denote participation in web repair, and values on the PC3 axis denote participation in web building. To assess whether individuals' traits were associated with task participation, we constructed three separate ANOVA models. The predictor variables in these models were individuals' body condition, body size, aggressiveness, and boldness. The response variables for these three models were PC1, PC2, and PC3. To account for the non-independence of individuals within each test colony, we included colony ID as a random effect in our models. For these analyses, we used Bonferroni-adjusted α values to reduce the experiment-wise type I error rate (Rice 1989). The adjusted level of significance was 0.017 ($\alpha = 0.05/3$).

To test whether individuals tended to engage in some colony maintenance tasks over others, or whether some individuals merely performed all colony maintenance tasks, we tested for correlations between individuals' scores along all three PCs. A positive correlation would suggest that some individuals tend to perform many/all colony maintenance tasks (e.g., "elites"), and a negative correlation would be consistent with division of labor.

To determine the drivers of colony-level personality, we performed independent model selection procedures for each of three response variables: latency of emergence, latency of attack, and number of individuals out at night during our web-building assays. For each model,

we average colonies' response across all of their observations. To find the best model, we used Akaike Information Criterion (AICc) and Akaike Weights to compare models (Akaike 1987; Burnham and Anderson 2002). We included the following predictor variables in our models: the average body size of colony constituents, the average aggressiveness of colony constituents, the average boldness of colony constituents, and the within-colony variation of all three traits (i.e., variance in body size, aggressiveness, and boldness within each colony). We present here the best three models from each of our model selection procedures. All analyses were completed using JMP 9.0 (JMP 9.0; SAS Institute, Cary, NC, USA).

2.3 RESULTS

2.3.1 Individual personality assays

Spiders exhibited consistent individual differences in their boldness ($F_{180,541} = 2.03$, $p < 0.0001$, repeatability = 0.63; Table 1; Distribution: Fig. 1) and aggressiveness ($F_{178,531} = 1.42$, $p = 0.002$, repeatability = 0.55; Table 1; Distribution: Fig. 1). We also detected a negative association between individuals' average aggressiveness and their average boldness score (Spearman's $\rho = -0.17$, $p = 0.03$). That is, bolder individuals were generally less aggressive. We also found that individuals with larger prosoma widths were less bold ($F_{1,180} = 5.77$, $p = 0.02$, $R^2 = 0.03$) and more aggressive ($\chi^2 = 5.29$, $df = 1$, $p = 0.02$, Nagelkerke $R^2 = 0.01$). Body condition was not significantly associated with boldness ($F_{1,180} = 3.11$, $p = 0.08$, $R^2 = 0.02$), but individuals which were in better body condition were more aggressive ($\chi^2 = 5.16$, $df = 1$, $p = 0.01$, Nagelkerke $R^2 = 0.01$).

2.3.2 Task performance

Our combined model predicting individuals' participation in prey capture tasks was highly significant ($F_{4,174} = 3.97$, $R^2 = 0.09$, $P = 0.004$, Table 2). Individuals with high body condition indices were less likely to participate in prey capture (PC1; $F_{1,174} = 12.8$, $R^2 = 0.08$, $p = 0.0005$; Fig. 2). However, individuals' tendency to engage in prey capture behavior was not associated with their personality traits (Boldness: $F_{1,174} = 0.03$, $p = 0.86$; Aggression: $F_{1,174} = 0.72$, $p = 0.40$) or prosoma widths ($F_{1,174} = 1.04$, $p = 0.31$).

Our combined model predicting individuals' participation in standard web building behavior was significant ($F_{4,174} = 2.83$, $R^2 = 0.06$, $P = 0.03$, Table 2). Here, larger individuals were less likely to engage in web construction than smaller individuals (PC3; $F_{1,174} = 8.59$, $p = 0.005$; Fig. 3). No other predictor variables were significant in our model (all $p > 0.5$).

No individual characteristics influenced participation in web repair behavior (PC2; $F_{4,174} = 1.04$, $R^2 = 0.02$, $P = 0.39$, Table 2).

We failed to detect an association between any of our three PC axes (all $p > 0.87$). That is, an individual's propensity to participate in one task had no relationship with its propensity to participate in other tasks. Therefore, the evidence suggests that tasks are not partitioned among individuals, nor are the individuals engaged in one set of tasks significantly more likely to engage in others.

2.3.3 Colony level personality assays

Across 10 days of observation, colonies exhibited consistent differences in their collective behavior (Latency of emergence: $F_{14,63} = 4.21$, $p < 0.0001$, repeatability = 0.74;

Latency to attack: $F_{14,63} = 4.20$, $p < 0.0001$, repeatability = 0.74; Number of spiders nocturnally active: $F_{14,63} = 3.24$, $p = 0.001$, repeatability = 0.69). In our best models predicting colony-level personality, colonies' latency of emergence, latency to attack, and the number of spiders engaged in standard web construction were not influenced by the average body size or body condition of the spiders therein (Table 3). The model which best explained the latency of emergence included within-colony variance in individuals' prosoma width and no other predictor variables ($F_{1,14} = 5.72$, $R^2 = 0.31$, $p < 0.05$; Table 3, Fig. 4). The model which best explained colonies' latency of attack included only within-colony variance in aggression and no other predictor variables ($F_{1,14} = 5.68$, $R^2 = 0.30$, $p < 0.05$; Table 3, Fig. 5). There were no models in which any element of colony composition effectively predicted the number of individuals engaged in standard web construction (i.e., all $P > 0.05$, Table 3), but the single best non-significant model included within-colony variance in boldness and no other variables ($R^2 = 0.05$, $p = 0.41$, Table 3).

2.4 DISCUSSION

Understanding how the traits and actions of individuals unite to determine social organization and collective behavior is vital to our understanding how such behaviors arise. In particular, the interplay between individual variation, task differentiation, and colony-level behavior/success has been a driving force in the social insect literature for more than four decades. Here we provide evidence that variation in individuals' body size and condition influence their propensity to perform a variety of colony maintenance tasks. This result is intriguing because this trait variation and its resulting task differentiation arises in a cooperative breeding society composed of highly-related, inbred individuals of nearly identical age which

develop together in synchrony. The once-conventional reasoning in the social spider literature was that these conditions will beget minimal inter-individual trait variation and insignificant levels of task differentiation (Darchen and Delage-Darchen 1986; Whitehouse and Lubin, 2005). Our data, in addition to others (e.g., Salomon et al., 2008) undermine this view. Moreover, the best predictors of colonies' collective behavior (i.e., colony-level personality) were always their degree of within-colony trait variation (Table 3): colonies with greater within-colony variation in body size emerged more slowly in response to prey; colonies with greater within-colony variation in aggressiveness took longer to attack prey; and, within-colony variation in boldness was (non-significantly) associated with more individuals engaged in standard web building behavior. Taken together, our data suggest that inter-individual variation can still play an important role in social organization and collective behavior in societies where basic intuition might predict its absence.

2.4.1 Task differentiation

Task differentiation is argued to be a vital innovation behind the success of the social insects and, by extension, one might predict an absence of such organizational mechanisms in other societies. However, here we show that task differentiation of a strikingly similar nature exists in an “egalitarian” social spider. That is, individuals differed in their propensity to perform various tasks and this was associated with individuals' traits. However, we found no evidence for division of labor or the presence of “elite” workers, which characterize the work forces of social insect societies (Robson and Traniello 1999; Pinter-Wollman et al. 2012) In other words, the propensity of an individual to participate in one task (e.g., web building) was neither negatively nor positively associated with its likelihood to participate in other tasks (e.g., prey capture).

We found that individuals' tendency to perform different tasks were associated either with their body size (i.e., prosoma width) or with their body condition, but not their personality types. This is surprising, because other studies on *Stegodyphus* have found that individuals' participation in foraging tasks is associated with both their boldness and their body size (Grinsted et al. 2013; Pruitt et al. 2013; Settepani et al. 2013). In contrast, in this study, body condition was the best predictor of individuals' tendency to participate in prey capture, where lower body condition was associated with more foraging activity (Figure 2). We reason that this may be the result of individuals' recent foraging success, and that satiation diminishes individuals' willingness to engage in further risk-associated behaviors. Additionally, spiders with smaller prosoma widths were more likely to engage in ambient web building behavior (Figure 3). Why small individuals become active at night is unknown, but this nocturnal activity pattern may be associated with risk aversion, since the majority of predation on *Stegodyphus* occurs during the day (Henschel 1998) and smaller individuals may be more susceptible to predation. A similar size-based task differentiation has been observed in *Atta* leaf-cutting ants, where small individuals are thought to increase their nocturnal activity as to avoid predation by parasitoid flies (Orr 1992). Lastly, some of the variation in task participation may also be due to differences in the behavior of adjacent life-stages. Although *S. dumicola* colonies are age-structured, the colonies collected in the field likely contained both adults and late-stage subadults (i.e., penultimate juveniles) which may differ in their propensity to perform foraging-related tasks.

2.4.2 Colony-level personality

We detected consistent colony-level variation in collective foraging and web-building behavior. And, the best predictor of colony-level behavior was always colonies' degree of

within-colony trait variation. First, we implicated within-colony variation in body size as the single best predictor of colonies' latency of emergence. This variable alone explained 31% of the variation and a modest Akaike weight of 0.42. The second (weight = 0.38) and third (weight = 0.20) best models predicting colonies' latency to emerge again included within-group variation in body size as well as variation in aggressiveness (Table 3). Likewise, within-colony variation in aggressiveness alone explained 30% of the variation observed in the colonies' latency to attack a prey item (Table 3) and this univariate model boasted an Akaike weight (0.55) twice that of either rival model. Taken together, we argue within-colony trait variation is a more informative indicator and important driver of collective behavior than mere average trait values in this species, and thus, differences in within-colony trait variation could have important implications for colony success.

The success of insect societies is often attributed to within-colony trait variation and the resulting division of labor (Oster and Wilson 1979; Wilson 1987). Consistent with this prediction, recent studies on animal personality have shown that within-group variation in behavior and body size is positively associated with productivity: ants (Modlmeier and Foitzik 2011; Modlmeier et al. 2012), social spiders (Pruitt and Riechert 2011a), invasive populations of mosquito fish (Fogarty et al. 2011). Our data here suggest that the positive relationship often described between within-group variation and group success in diverse social taxa (e.g., Swanson et al., 2003; Pruitt and Riechert 2011a; Modlmeier et al. 2012) may be mediated by the consequences of within-group variation on collective behavior. Collective behavior is, fundamentally, a product of the variation in the behavior and/or morphology of individual group members. And, for many systems, variation in collective behavior is a major driver of group success (e.g., Dussutour et al. 2008; Pruitt 2013; but see Jandt and Dornhaus, 2013). However,

the precise reasons why within-group variation is correlated with differences in colony-level behavior in this system remain a mystery.

2.4.3 Synthesis of social spider literature

A growing body of literature demonstrates the utility of social spiders for studies on the relationship between group composition, collective behavior, and colony success. This is because, although social spiders are not enormously successful in terms of their evolutionary or ecological success (Agnarsson et al. 2006; Agnarsson et al. 2013), they do lend themselves superbly to manipulative experiments on group composition in the laboratory and in the field. To date, studies on group composition, task differentiation, and colony success have been conducted on nine different species across three genera and two families (Table 4), which together represent an estimated eight independent derivations of sociality (Agnarsson et al. 2006). This diversity of test systems is powerful, because it provides us with the opportunity to explore whether generalizable patterns in social organization and colony success emerge iteratively with the evolution of sociality in these organisms. In this final section, we briefly summarize the data available from various social spiders and remark on similar patterns where they occur.

Similar relationships between task differentiation, group composition, and collective behavior/success have been observed in two of the world's most speciose genera of social spiders: New-world *Anelosimus* and Old-world *Stegodyphus*. Significant levels of task differentiation have been noted in eight or nine out of the nine species studied to date, with *S. mimosarum* having conflicting results among studies. Individual personality is the trait most commonly associated with an individual's propensity to perform different tasks (66% of

species), followed by body size (33% of species). Personality is also the most phylogenetically widespread predictor of task differentiation, encompassing both *Stegodyphus* and *Anelosimus* species, whereas body size is only a significant predictor among social *Stegodyphus*. The fact that similar relationships between individuals' traits and task participation emerge iteratively with the evolution of sociality in spiders either implies that similar trait-task associations evolve *de novo* with the evolution of sociality or that such patterns emerge as a mere epiphenomenon of group living in spiders. Studies that stage associations among normally subsocial species will help untangle these alternatives.

Group composition has a significant impact on collective behavior in seven of the nine species studied, including both *Anelosimus* and *Stegodyphus*. Here, within-group variation in personality has been associated with collective foraging tasks in all seven species, and variation in body size has been associated with collective foraging behavior in *S. dumicola* only. We argue that these results imply similar mechanistic underpinnings to the collective behavior in diverse spider societies and that personality, in particular, is a major driver. Finally, group composition has notable impacts on colony success in six out of nine of the species studied, including representatives from both *Anelosimus* and *Stegodyphus*. Here again, variation in colonies' personality composition is a consistent driver of colony success in all six species: boasting effects ranging from increased/decreased group feeding success to mediating the productivity and extinction risk of entire societies. Taken together, we argue that personality appears to play a similar orchestrating role in the social organization and success of spider lineages to that of morphological castes in the social insects. If this is indeed the case, then these results could bode well for the continued growth and cross-fertilization of theory and data derived from both literatures, which have until recently remained largely disjointed.

2.4.4 Conclusions

In the work presented herein, we have (I) identified task differentiation between members of *S. dumicola* colonies, (II) confirmed that colonies vary consistently in their collective behavior, and (III) demonstrated that the relationship between within-colony variation and collective behavior is robust. As noted above, similar relationships between task differentiation, group composition, and colony-level impacts have now been noted in numerous species of social spider, including *S. dumicola*. In our view, the combined weight of data effectively refutes the long-held notion that social spider societies are homogeneous and egalitarian (Whitehouse and Lubin 2005). Instead, the repeated pattern of social organization observed in these systems bears more striking resemblance to classical views of the social Hymenoptera. Thus, these seemingly divergent societies (insects and spiders) may share more behavioral and organizational homoplasies than would have been predicted as recently as two years ago.

Table 2-1 (a) Tests for repeatability in individual and colony-level behavioral assays.

(b) Associations between individual-level personality and individuals' body size or condition.

(a)	Repeatability	<i>F</i>	<i>df</i>	<i>p-value</i>
Individual spiders				
Boldness	0.63	2.03	180, 721	< 0.0001
Aggression	0.57	1.42	178, 709	0.002
Colony collective behaviors				
Latency of emergence	0.74	4.21	14, 63	< 0.0001
Latency to attack	0.74	4.20	14, 63	< 0.0001
Number of web repairers	0.69	3.24	14, 63	0.001
(b)	R^2	F, χ^2	<i>df</i>	<i>p-value</i>
Individual spiders				
Boldness vs body condition	0.02	3.11	1, 180	0.08
Boldness vs Prosoma width	0.03	5.77	1, 180	0.02
Aggression vs body condition	0.01	5.16	1	0.01
Aggression vs prosoma width	0.01	5.29	1	0.02

Table 2-2. Results of three ANOVAs predicting the relationship between individuals' traits (behavioral tendencies, body size, body condition) and their participation in various colony tasks.

Independent Variable	PC1		PC2		PC3	
	F_{4,174}	p-value	F_{4,174}	p-value	F_{4,174}	p-value
Whole Model	3.97	0.004	1.03	0.39	2.83	0.03
Boldness	0.03	0.86	1.42	0.24	0.02	0.88
Aggression	0.72	0.40	0.05	0.82	0.39	0.53
Prosoma width	1.04	0.31	2.03	0.16	8.60	0.005
Body Condition	12.8	0.0005	0.04	0.85	0.27	0.6

Table 2-3. Model comparison of those obtained via independent model selection procedures predicting latency of emergence, latency of attack, and number of individuals participating in ambient web-building in experimental colonies.

Model variables	<i>R</i> ²	<i>P</i>	<i>RMSE</i>	<i>AICc</i>	<i>Akaike Weight</i>
Latency of emergence					
Variance in prosoma width	0.31	< 0.05	91.9592	184.2438	0.42
Variance in prosoma width, Variance in aggression	0.46	< 0.05	84.7293	184.4049	0.38
Average colony aggression, variance in prosoma width, Variance in aggressiveness	0.56	< 0.05	79.3683	185.8055	0.19
Latency to attack					
Variance in aggression	0.30	< 0.05	123.3575	193.0561	0.55
Variance in prosoma width, Variance in aggression	0.40	< 0.05	119.2152	194.6489	0.25
Average colony boldness, Variance in prosoma width, Variance in aggression	0.55	< 0.05	108.2333	195.1112	0.20
Number of individual nocturnally active					
Variance in boldness	0.05	0.41	0.1113	-17.2546	0.77
Average body condition, Variance in boldness	0.10	0.51	0.1128	-14.2508	0.17
Average body condition, Average colony aggression, Variance in aggression	0.23	0.38	0.1089	-11.943	0.05

Table 2-4. Comparisons in the relationship between individual traits and colony social organization and success across nine species of social spider, representing eight independent derivations of sociality.

Taxon	Range/habitat	Predictors of task participation	Relationship between colony composition and collective behavior?	Relationship between colony composition and colony success?	References
Genus: <i>Stegodyphus</i>					
<i>S. dumicola</i>	Southwest Africa; arid scrub and bushveld	Body size, body condition	<i>Yes</i> - within-colony trait variation and colony members with extreme phenotypes influence collective behavior	<i>Yes</i> -Colonies containing one very bold individual exhibit greater mass gain and survivorship in laboratory	This paper; Pruitt & Keiser <i>in review</i>
<i>S. mimosarum</i>	East Africa; arid scrub and bushveld	None or body size	<i>Unknown</i>	<i>Unknown</i>	Ward and Enders 1985; Wickler and Seibt 1993; Ainsworth et al. 2002); Grinsted & Settepani <i>Unpublished Data</i>

<i>S. sarasinorum</i>	Southcentral Asia, arid scrub and bushveld	Body size, personality	<i>Yes</i> - the boldness of colonies' boldest constituents is associated with collective behavior	<i>Unknown</i>	Settepani et al. 2013; Pruitt et al. 2013
Genus: <i>Anelosimus</i>					
<i>A. studiosus</i>	North and South America; Temperate deciduous forests	Personality	<i>Yes</i> - colonies with more aggressive individual deploy a greater number of foundresses each year and exhibit greater defensive behavior	<i>Yes</i> - frequency of aggressive females begets nine-fold differences in extinction risk and four-fold differences in productivity	Pruitt 2012, 2013
<i>A. eximius</i>	Central and South America; lowland rainforest	Weight, nutrition, personality	<i>Yes</i> - colonies with aggressive individuals are more effective at capturing prey	<i>Yes</i> - colonies of mixed docile/aggressive composition are more effective at group feeding	Ebert 1998; Pruitt et al. 2012
<i>A. guacamayos</i>	Central and South America; Mid-elevation cloudforest	Personality	<i>Yes</i> - colonies with aggressive individuals are more effective at capturing prey	<i>Yes</i> - colonies of mixed docile/aggressive composition are more effective at group feeding	Pruitt et al. 2012

<i>A. oritoyacu</i>	Central and South America; Mid-elevation cloudforest	Personality	<i>Yes</i> - colonies with aggressive individuals are more effective at capturing prey	<i>Yes</i> - colonies of mixed docile/aggressive composition are more effective at group feeding	Pruitt et al. 2012
<i>A. rupununi</i>	Central and South America; lowland rainforest	Personality	<i>Yes</i> - colonies with aggressive individuals are more effective at capturing prey	<i>Yes</i> - colonies of mixed docile/aggressive composition are more effective at group feeding	Pruitt et al. 2012
Genus: <i>Achaearanea</i>					
<i>Ac. wau</i>	Montane Rainforests, Papua New Guinea	Age	<i>Unknown</i>	<i>Unknown</i>	Lubin 1995

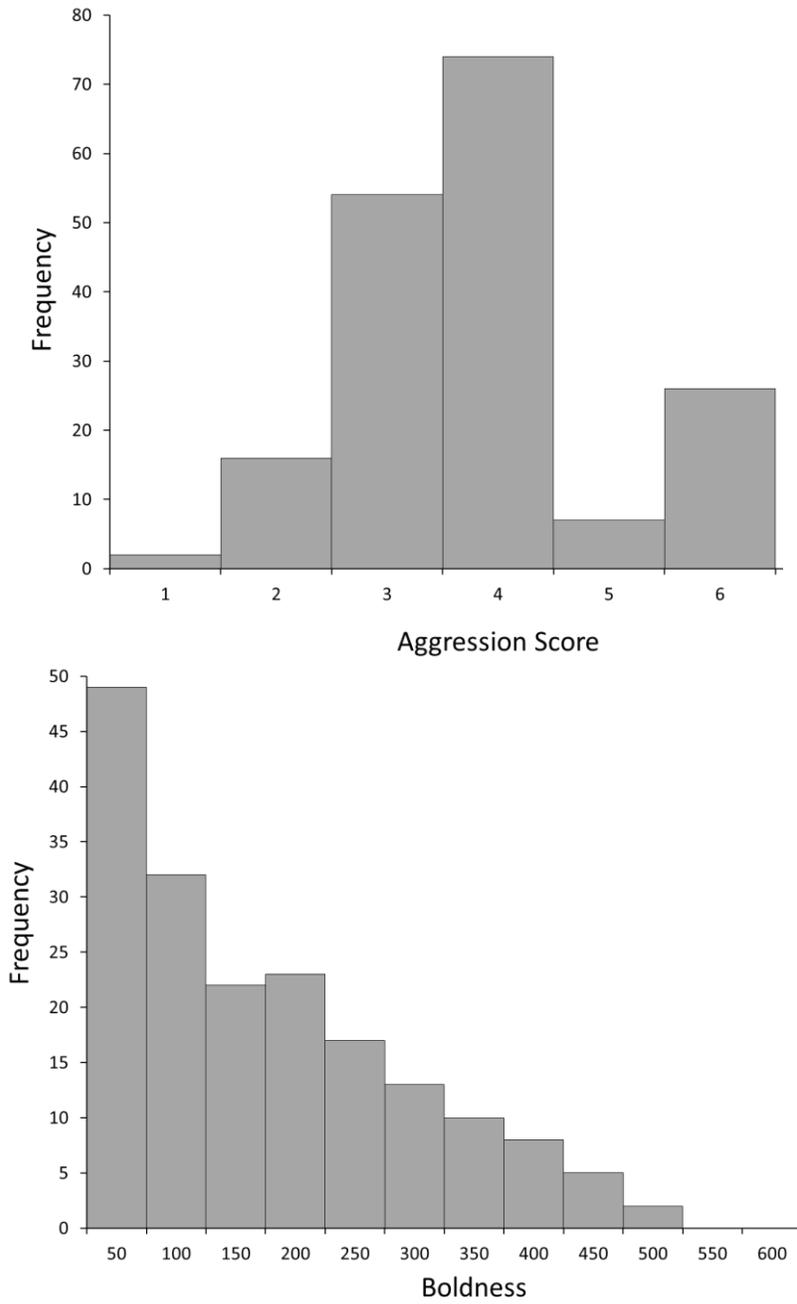


Figure 2-1. Frequency histograms depicting the average personality traits exhibited by individual female *S. dumicola*.

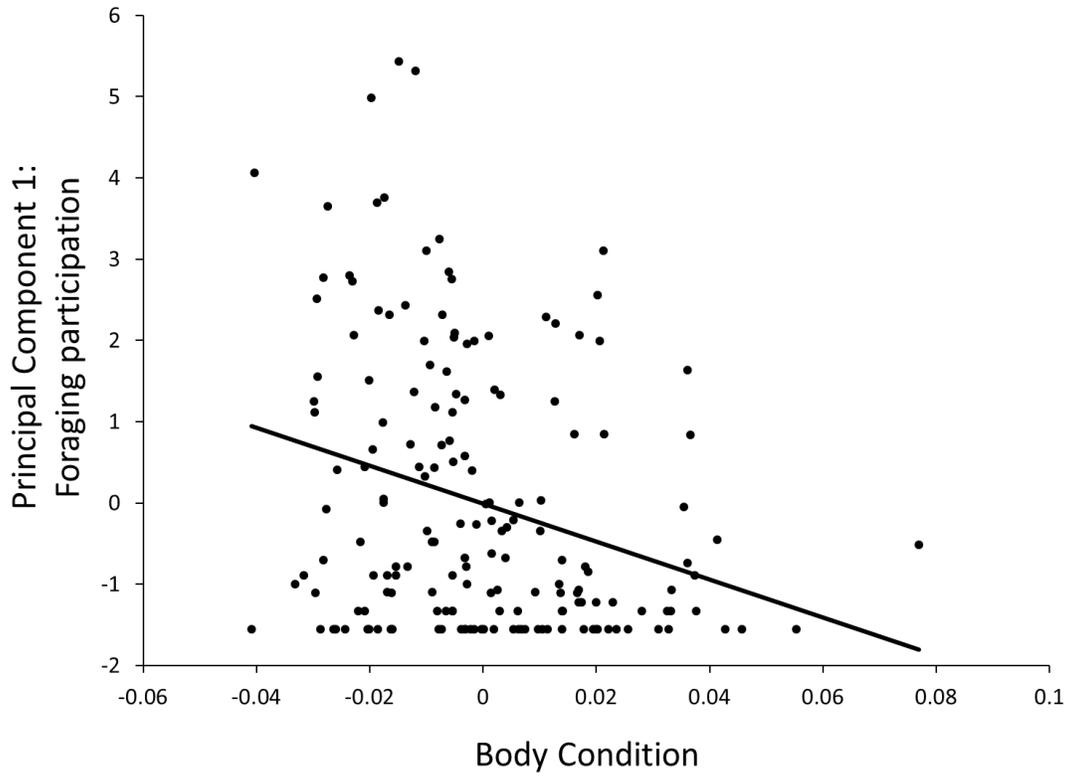


Figure 2-2. Individuals with larger than average mass for their body size, an indication of body condition, were less likely to participate in foraging-related tasks.

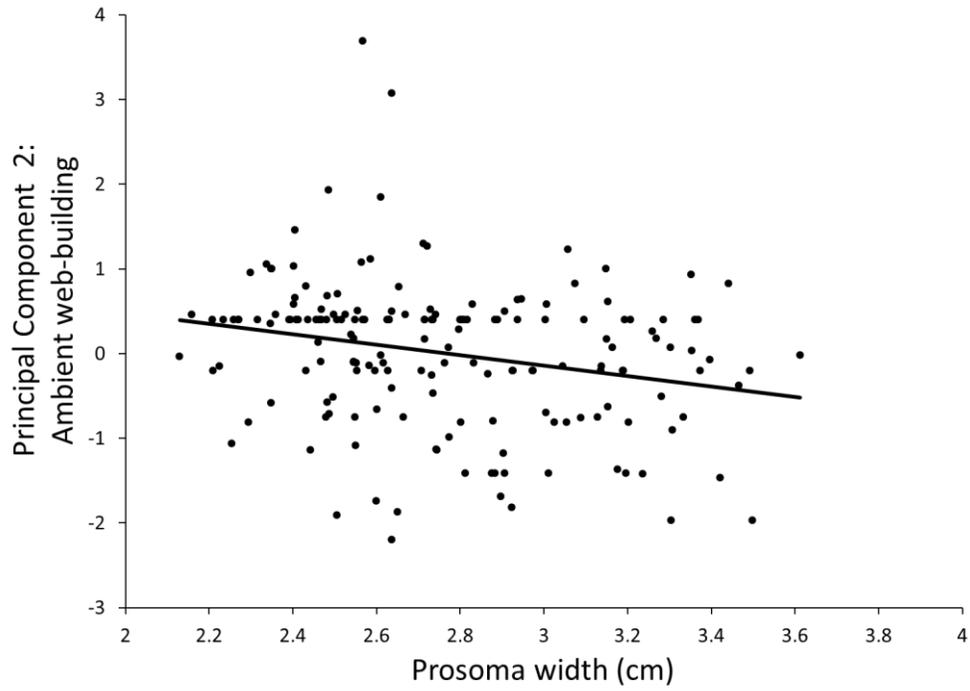


Figure 2-3. Larger individuals were less likely to participate in ambient web construction after during colony expansion into a larger environment.

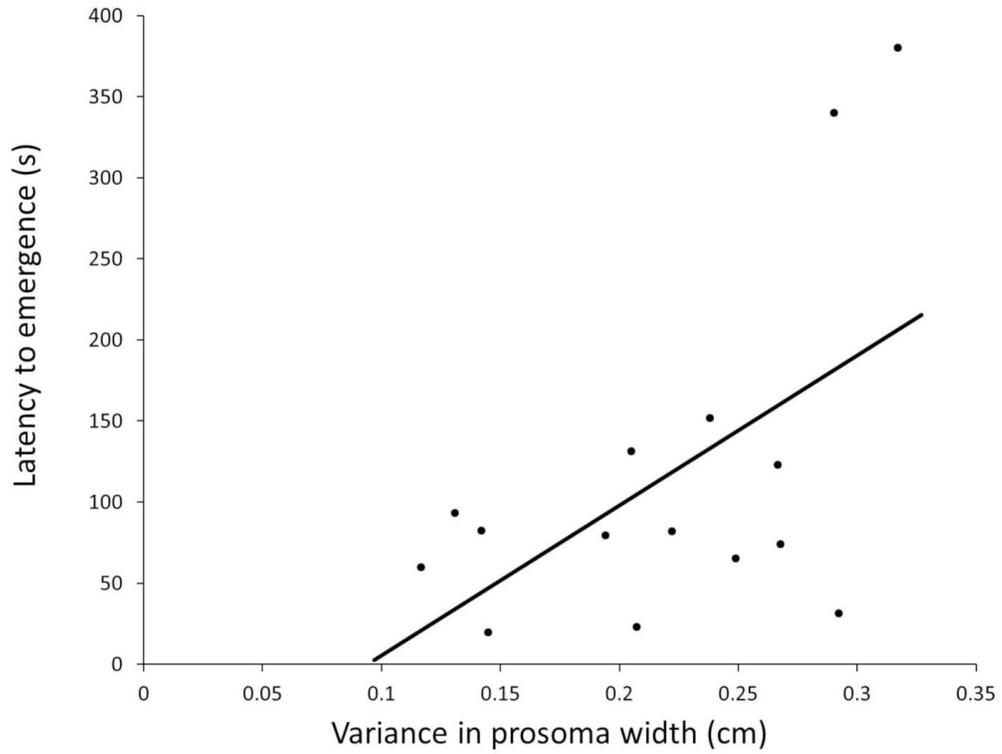


Figure 2-4. The latency of emergence from the nest after prey stimulus increased along with within-colony variance in individuals' prosoma width.

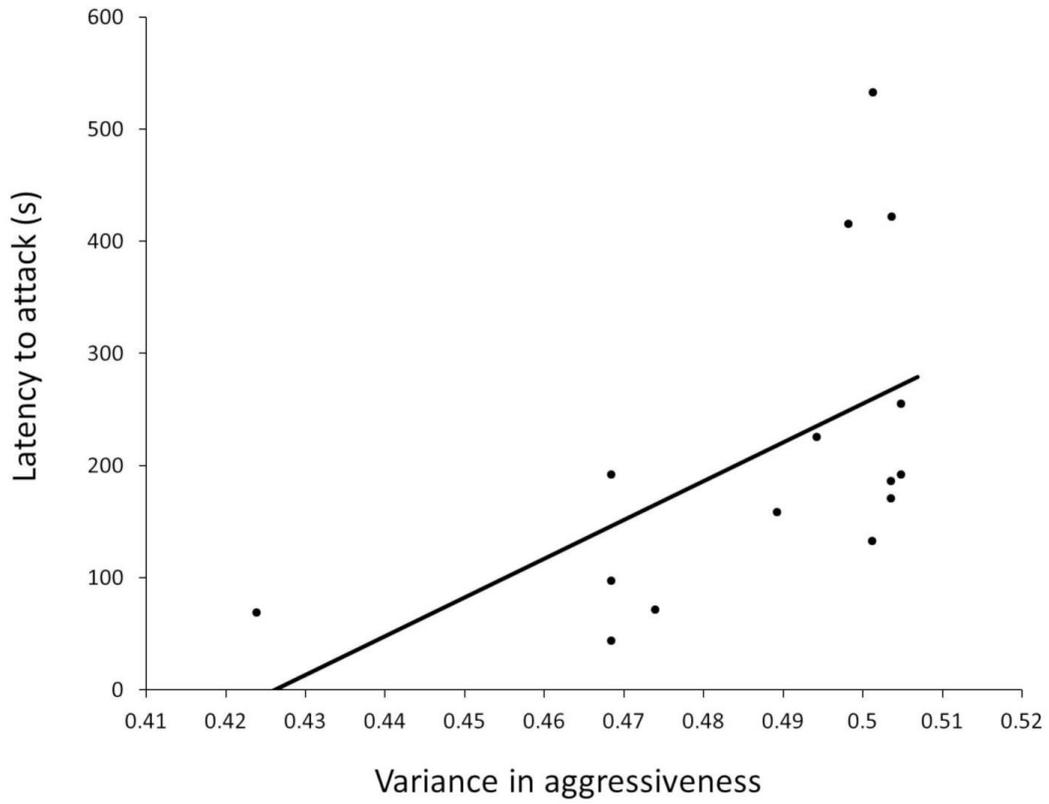


Figure 2-5. Colonies' latency to attack a prey item increased along with within-colony variance in aggression.

3.0 PERSONALITY COMPOSITION IS MORE IMPORTANT THAN GROUP SIZE IN DETERMINING COLLECTIVE BEHAVIOR IN THE WILD

Keiser CN and **Pruitt JN**. 2014. Personality composition is more important than group size in determining collective foraging behaviour in the wild. *Proceedings of the Royal Society B: Biological Sciences*. 281: 20141424.

3.1 INTRODUCTION

Understanding the factors that shape collective behavior is vital for our understanding of animal societies because they can be key drivers of group success (Gordon 2013; Ingram *et al.* 2013). The tendency for individuals to differ consistently in their behavior through time and across contexts (i.e., “animal personality”; (Sih, Bell & Johnson 2004; Sih *et al.* 2012a; Kralj-Fišer & Schuett 2014) is often a major determinant of collective behaviors (Brown & Irving 2014; Keiser *et al.* 2014a), colony productivity (Modlmeier, Liebmann & Foitzik 2012), and survival (Pruitt 2012; Pruitt 2013) in a variety of taxa (fish: Dyer *et al.* 2009; Harcourt *et al.* 2009; birds: Kurvers *et al.* 2010). This is in part because individual traits, like personality, influence task participation (e.g., Grinsted *et al.* 2013; Pruitt, Grinsted & Settepani 2013b; Settepani *et al.* 2013), individual aptitudes for those tasks (but see Dornhaus 2008; Wright, Holbrook & Pruitt In press), and the manner in which tasks are executed (Chang & Sih 2013).

Yet, how group personality composition interacts with other more familiar social factors like group size remains poorly resolved. Here we explore the question: Is the presently fashionable trait of animal personality as informative or valuable as classic social traits, like group size, in predicting collective behavior?

Countless studies have demonstrated an effect of group size in the development and emergence of collective behaviors (Clark & Mangel 1986; Leticia Avilés & Paul Tufiño 1998; Krause & Ruxton 2002; Dornhaus, Powell & Bengston 2012). For example, it has been shown that increased colony sizes can beget increased behavioral differentiation and task elitism (Gautrais *et al.* 2002) and division of labor often scales positively with colony size (Jeanson *et al.* 2007; Holbrook, Barden & Fewell 2011). Group size can even act as a facilitator of collective behavior in human crowds, from social facilitation (Milgram, Bickman & Berkowitz 1969) to social loafing (Karau & Williams 1993). Comparatively fewer studies, however, have accounted for the composition of behavioral phenotypes within groups of different sizes. Yet, as group size and composition could be intrinsically-related social factors, simultaneously studying both attributes should provide a more complete understanding of social trade-offs, optimal group size, and the execution of cooperative behaviors (Krause & Ruxton 2002). Most importantly, the outcomes that we have commonly attributed to group size may actually be a result of group composition, since increases in group size will frequently increase the phenotypic diversity within groups and alter their composition. Thus, classical manipulations of group size have almost universally conflated group size with group composition and/or within-group trait variation (but see: Jeanson & Fewell 2008).

Although addressing group size and group composition independently has led to a deeper understanding of animal societies, the majority of behavioral studies are conducted under

laboratory conditions devoid of ecological challenges like predation risk and abiotic stressors (e.g., Purcell & Avilés 2007; Keiser *et al.* 2014a). How, then, might habitats containing natural pressures influence the emergence of collective behavior across groups of different sizes and personality compositions? Here we focus on collective prey capture behavior in a highly tractable animal model, the social spider *Stegodyphus dumicola*. We focus on collective foraging in particular because the success of social spiders has often been attributed to their ability to cooperate to subdue larger and more profitable prey (Yip, Powers & Avilés 2008; Harwood & Avilés 2013).

With the experiment herein, we examine the relative contribution of colonies' personality composition versus group size in predicting collective foraging behavior, web architecture, and anti-predator behavior in *Stegodyphus dumicola*. We offer three hypotheses:

Hypothesis 1: Colonies composed of a higher proportion of bold individuals will attack prey more rapidly, while both larger and bolder colonies will attack with a greater number of attackers.

Hypothesis 2: Larger and bolder colonies will build larger capture webs, though the predictive power of colony composition will be greater than that of group size.

Hypothesis 3: Colonies composed of a higher proportion of bold individuals will take longer to escape an aversive stimulus by evacuating the capture web into the colony retreat.

Together these hypotheses are designed to probe the (oft conflated) influences of group size and personality composition *in situ* in a manner that is rarely achievable in other test systems.

3.2 METHODS

3.2.1 Study species and field site

Stegodyphus dumicola (Araneae: Eresidae) is an Old World social spider that lives in colonies of tens to hundreds of females that cooperate in shared web building, prey capture, and alloparental care (Aviles 1997; Lubin & Bilde 2007a). We collected colonies of *S. dumicola* in *Acacia mellifera* trees and along roadside fences in the southern Kalahari Desert, South Africa in January 2014. We transported colonies to our field site in Griekwastad, Northern Cape, South Africa. This site is an arid thornveld dominated by *Acacia mellifera*.

3.2.2 Individual personality assays

Prior to personality assays, we measured the body mass and prosoma width of each spider with a digital scale and digital calipers, respectively. To determine spiders' personality we tested their boldness by assessing their response to an aversive stimulus. Boldness is defined here as the latency to resume movement after an aversive stimulus. This metric is both highly repeatable at the individual level (repeatability = 0.63; Keiser *et al.* 2014a) and associated with task participation (Grinsted *et al.* 2013; Keiser *et al.* 2014a), collective behavior (Pruitt, Grinsted & Settepani 2013a), and social stability (Laskowski & Pruitt 2014) in this and other social *Stegodyphus*.

Spiders were placed in a black plastic arena (diameter = 12 cm, height = 4 cm) and given a 60s acclimation period beneath an opaque plastic cover object. After 60s the cover object was removed and two rapid puffs of air were administered to the spider's anterior prosoma using an

infant ear-cleaning bulb. We then measured the time until the spider moved one full body length. Trials were terminated after 600s. Individuals with long latencies to resume movement (400s-600s) are deemed “shy”, individuals which resume movement rapidly are termed “bold” (1s-200s), and individuals which resume movement between 200s and 400s are considered “average” (Supplementary Fig. 1). After their personality assays, we gave each individual spider a unique 3-color ID mark with acrylic paint atop their abdomen to permit individual identification.

3.2.3 Collective behavior assays

We constructed artificial colonies of *S. dumicola* of two different sizes (10 or 30 individuals) and four different personality compositions (all bold, all shy, 50:50 bold and shy, or all individuals of “average” boldness) in a fully factorial design ($n = 64$ experimental units, $n \approx 8$ of each treatment combination). These experimental colony sizes fall within the natural range of sizes found in the southern Kalahari (1 - ~700 spiders, *unpubl. data*). In these populations, all colonies sampled contain a majority of shy individuals, while the remaining individuals exhibit varying distributions ranging from average to bold (Supplementary Fig. 1). We allowed each colony 24 hours indoors to produce a silken retreat in 240ml plastic cups. We divided our collective foraging assays into two parts. We first tested each colony three times before they produced a capture web. Thus, each colony was operating on a web of the same volume (i.e., the size of their cup). These collective foraging assays were performed in the field under ambient temperature and natural light: dark cycles. Collective foraging assays were initiated by placing a small piece of white paper (1.5cm^2) in the center of the web and allowing a 20s acclimation

period. We then used a battery-powered handheld vibratory device to vibrate the piece of paper to simulate a prey item caught in the silk. We recorded the latency for the first spider to emerge from the retreat, the latency for the first spider to attack the paper, and the total number of attackers that participated in the prey capture event.

After the three initial assays, each colony container was fastened to a hookbush acacia (*Acacia mellifera*) branch with clothespins in the early evening hours (2000-2130). This particular site contains an abundance of the widely-foraging predatory ant, *Anoplolepis custodiens* (2-8 nest entrances per m²). This ant, even in small numbers, is as a top predator responsible for colony-wide death in *S. dumicola* in the nearby Namib Desert (Henschel 1998). The following morning (0545-0600) we measured the capture web area produced by estimating its general shape (triangle, rectangle, etc.) and then recording the appropriate dimensions to estimate its area using a tape measure. Although relatively rare (23/64 cases), we also counted the number of individuals which had “dispersed” from the plastic cup and had produced a smaller retreat on another branch. Such ancillary nests were connected to the central nest via a shared capture web. At 0600 and 1800 the following days we tested the collective foraging of each colony. The foraging behavior of each colony was measured between 3 and 16 times total. After each collective foraging event we tested the speed at which spiders evacuated into the retreat following an aversive stimulus. The aversive stimulus was implemented by striking the branch to which the colony was attached with a blunt probe. This stimulus sends a vibration throughout the web which caused spiders to disperse from the simulated prey item and run back into their retreat. We measured the latency for the first and the last individual to enter the retreat following this stimulus.

3.2.4 Statistical analyses

The web size data were analyzed with general linear mixed-model ANOVA with the following predictor variables: group size, colony composition, and group size \times colony composition. We also included source colony ID as a random effect. We analyzed the number of dispersing spiders with ANOVA with the same predictor variables and random effects as the web size data.

The latency to emerge and latency to attack data were log transformed to meet model assumptions. We used independent general linear mixed-models to predict three response variables (latency to emerge, latency to attack, # of attackers) with the following predictor variables: source colony ID (random), group size, colony composition, web presence (With/Without capture web), group size \times colony composition, group size \times web presence, composition \times web presence, and group size \times colony composition \times web presence. Post-hoc tests were performed using Tukey's HSD.

3.3 RESULTS

3.3.1 Web architecture and dispersal

The combined model predicting capture web size was highly significant ($F_{16,37}=4.84$, $R^2=0.65$, $P=0.007$). Groups of 30 spiders produced capture webs three times larger than those created by groups of 10 individuals ($F_{1,42.9} = 39.32$, $p < 0.0001$) while group composition had no

detectable influence on total web area ($F_{3,40.1} = 1.16$, $p = 0.33$). The effect of group size did not differ for different group compositions ($F_{3, 40.4} = 1.46$, $p = 0.23$).

Larger groups also had more individuals which left the colony to create small retreats along other sections of the same capture web ($F_{1,23} = 4.32$, $p = 0.05$). The number of dispersers, however, was not influenced by group composition ($F_{3,23} = 1.11$, $p = 0.37$) and the interaction term between size and composition was also not significant ($F_{3,23} = 0.97$, $p = 0.42$), meaning that the number of individuals that dispersed at each colony size did not differ based on the group composition. The number of dispersers was also not influenced by the identity of the source colony from which spiders were collected ($P > 0.05$).

3.3.2 Prey capture

Our combined model predicting colonies' latency to emerge was highly significant ($F_{23,253}=4.85$, $R^2=0.37$, $P<0.0001$). Group composition but not group size had a significant effect on colonies' latency to emerge in response to prey (Table 1). However, the effect of group composition differed in the presence vs. absence of the capture web. In the absence of a capture web, colonies composed of all bold individuals were 2.2 times faster in emerging from their retreat compared to other compositions, regardless of group size (Table 1, Fig. 1). However, we failed to detect a relationship between latency to attack and colonies' personality composition when capture webs were present (Fig. 1).

Our combined model predicting colonies' latency to attack was also highly significant ($F_{23,253}=5.43$, $R^2=0.35$, $P=0.004$). As with latency to emerge, we detected a significant effect of group composition but not group size on colonies' latency to attack (Table 1). However, the

effects of group composition differed depending on whether the capture web was present versus absent. Post hoc tests revealed that, in the absence of a capture web, colonies of shy spiders were 2.6 times slower to attack the prey stimulus compared to all other colony compositions (Fig. 2). We failed to detect any significant effects of group size or composition when colonies were permitted to construct capture webs (Fig. 2).

The combined model predicting the total number of attackers was significant ($F_{23,253}=6.14$, $R^2=0.39$, $P<0.0001$). This attribute was impacted by both the composition of the group and the group size (Table 1). Here, however, the effects of either attribute were indistinguishable in the presence vs. absence of the capture web. Groups composed of only bold individuals attacked prey with 1.8-2.6 times the number of attackers as rival colonies. Whereas, colonies of 30 individuals attacked prey with 1.26-2.1 times the number of attackers as colonies of only 10 individuals. Notably, the number of attackers participating in prey capture increased more slowly than group size, likely a result of the behavioral composition of the colony. For comparison, the effect size of having a colony composed of only bold individuals ($\beta=0.39$, $SE=0.05$) was far greater than that of having a colony of 30 individuals ($\beta=0.15$, $SE=0.03$).

Finally, the identity of the source colony from which individual spiders were obtained had no significant effect on any of the collective traits assessed here (all 95% confidence intervals overlapped zero).

3.3.3 Web evacuation

No independent variables (i.e., composition, size, etc.) had a significant influence on the latency for the first individual (all $p \geq 0.12$) or the last individual (all $p \geq 0.18$) to evacuate the capture web following an aversive stimulus.

3.4 DISCUSSION

Determining the traits that underlie collective behavior is important, in part, because it provides clues to how evolution can hone the collective and/or emergent traits of groups. Given its intuitive appeal and ease of manipulation, group size has been manipulated in an extraordinary number of systems, and it seems to be important in determining a variety of collective traits. However, groups of different sizes are inherently different in other, more cryptic traits. For example, larger groups might also contain more informed individuals (Sumpter *et al.* 2008) or have an increased likelihood that keystone individuals will be present in the mélange (Modlmeier *et al.* 2014a). Here, we demonstrate that the relative importance of personality composition vs. group size varies depending on the trait under consideration. And, in some cases, the effects of personality composition effectively dwarf those attributable to group size.

3.4.1 Web architecture and dispersal

For web architecture the only significant predictor was group size. Here the estimated effect of group size was three times that of personality composition, where groups of 30 individuals produced webs three times larger than colonies of only 10 individuals. Thus, group size has a roughly additive effect (i.e., linear scaling) on capture web size. For this collective trait, the influence of group size is far greater than that of personality composition. This corroborates previous evidence in this social system which suggest that individual personality does not influence the propensity for individuals to produce capture web silk, though smaller individuals are more likely to participate in web-building (Keiser *et al.* 2014a). In other social spiders, larger capture webs increase prey capture rate but decrease per capita food intake (Yip,

Powers & Avilés 2008), so a linear scaling between group size and capture web size does not confer per capita foraging benefits.

Similarly, the number of individuals which dispersed to created new retreats along the capture web was influenced only by group size, where larger groups contained more dispersers. Colonies of *S. dумicola* in the field are often polydomous (i.e., containing multiple retreats), where smaller colonies will “bud” off of the natal colony and share a single large capture web (Henschel 1998). Although it is unknown if these budding individuals are also more likely to engage in long-distance dispersal to found new clusters of colonies (Schneider *et al.* 2001), evidence from mitochondrial DNA suggest that colonies in close proximity are formed from single matri-lineage propagules and thus local population dynamics are strongly influenced by individual behavioral decisions (Johannesen *et al.* 2002). The propensity for individuals to leave the colony, in turn, seems to be influenced by group size (like in other social groups (Smith *et al.* 2014)), but an individual’s decision to bud off from the natal colony may be influenced by individuals’ traits (i.e., hunger state, personality).

3.4.2 Prey capture

The effects of personality composition were larger than group size for collective foraging behavior. Admittedly, it is not terribly surprising that both group size and personality composition impact some aspects of collective foraging behavior. Dozens of studies from a diversity of systems have demonstrated these sorts of associations. The surprising findings from our data are the sizable differences in effect size. Personality composition was the only trait significantly associated with colonies’ latency to emerge or latency to attack under any

condition. Without a capture web, colonies of bold spiders attacked prey 3-8 times more rapidly than other compositions. As for the number of attackers, the effect size of personality composition was more than twice that of group size. To place this in relative terms, colonies of ten bold spiders are predicted to attack prey with as many attackers as colonies of 110 spiders with only average boldness, assuming linear relationships (Fig. 4). Given that the foraging success of social spiders is often linked to the number of attackers (Nentwig 1985; Yip, Powers & Avilés 2008; Pruitt & Keiser 2014), our results suggest that foraging success may hinge more heavily on the *types* of individuals in the colony rather than the mere number of members.

These results are exciting because countless studies on collective behavior have overlooked personality composition, in part, because it is a cryptic aspect of trait variation and can be difficult to manipulate in the field. However, our results suggest that future studies should attempt to account for this variation. Moreover, classic studies that considered group size alone may have inadvertently attributed effects to group size that are actually the effect of differences in group composition or within-group behavioral variation. This might also explain why different studies often finding conflicting effects of group size (reviewed in Dornhaus, Powell & Bengston 2012): because by manipulating group size, studies are simultaneously and unknowingly shifting groups' personality compositions.

Collective foraging behaviors differed drastically based on whether or not colonies had produced a capture web. In fact, when colonies were allowed to produce a capture web in an *Acacia* tree, the effect of both group size and composition were lost on two of the three collective foraging behaviors measured. Importantly, even before we allowed colonies to produce a capture web, they were still exposed to many environmental cues (e.g., wind, olfactory cues, light/dark cycles). However, they admittedly lacked any potential interactions with live prey or predators in

these initial assays. In contrast, when colonies were permitted to construct a capture web, we noted live prey in nearly every web and we often observed individuals and groups of spiders interacting with predatory ants. Although mostly speculative, we propose that antagonistic species interactions may decouple the influence of both group size and group composition on some collective behaviors but not others, as has been observed in some other systems (Cote *et al.* 2013).

Contrary to the seemingly linear relationship between group size and some collective behaviors (e.g., web production), the strong effect of group composition on collective behavior may be subtle and non-linear. For instance, in some extreme examples, colonies' collective behavior can be driven by the traits of one or a few highly influential group members [39, 44]. Under such circumstance, the effects of group size on collective behavior are small or undetectable (Pruitt, Grinsted & Settepani 2013b). Data from laboratory studies suggest that the presence of bold *Stegodyphus* is particularly important in determining colonies' behavior because bold spiders somehow instigate or catalyze more aggressive foraging behavior in their fellow (often shy) colony mates (Pruitt & Keiser 2014).

3.4.3 Web evacuation

Neither colony composition nor group size affected the latency for individuals to evacuate the capture web following a vibratory aversive stimulus. This is surprising because one would intuitively predict that web evacuation, which is a measure of colonies' collective boldness/fear, would be intimately associated with the boldness of the colony constituents. Thus, it appears that the relationships between the collective behavior of colonies and the personalities

of their constituents are not always easy to predict and may easily vary depending on the ecological context (e.g., foraging vs. anti-predator behavior) under consideration and/or how we measure them.

3.4.4 Conclusions

The results of separate studies on either personality composition or group size have often attributed similar findings to either mechanism independently (e.g., invasion biology: Hee *et al.* 2000; Fogarty, Cote & Sih 2011), while others have profitably considered the eco-evolutionary consequences of both colony composition and size simultaneously (Jeanson & Fewell 2008; Pruitt 2013). In our system the relative influence of group size and personality composition depended on the collective trait being considered. In some cases the differences in effect sizes were considerable and counterintuitive. As such, we urge that future studies on common models of collective behavior (e.g., fish schools, bird flocks, ant colonies, etc.) should manipulate both personality composition and group size simultaneously to elucidate their interplay and relative contributions on a variety of collective behaviors. In *S. dumicola*, naturally occurring colonies vary considerably in their size:composition relationship, where larger groups generally contain a larger proportion of bold individuals ($F_{1,15} = 16.3$, $p = 0.001$, $r^2 = 0.54$; Supplementary fig. 2). Furthermore, colony composition is likely not driven by within-group relatedness, since variable colony compositions arise among colonies that share similar levels of within-group relatedness ((Johannesen *et al.* 2002)). We further wonder whether or how group size and group compositions naturally covary in diverse systems, for instance, along environmental gradients. We argue that these are vital next steps towards a comprehensive understanding of the behavioral and evolutionary ecology of collective behaviors.

Table 3-1. Summary of effect tests from three general linear models predicting three aspects of colonies' collective foraging behavior in the wild. An asterisk denotes significant values.

Latency to Emerge				
Source	DF	DF_{Den}	F	P-value
Size	1	260.6	2.56	0.12
Composition	3	257.5	4.17	0.009*
Web presence	1	255.5	1.88	0.31
Size × Composition	3	259	0.42	0.85
Size × Web presence	1	254.4	1.76	0.41
Composition × Web presence	3	254.8	3.81	0.007*
Size × Composition × Web presence	3	254.6	1.05	0.23
Latency to Attack				
Source	DF	DF_{Den}	F	P-value
Size	1	260.6	2.74	0.09
Composition	3	257.5	4.56	0.004*
Web presence	1	255.5	1.55	0.21
Size × Composition	3	259	0.32	0.81
Size × Web presence	1	254.4	1.62	0.2
Composition × Web presence	3	254.8	3.94	0.009*
Size × Composition × Web presence	3	254.6	1.85	0.14
Number of Attackers				
Source	DF	DF_{Den}	F	P-value
Size	1	257.5	26.25	< .0001*
Composition	3	258.2	17.99	< .0001*
Web presence	1	256.7	14.1	0.0002*
Size × Composition	3	256.1	5.12	0.002*
Size × Web presence	1	254.1	0.16	0.69
Composition × Web presence	3	254.9	0.43	0.73
Size × Composition × Web presence	3	254.7	2.46	0.06

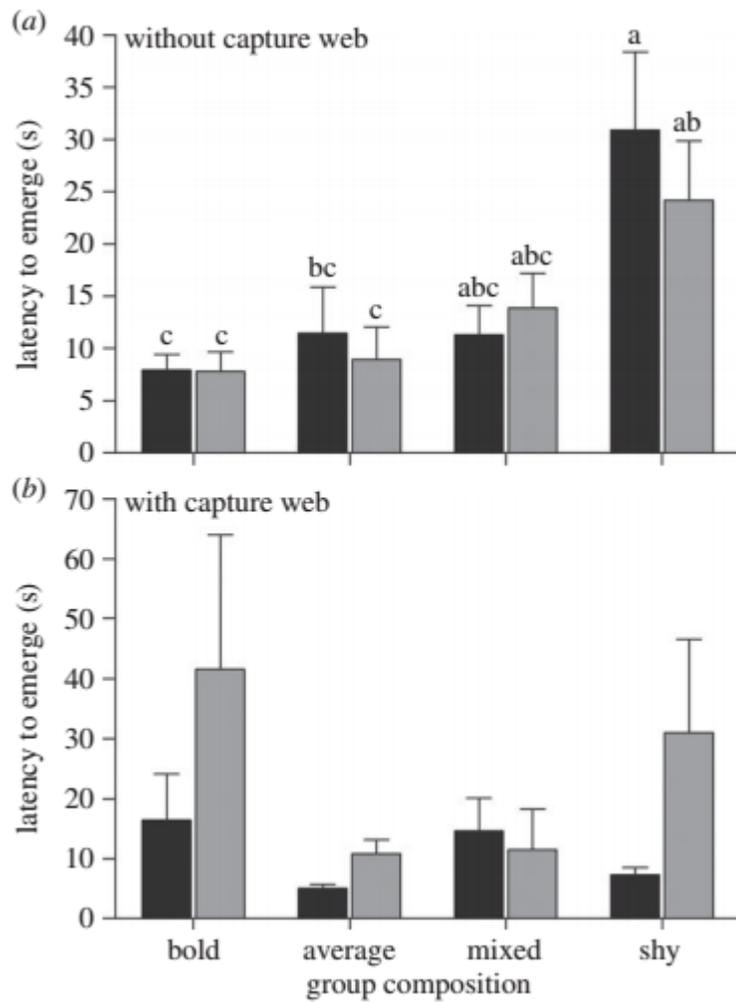


Figure 3-1. Without a capture web, the latency for the first individual to emerge from the retreat after prey stimulus was a product of the colony's personality composition. Values significantly different from each other are represented by different letters within each panel

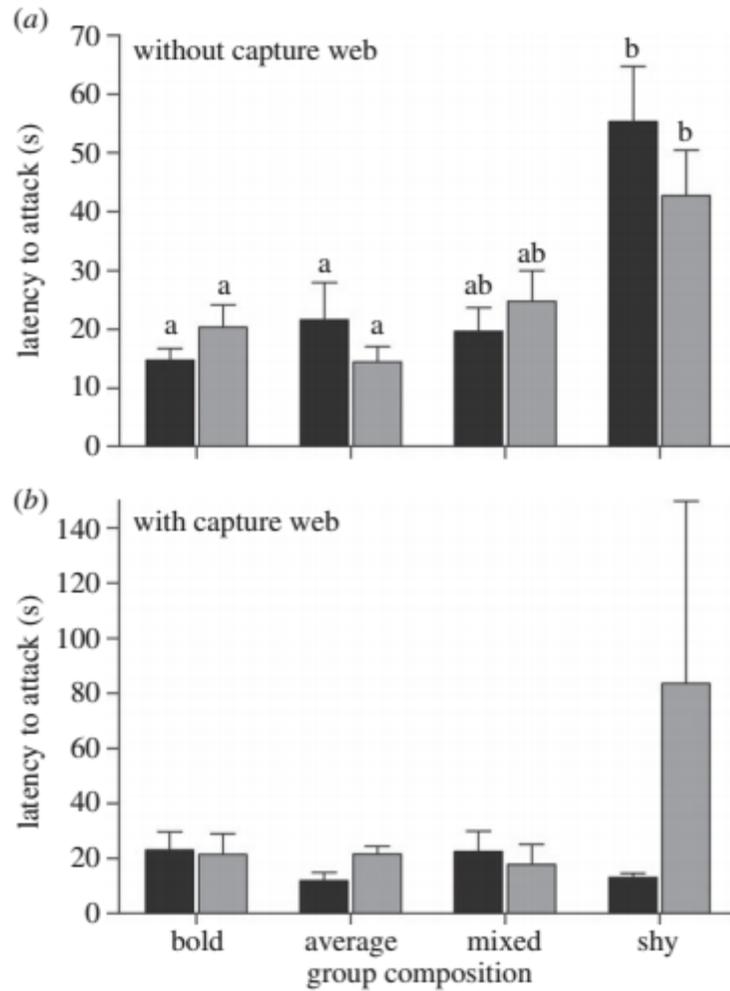


Figure 3-2. Without a capture web, colonies composed of all shy individuals were 1.3 times faster in attacking the prey stimulus when their colony size was thirty as opposed to ten. Values significantly different from each other are represented by different letters within each panel.

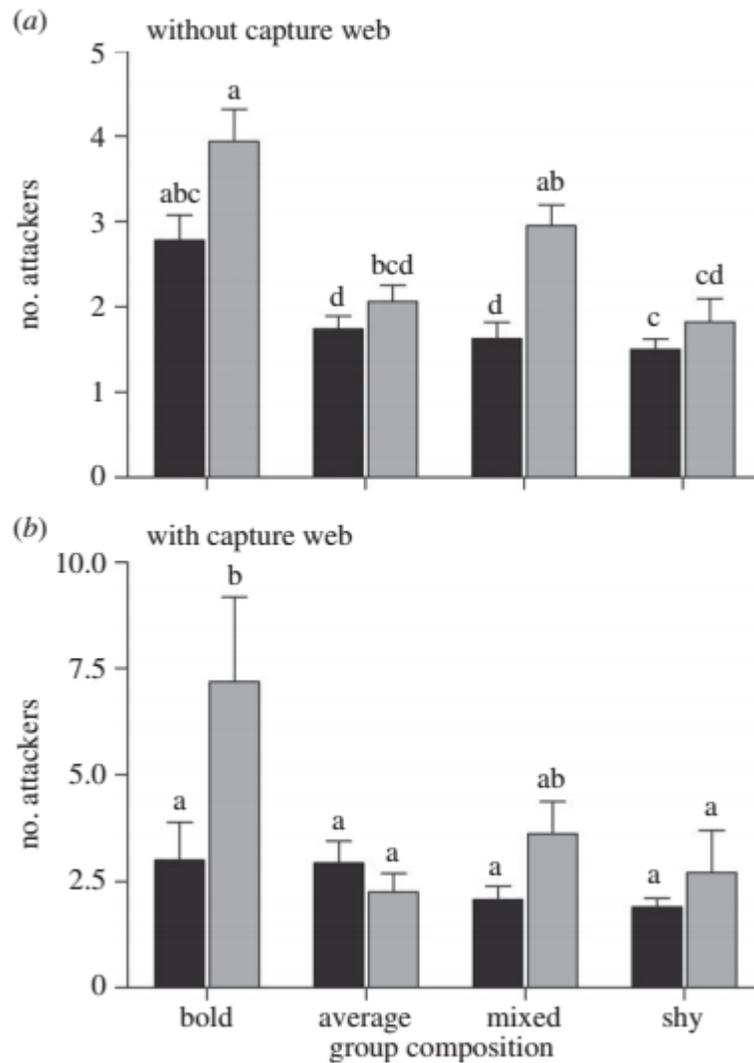


Figure 3-3. The number of individuals which participated in the attack was influenced both by the composition of the group and the group size. Values significantly different from each other are represented by different letters within each panel.

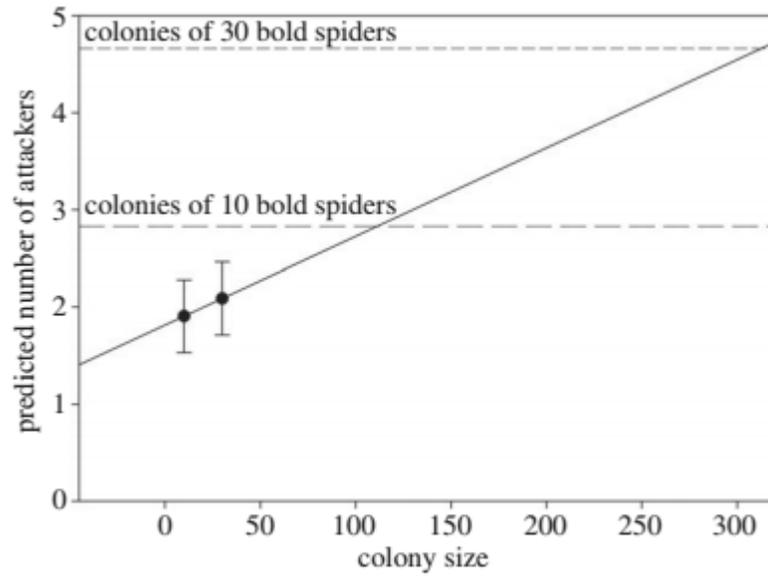


Figure 3-4. A speculative depiction of the predicted relationship between colony size and participation in collective prey capture for shy/average colonies (solid line).

4.0 INCREASED BACTERIAL LOAD ON KEYSTONE INDIVIDUALS ALTERS GROUP COLLECTIVE BEHAVIOUR

Keiser CN, Wright CM, and Pruitt, JN. 2016. Increased bacterial load on keystone individuals alters group collective behaviour. *Animal Behaviour*. 114: 211-218.

4.1 INTRODUCTION

The collective behaviors 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 multi-level sociality has been described as one of the great evolutionary transitions in biological organization, by allowing animal societies to perform feats which are unachievable by solitary individuals (Smith & Szathmary 1997). Although theorists have classically maintained an egalitarian view regarding the organization of collective behaviors for many animal societies, behaviorists 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 *et al.* 2010) or knowledgeable matriarchs in elephant herds (McComb *et al.* 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 (Paine 1969; Modlmeier *et al.* 2014a).

There are myriad examples demonstrating how the presence of keystones can augment collective behaviors or enhance group productivity and survivorship (reviewed in Modlmeier *et al.* 2014a). However, circumstances also exist where the presence of key individuals can dampen collective behaviors 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 “hyper-aggressive males” can depress the reproductive success of entire groups of water striders (Sih & Watters 2005; Chang & Sih 2013). A similar phenomenon had also previously been observed in yellow baboons (Alberts, Sapolsky & Altmann 1992). Other examples exist where the collective exploratory behavior of feral guppy schools is restricted by the least active member of the group (Brown & Irving 2014), and the foraging preferences of dominant pairs of brant geese 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 aging, injury, or reproductive status (Brent *et al.*; Horner *et al.* 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 behavioral traits like social dominance (Sapolsky 2005), aggressiveness (Jin *et al.* 2013), and exploratory behavior (Boyer *et al.* 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 *et al.* 2005). Unfortunately, superspreaders are often only identified *a posteriori* as the index cases of larger epidemics or local transmission events in human (Gibbins 1998; Shen *et al.* 2004; Gahr *et al.* 2014) and animal populations (Matthews *et al.* 2006; Kao *et al.* 2007; Hampson *et al.* 2009). Further, cases where formerly-influential keystone individuals like 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 behavior of one or a few key individuals (Pruitt, Grinsted & Settepani 2013b; 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 *et al.* 1994). Pruitt and Keiser (2014) recently demonstrated that the presence of just one extremely bold individual can enhance the foraging aggressiveness of entire colonies, increasing the average mass gained by their colony-mates. These keystone individuals initiate more foraging bouts than their less-bold colony mates, though their presence seems to eventually catalyze 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 *et al.* 2013; Pruitt, Grinsted & Settepani 2013b; Keiser *et al.* 2014b; Keiser & Pruitt 2014a; Pruitt & Keiser 2014), this system represents a superb model to test questions on how interactions between keystone individuals and microbes can change the collective behavior of their colonies. In *S. 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, though there is no evidence that cuticular microbes alter host behavior (Keiser *et al.* 2016).

Here, we test the collective behaviors 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 aim to address the following questions: (1) To what degree will a putative keystone individual's prior exposure to harmful bacteria alter the collective behavior of the colony? (2) To what extent will the personality type (boldness) of the keystone alter the association between its bacterial exposure history and colonies' collective behavior? (3) Does a putative keystone individual's propensity to participate in collective behavior 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.

4.2 METHODS

4.2.1 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 (Henschel, Lubin & Schneider 1995; Henschel 1998; Avilés, Varas & Dyreson 1999). 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 3-dimensional dense silken retreat and one or a few 2-dimensional capture webs where spiders interact with prey. Spiders repair damage to this capture web nightly (Keiser *et al.* 2014a). 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 (S28°54'32.0" E23°24'33.7") where the colonies were maintained indoors in 500ml plastic cups at ambient temperature and natural light:dark cycle. Prior to experimentation, we isolated each adult female from the colony into 1ml plastic condiment cups.

4.2.2 Bacterial 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 LB broth. Bacteria were identified using 300bp 16S ribosomal DNA sequencing and MicroSeq[®] BLAST Software (SeqWright Genomic

Services Houston, TX 77054). 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 1ml 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).

4.2.3 Experimental bacterial exposure

24hr prior to their introduction into experimental colonies, putative keystone individuals were exposed to bacteria by placing the spider in 1ml of either the bacterial cocktail or sterile LB agar and shaking the spider in the solution at 1500rpm for 3s 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 vs. control spiders = 27 days; Keiser *et al.* 2016). Prior to bacterial exposure, keystone individuals were given a dot of non-toxic blue acrylic paint atop their cephalothorax so we could track their behavior within the colonies.

4.2.4 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 4 spiders per day for 4 days from each treatment group by vortexing the spiders in 1ml of LB broth at 2000rpm for 10s. We then performed four 10-fold serial dilutions of this solution in LB broth and plated 100 μ l 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 63103). These plates were incubated for 24hr at 30°C and the number of bacterial colonies visible to the naked eye was counted 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 calipers and an analytical balance (Model P-114, Denver instruments, Bohemia, NY 11716), 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).

4.2.5 Experimental colony construction

We collected adult female spiders from 5 different source colonies for the collective foraging experiment and 7 different source colonies for the web maintenance experiment. We measured each spider's mass and prosoma width, and then subjected them to an antipredator behavioral 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 behavioral metric (repeatability $\approx 0.5 - 0.7$) (Pruitt, Grinsted & Settepani 2013b; Keiser *et al.* 2014b; Keiser *et al.* 2014c) that is linked with individuals tendencies to perform a variety of tasks (Keiser *et al.* 2014a; Wright, Keiser & Pruitt 2015).

We used an assay developed by Riechert and Hedrick (1993), where the spider is placed in a black plastic arena (12cm diameter \times 4cm height) and given a 30s 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 600s 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 seconds $-$ spider latency) to make the interpretation of results more intuitive. That is, a higher boldness score represents bolder behavior (i.e., spiders that resume activity faster after the stimulus).

We then constructed artificial colonies containing 9 "shy" spiders (each with a latency to move score of 600) and later added one bolder spider, the putative keystone individual (latency to move scores ranged from 1 to 600 in both treatment groups), in 240ml clear plastic cups. keystones were assigned to treatment groups (exposed vs. unexposed) and experimental colonies

haphazardly, and individuals were not mixed from different source colonies, as to maintain naturally-occurring levels of within-colony familiarity (Modlmeier *et al.* 2014b) and relatedness (Lubin & Bilde 2007b).

4.2.6 Collective foraging assay

Experimental colonies (n = 30 exposed keystone; n = 29 control keystone) containing 9 shy spiders were hung in *Acacia mellifera* trees with clothespins at 20:00hr to allow capture web construction/expansion overnight. At 06:00hr and 18:00hr the following day, we measured their collective foraging by placing a 1.5cm² piece of paper in the center of the capture web, allowing a 30s acclimation, and vibrating the paper with a wire attached to a handheld vibrator (Model: Flamenco Purple #4, Golden Triangle). The vibrator was set to a low-frequency “pulse” setting with a pulse frequency of approximately 3 pulses/sec. This stimulus is meant to simulate the fluttering behavior of a prey item captured in the web. We then recorded the latency for the first spider to (1) emerge from the retreat, (2) 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 20:00hr. The keystone individuals had been either exposed to the bacterial cocktail described above or a control treatment of sterile LB broth. We measured the collective foraging as before twice per day for the next three days (six measurements total) and noted whether or not the keystone participated in the attack.

4.2.7 Web maintenance assay

We constructed an additional 34 experimental colonies containing 9 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 24hr to build a retreat in 240ml plastic cups, and then placed the colonies in *A. mellifera* trees at 20:00hr as before. At 05:00hr 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 (cm²). We scanned each colony between 19:00hr and 20:00hr 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.

4.2.8 Statistical analyses

Bacterial load estimates: Bacterial load data were log-transformed and analyzed with a general linear mixed model with keystone exposure, spider prosoma width, days since exposure, and a days since exposure \times 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 vs. control keystones was associated with a change in the collective behavior of colonies, we performed two separate GLMMs (control vs. exposed colonies separately) with absence vs. presence of a keystone as an

independent variable predicting (1) the latency for the first spider 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 behavior of their colony-mates. To further analyze 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 vs. control), keystone boldness score, and a keystone exposure \times 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 behavior of other colony members, rather than on the keystone individual itself.

Collective web maintenance: To analyze 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 \times boldness interaction term as independent variables. Assay # 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 analyzed with binomial logistic regressions with keystone boldness, keystone exposure, observation #, keystone exposure \times observation #, and a keystone exposure \times boldness interaction term as independent variables. Response variables were whether or not the individual participated in the task (participate vs. 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, USA).

4.3 RESULTS

4.3.1 Bacterial load

On average, exposure to the bacterial cocktail was associated with an increased cuticular bacterial load 2-3 orders of magnitude greater than that estimated on the cuticles of control spiders, which were not significantly different from that of spiders which were untreated ($F_{2,28.6} = 22.0$, $p < 0.0001$; Fig. 1; Table S1). 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 ($F_{1,25.8} = 0.004$, $p = 0.95$).

4.3.2 Collective foraging

Colonies that were assigned keystones of different bacterial exposure statuses did not differ in terms of their collective foraging behavior 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 \times 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 \times 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 colonies' 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 compared to those with an exposed keystone ($F_{1,36.3} = 13.24$, $p = 0.0008$; Fig 2B). A nearly identical number of individuals were observed participating in collective foraging prior to the addition of a keystone compared to those where an exposed keystone was added. 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).

4.3.3 Web maintenance

Colonies' capture web area (cm²) 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 than 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).

4.4 DISCUSSION

The successful execution of group's collective behaviors can often depend on the actions of just one or a few keystone individuals. Though, how such individuals' conditions or recent experiences affect group behaviors remains little-explored. Here, using the social spider *S. dumicola*, we demonstrate that recent increases in keystone individuals' cuticular bacterial load can alter the collective behavior 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 behavior of their colony changed based

on the keystone's recent bacterial exposure history. That is, colonies containing bolder keystones attacked prey more quickly than those 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 data suggest that a keystone individual's recent exposure to bacteria can have context-dependent effects on the collective behavior of their colonies.

The success of social spider colonies is often dependent on their ability to capture and consume large and particularly profitable prey items (Nentwig 1985; Rypstra & Tirey 1991; Powers & Avilés 2007). 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 (Rypstra & Tirey 1991; Harwood & Avilés 2013). Thus, we argue that the differences in collective foraging behavior displayed by colonies with exposed vs. unexposed keystones could result in reduced foraging efficiency (Ward & Enders 1985; Krafft & Cookson 2012) and potentially lower colony success. Consistent with this extrapolation, previous laboratory experiments (Pruitt & Keiser 2014) showed that colonies that contained bolder keystone individuals attacked prey with a greater number of attackers and this was associated with heightened mass gains and survival rates for the whole colony.

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 2015b) and a field experiment

with a congener (Pruitt, Grinsted & Settepani 2013b) 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 this field experiment: 990 cm² vs. maximum capture web size in laboratory: ~100 cm²). 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 response to prey and the number of individuals that participate in web building are much clearer.

One wonders whether potential "sickness behaviors" 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 *et al.* 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 behaviors 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 behavior playing a role in their reduced involvement. At present, we still disfavor 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 individual's 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 (Carl N Keiser, DeMarco, Shearer, Robertson, & Pruitt, 2015), so this remains an unlikely explanation for the depressed collective behavior 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 non-keystones, detailed social interactions, and putative sickness behavior will truly resolve these explanations (Klein 2003; Lopes *et al.* 2012).

One could argue that the mere addition of one more exposed or unexposed colony member (keystone or otherwise) could be responsible 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 behavior 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 behavior of all other colony members and not the participatory behavior of the keystone itself. Thus, as documented in previous studies (Pruitt & Keiser 2014), the presence of a keystone appears to catalyze more aggressive foraging behavior 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 only subtly impact colony web-building behavior. 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 2014a). However, when an unexposed keystone was added to a colony we observed roughly twice as many spiders engaged in web-building behavior 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 behavior 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. Consistent with this hypothesis, we observed a general decrease in the number of web-builders that is coincident with the disappearance of the keystones' influence (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 behavior in diverse taxa (Zilber-Rosenberg & Rosenberg 2008; Cho & Blaser 2012; Ezenwa *et al.* 2012; McFall-Ngai *et al.* 2013; Newton *et al.* 2013). Further, 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; Scheuring & Yu 2012; Sanders *et al.* 2014). 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 behaviors. 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 behaviors in a variety of social groups (e.g., ant colonies, elephant herds, fish schools, etc.; Modlmeier *et al.* 2014a), our findings suggest that a single individual's experiences with microbes could play an important and unappreciated role in determining the collective behavior of social groups.

Table 4-1. 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.

Latency to Emerge				
Source	DF	DF _{Den}	F	P-value
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				
Source	DF	DF _{Den}	F	P-value
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				
Source	DF	DF _{Den}	F	P-value
Keystone Exposure	1	36.3	13.24	0.0008*
Keystone Boldness	1	32.2	0.09	0.76
Keystone Exposure × Boldness	1	38.1	2.6	0.12
Participation by keystone				
Source	DF	DF _{Den}	χ^2	P-value
Keystone Exposure	1	-	0.32	0.57
Keystone Boldness	1	-	0.006	0.94
Keystone Exposure × Boldness	1	-	1.30	0.25
Observation #	1	-	3.06	0.08
Keystone Exposure × Observation	1	-	0.009	0.92

Table 4-2. 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.

Capture web area				
Source	DF	DF _{Den}	F	P-value
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				
Source	DF	DF _{Den}	F	P-value
Keystone status	2	27.4	1.79	0.19
Night of observation (#)	4	95.2	6.41	0.0001*
Keystone status × Night #	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				
Source	DF	DF _{Den}	χ^2	P-value
Keystone status	1	-	0.78	0.38
Night of observation (#)	4	-	3.97	0.41
Keystone status × Night #	4	-	1.19	0.88
Keystone Boldness	1	-	0.02	0.90
Keystone status × Boldness	1	-	0.05	0.82
Night of observation (#)	4	-	3.99	0.41
Keystone status × Night #	4	-	0.43	0.98

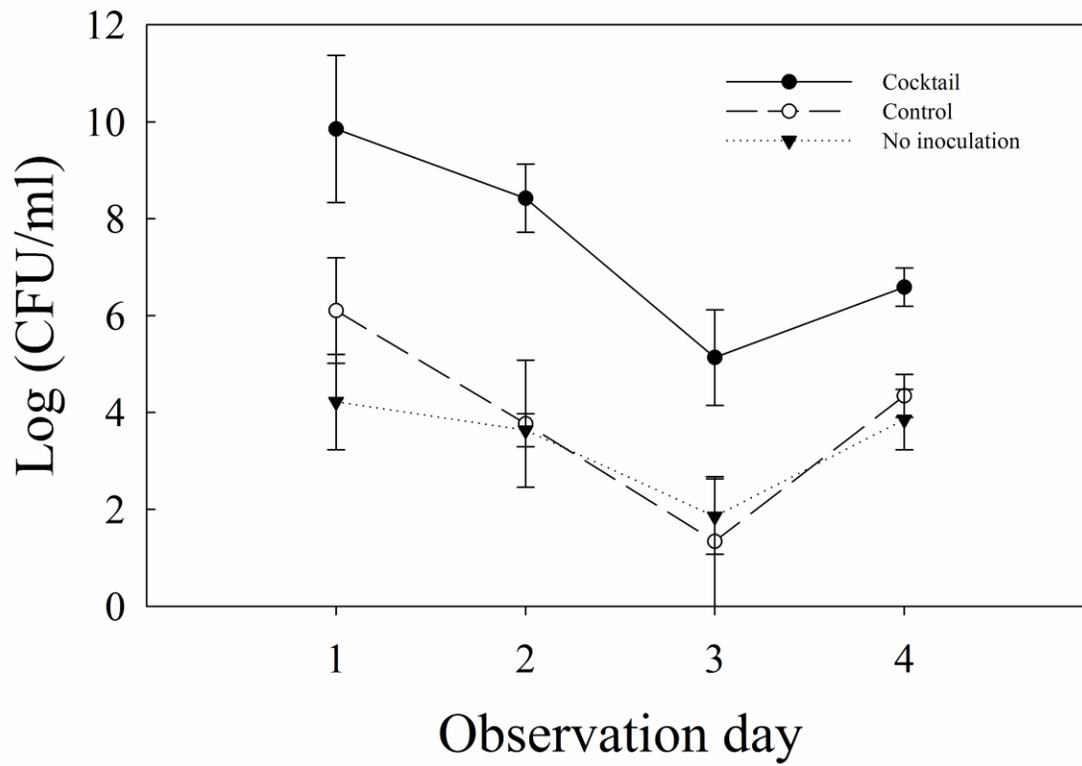


Figure 4-1. Bacterial exposure increases cuticular bacterial load.

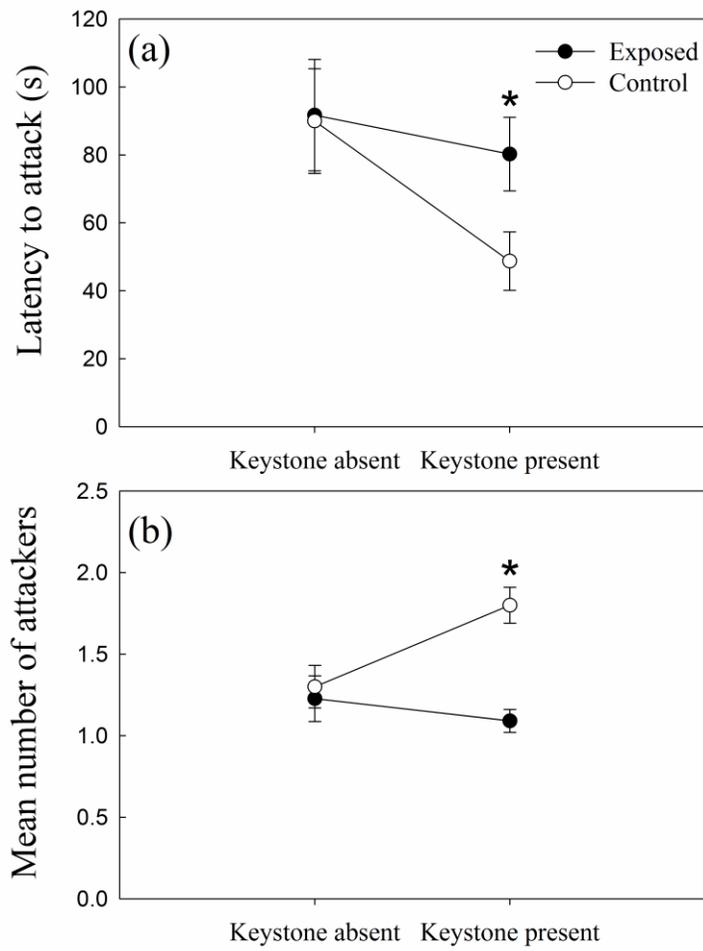


Figure 4-2. Bacterial exposure alters colony collective foraging.

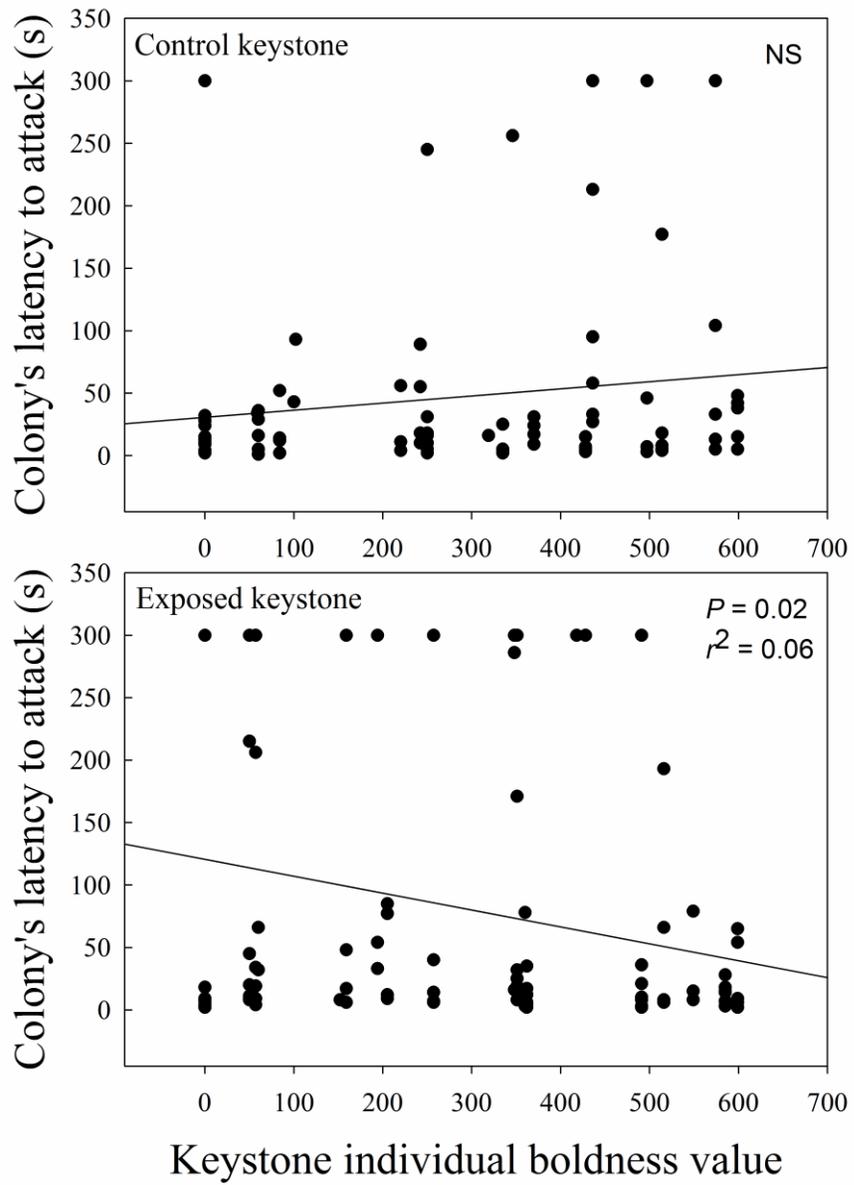


Figure 4-3. Keystone boldness, bacterial exposure, and collective foraging.

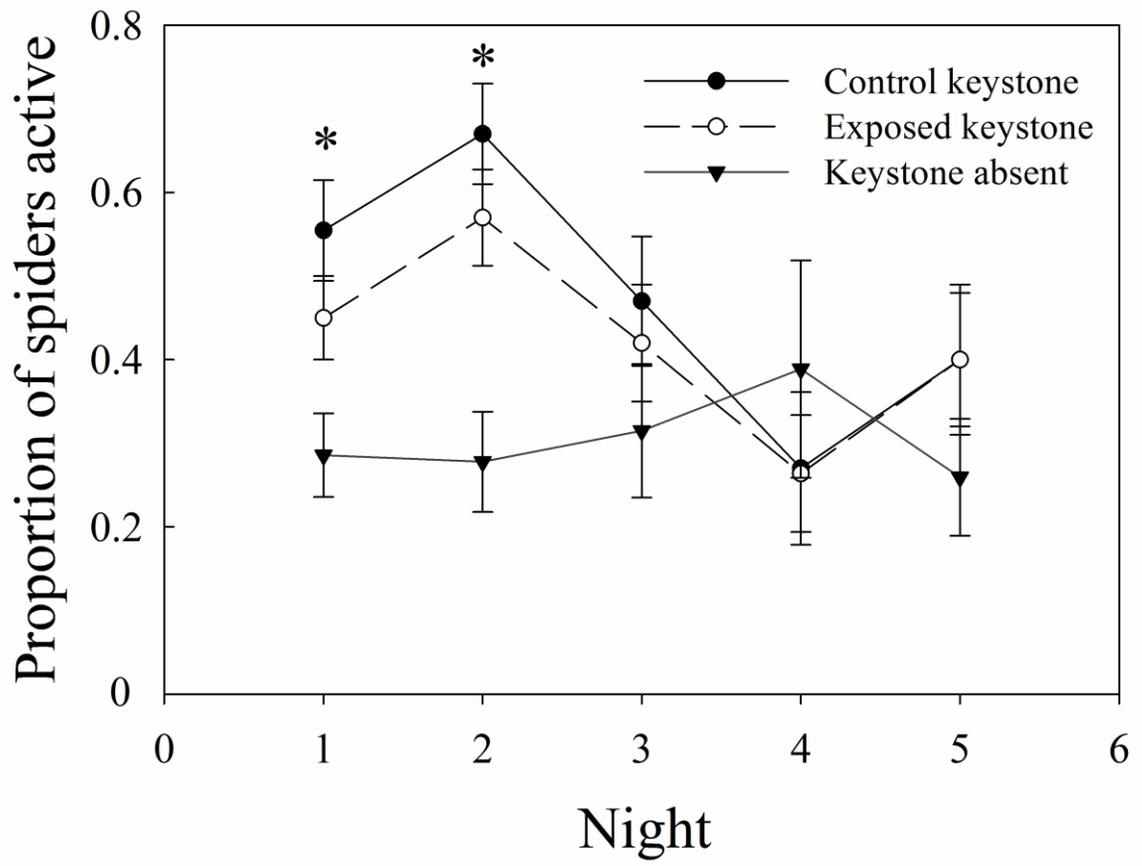


Figure 4-4. Bacterial exposure and web construction/maintenance.

5.0 INDIVIDUAL DIFFERENCES IN BOLDNESS INFLUENCE PATTERNS OF SOCIAL INTERACTIONS AND THE TRANSMISSION OF CUTICULAR BACTERIA AMONG GROUP-MATES

Keiser CN, Pinter-Wollman N, Augustine DA, Ziamba MJ, Hao L, Lawrence JG, and Pruitt JN. 2016. Individual differences in boldness influence patterns of social interactions and the transmission of cuticular bacteria among group-mates. *Proceedings of the Royal Society B: Biological Sciences*. 283: 20160457.

5.1 INTRODUCTION

The transmission of microorganisms from exposed to susceptible hosts represents one of the most crucial forces regulating infectious disease dynamics. Recent attention (McCallum, Barlow & Hone 2001; Begon *et al.* 2002), however, has scrutinized historical attempts to quantify and predict transmission dynamics. For example, a foundational principle of epidemiology, “the mass action principle”, relies on the assumption that the course of an epidemic is determined by the rate of random contacts between infected and susceptible individuals (e.g., Regoes *et al.* 2003). Of course, populations are not comprised of individuals who interact randomly, and interactions during epidemics do not always result in transmission (Blyton *et al.* 2014). Variation in host behavioral phenotypes and social interactions remain

putative explanations of the immense transmission heterogeneity observed in human and wildlife diseases (Woolhouse *et al.* 1997; Lloyd-Smith *et al.* 2005; White, Forester & Craft 2015).

Consequently, an important topic of current discourse in disease ecology has focused on understanding how consistent behavioral variation among individuals (i.e., behavioural types, syndromes, or personality Barber & Dingemanse 2010; Sih *et al.* 2012b) may influence the dynamics of microbial transmission (Kortet, Hedrick & Vainikka 2010).

Many of the most prevalent and deadly diseases threatening wildlife and human populations are characterized by intense variation in the degree to which infected individuals produce secondary cases of infection (Lloyd-Smith *et al.* 2005). In fact, host heterogeneity in infection susceptibility (Dwyer, Elkinton & Buonaccorsi 1997) and pathogen transmission (Woolhouse *et al.* 1997; Galvani & May 2005; Lloyd-Smith *et al.* 2005) are becoming increasingly emphasized in disease ecology and epidemiology. In addition, a group's behavioral composition may also have consequences for the spread of infectious agents (Pike *et al.* 2008). A recent study on behavioral variation and disease dynamics in house finches found that an individuals' propensity to use experimental feeders increased both the acquisition and transmission of a bacterial pathogen (Adelman *et al.* 2015). Such studies are sorely needed because they employ experimental infections and characterize inter-individual variation in behaviors which are predicted to influence transmission dynamics.

Variation among individuals in how frequently and with whom they interact has important implications for epidemiological dynamics. For example, individuals that interact with others more frequently can have an inordinately large effect on driving the spread of an epidemic (Lloyd-Smith *et al.*). Both individual-level (*e.g.*, infection status) and group-wide processes (*e.g.*, disease outbreaks) are influenced by the connectivity of individuals (Perkins *et al.* 2009; Sih,

Hanser & McHugh 2009; Gardy *et al.* 2011). The incorporation of social network theory into models of disease dynamics has shown that the structure of the interaction network and the processes that underlies its formation can affect transmission rate (Bansal, Grenfell & Meyers; Fefferman & Ng; Hock & Fefferman). Therefore, empirical studies of the processes that drive social network formation are crucial for our understanding of disease dynamics. A common mechanism of network formation is assortativity according to a certain trait, that is, individuals often tend to interact with others of similar phenotypes (Croft *et al.* 2009). Just as common is the complementary mechanism, disassortativity, where individuals tend to interact with individuals of opposing phenotypes (Newman 2002; Newman 2003). These interaction preferences will shape network structure and may influence the transmission rate of microbes among individuals (Fefferman & Ng; White, Forester & Craft 2015).

Some prior studies have identified natural relationships between host personality type and infection status (Boyer *et al.* 2010; Dizney & Dearing 2013; Seaman & Briffa 2015), though fewer studies have experimentally differentiated behavior-mediated infection from parasite manipulation of behavior (notable exceptions include: Kekäläinen *et al.* 2014; DiRienzo *et al.* 2015) (reviewed in Poulin 2013). Others report evidence for associations between host behavioral traits and virus transmission (Pontier *et al.* 1998), and experimental infections of laboratory animals have generated enormous variation in pathogen shedding rates associated with host traits like co-infection status and immunocompetence (Gopinath *et al.* 2013; Lass *et al.* 2013). Despite these advances, studies rarely, if ever, evaluate the role of behavioral phenotypes in both infected *and* susceptible individuals simultaneously to test their joint effects on multiple modes of transmission.

Here we examine how variation among individuals in consistent behavioral traits influences contact network formation and bacterial transmission in the social spider *Stegodyphus dumicola* (Araneae, Eresidae). In colonies of *S. dumicola*, the execution of collective behaviors like prey capture (Pruitt, Grinsted & Settepani 2013b; Keiser & Pruitt 2014b; Pruitt & Keiser 2014; Pruitt & Pinter-Wollman) and antipredator defenses (Wright, Keiser & Pruitt 2016) is associated with heterogeneity among individuals in their boldness, suggesting that boldness is a reliable indicator of individuals' role in these societies. Therefore, we examined assortativity according to boldness by observing marked individuals in experimental colonies. To examine how the boldness of both exposed and susceptible individuals influences the likelihood of direct and indirect (*i.e.*, environmental) transmission, we experimentally exposed individual spiders with a Green Fluorescent Protein (GFP)-transformed cuticular bacterium. We focused on cuticular bacteria because the integument represents the barrier between the host and constant bombardment by microbes from its environment, representing a primary line of defense against invading microbes, and body contact represents a likely mode of transmission for cuticle-associated microbes (Brey *et al.* 1993; Vallet-Gely, Lemaitre & Boccard 2008). We hypothesize that cuticular bacterial transmission will be more likely to occur between individuals who are more likely to interact in observed contact networks. That is, if colony contact networks are assortative, then cuticular bacterial transmission should be more likely between similar individuals. In contrast, if colony networks are disassortative, then transmission should be more likely to occur between dissimilar individuals. Lastly, we hypothesize that cuticular bacteria will be primarily transmitted through direct interactions (*i.e.*, bodily contact) rather than indirectly through the environment.

5.2 METHODS

5.2.1 Animal collection and maintenance

Stegodyphus dumicola is a southwestern African social spider that lives in age-structured colonies of up to several hundred individuals that exhibit cooperative behaviors and alloparental care, and spend the majority of their time in close contact with colony-mates, either in the colony retreat or during co-feeding (Henschel, Lubin & Schneider 1995; Henschel 1998; Avilés, Varas & Dyreson 1999; Bilde *et al.* 2007). We collected 16 *S. dumicola* colonies along roadside Acacia trees in the Northern Cape of South Africa in January 2015. After transport to the laboratory, individual adult females were isolated into 30ml plastic cups containing a piece of chicken wire to facilitate web-building. Only adult female spiders were used in the present study. Spiders were each fed one 2-week old cricket weekly until the onset of behavioral assays.

5.2.2 Behavioral assays

In *Stegodyphus*, “boldness” (the latency to resume movement after experiencing an aversive stimulus (Sloan Wilson *et al.* 1994)) and aggressiveness are highly consistent behavioral metrics (repeatability ≈ 0.63 and 0.55 , respectively; Keiser *et al.* 2014a) and are linked with an individual’s propensity to participate in a variety of collective tasks (Grinsted *et al.* 2013; Settepani *et al.* 2013; Keiser *et al.* 2014a; Wright, Keiser & Pruitt 2015).

To determine individuals’ boldness, we subjected them to an antipredator behavior assay developed by Riechert and Hedrick (Riechert & Hedrick 1993). The spider is placed in a clear

plastic arena (12cm diameter), given a 30s acclimation period, and then administered two rapid puffs of air to the anterior prosoma using an infant nose-cleaning bulb which causes them to “huddle” by halting movement and pulling the legs close to the body. We then measured the latency for spiders to “unhuddle” and move one full body length. “Bold” individuals unhuddle and resumed movement more quickly, while “shy” individuals have longer latencies to resume activity. We subtract the latency for a spider to resume movement from the maximum latency allowed (600s) such that a higher boldness score represents bolder behavior.

For the bacterial transmission experiments, we also assessed individuals’ aggressiveness by placing the spider in a plastic arena (12cm diameter), allowing it a 30s acclimation period, and then prodding their foremost left leg with a blunt metal probe. We scored their immediate response to this stimulus with a nominal categorization described previously (Grinsted *et al.* 2013; Keiser *et al.* 2014a). “Non-aggressive” behaviors included a “huddle” response and moving away from stimulus, while “aggressive” behaviors included turning or walking towards the stimulus, raising their anterior legs, and shifting the abdomen in place. Aggressiveness assays took place the same day as boldness assays, approximately 6h later.

5.2.3 Social interactions

We observed the interactions of laboratory colonies to determine whether individuals assort according to boldness and whether there is a relationship between boldness and the number of associates of resting spiders. Nine colonies of 10-30 adult female spiders of known boldness (see boldness assay above), and individually marked with acrylic paint dots atop their dorsal abdomen, were kept in plastic containers with chicken wire that allowed them to build a retreat and a capture web. Each experimental colony was constructed from a different source

colony, without mixing individuals from multiple source colonies. These spiders spend much of their time resting in the colony retreat, often in groups. We defined interactions between resting group members as a physical contact between any body parts of two spiders. We manually noted the resting interaction patterns of all individuals in each colony 2-4 times a week (e.g., Fig. 1). Repeated observations of the same colony occurred either on different days or on the same day if the colony had an opportunity to re-assort. For example, an observation was conducted before a colony was fed or provided with water and, if the colony responded to the prey or water, another resting network observation was conducted after the collective response ended and the spiders resumed resting.

5.2.4 Bacterial exposure

We used electroporation (Li *et al.* 1999) to transform a strain of *Pantoea* (CNK01) collected from the cuticle of an adult female spider in the field in January 2014 (methods described in Keiser, *et al. In press*) with the *pGLO* plasmid (BioRad, Hercules, CA) that encodes β -lactamase (conferring ampicillin resistance) and Green Fluorescent Protein (henceforth CNK02). Experimental bacterial cultures were prepared by selecting a single colony of CNK02 and growing it in 1ml LB broth supplemented with 100 μ g/ml ampicillin and 20% arabinose (“LB amp/ara”) for 15h with agitation. This solution was vortexed at 2500rpm for 25min, washed with 1ml phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich, St. Louis, MO 63103), and then diluted in 1ml PBS. Ninety spiders marked with a blue paint dot were placed in 1ml of liquid culture of CNK02 at approximately 10^9 CFU/ml, and shaken at 1500rpm for 3s with a vortex to disrupt the hydrophobic barrier of the spider’s cetae. These bacteria remain viable and detectable on the cuticles of spiders at least 72h after exposure. Henceforth, we will

refer to experimentally exposed individuals as “exposed” and those that were exposed only to PBS and marked with a green paint dot as “susceptible”. Prior to bacterial exposure, we measured each spider’s mass and prosoma width. All bacterial exposures were carried out by the same two researchers (CNK and DAA) to minimize methodological inconsistencies.

5.2.5 Transmission experiments

To test for the transmission of bacteria between exposed and susceptible individuals when allowed to interact directly (*i.e.*, via cuticle-to-cuticle contact), we exposed one spider as described above, and allowed it 24h in its housing container to dry. We then transferred the exposed individual to the housing container of a susceptible individual with a different boldness value than the exposed individual, and allowed them to interact naturally for 24h (n = 66 pairs). The identities of the spiders chosen for each pair were chosen using a random-number generator, and paired spiders always originated from the same source colony. Care was taken to place the exposed individual away from the susceptible individual in its home container to allow natural contact between spiders. After 24h, we removed the susceptible individual and vortexed it in 1ml sterile LB amp/ara broth for 10s. We removed the spider, transferred 40µl of this solution onto LB amp/ara agar, and incubated this plate and the remaining solution (960µl) at 30°C for 20hr. We visually counted the number of LB amp/ara broth solutions that fluoresced under a long-wave UV light pen (BioRad, Hercules, CA) to assess successful transmission, and counted the number of colony forming units (CFUs) that grew and fluoresced on each LB amp/ara plate to approximate the relative bacterial load that had been transferred to susceptible individuals. We performed a series of side experiments to verify that (1) the pGLO plasmid is required to observe

fluorescence from spider-collected bacterial cultures, (2) that amplification at 30°C is required to visually detect fluorescence from GFP-transformed *Pantoea* collected from spiders, (3) that 20h of amplification under 30°C is sufficient to detect fluorescence of bacteria collected from bold and shy spiders, and that (4) fluorescent bacteria are detectable on the cuticles of spiders for at least 72h after exposure.

To test for the occurrence of bacterial transmission from spider to spider indirectly via silk, we exposed a spider to CNK02 as above and allowed it 24h to dry in its housing container. During this time, a susceptible individual was housed in a different container and allowed to build a web. We then removed the susceptible individual and transferred an exposed individual to its housing container. We allowed the exposed individual to interact with the silk of the susceptible individual for 24h (n = 33 pairs). After 24h, we removed the exposed individual, replaced it with the susceptible individual, and allowed it to interact with the exposed silk for 24h. Then, we removed the susceptible individual and tested for the presence of CNK02 on its cuticle as described above. We also gathered the silk from the susceptible individuals' containers with sterile wooden rods and placed them in LB amp/ara at 30°C for 20h to test for the presence of viable CNK02 on the silk.

5.2.6 Statistical analyses

Social network analysis: To determine assortativity according to boldness of the resting networks, we used the *igraph* package in R (R Core). Positive assortativity values indicate assortative networks (*i.e.*, homophily) and negative assortativity values indicate disassortative networks. To determine whether the observed assortativity values are different than expected at

random we created 10,000 randomized networks for each observed network maintaining the connectivity (degree) of each individual (node) and permuting who it interacted with. We then calculated the probability that an observed assortativity value differed from the 10,000 randomized assortativity values as the proportion of cases in which the absolute value of the observed assortativity was smaller than the absolute value of the randomized assortativity. To determine whether the difference between observed and randomized assortativity values was statistically significant we compared the average assortativity of all observed networks with the average assortativity of each of the 10,000 sets of randomized networks. We deemed the observed assortativity significantly different from random if the absolute value of the observed average assortativity was smaller than the absolute value of the average randomized assortativity in less than 0.05 of the 10,000 randomizations. We examined the relationship between connectivity (degree) and boldness in each interaction network using a Pearson's correlation and a Bonferroni correction for multiple hypotheses testing which set the significant p-value at 0.002.

Transmission experiments: We used two nominal logistic regressions and one GLMM with a (log link function) with identical independent variables to test for transmission via direct interaction, indirect transmission, and the number of CFUs/ml collected from susceptible individuals in the direct interaction experiment, respectively. The independent variables in each model were the difference in boldness between the two individuals, the aggressiveness of the exposed individual, the aggressiveness of susceptible individual, the body condition of susceptible individual, and the body condition of exposed individual. Values for the difference in boldness between exposed and susceptible individuals ranged from -600 to 600. Body condition was estimated using the residuals of a linear regression of individual body mass and body size (Jakob, Marshall & Uetz 1996). We treated aggressiveness as a categorical variable here

(following the methods of: Keiser *et al.* 2014a) In a supplemental analysis, we treated aggressiveness as an ordinal variable (following the methods of: Grinsted *et al.* 2013), and used a “difference in aggressiveness” value for each spider pair. We tested for correlations between measures of boldness and aggressiveness with nominal logistic regression. Experimental pair ID nested in source colony ID was included as a random effect in each model.

5.3 RESULTS

5.3.1 Social network analyses

Observed networks were significantly more disassortative than expected at random. The observed average assortativity value was significantly smaller than that obtained from 10,000 randomizations (Fig. 2). Of the 36 resting interaction networks, 35 exhibited disassortative mixing, where individuals preferentially interacted with individuals of different boldness than their own. We did not detect a significant relationship between the boldness of an individual and the number of individuals it contacted while resting in any of the interaction networks.

5.3.2 Bacterial transmission

Transmission of cuticular-bacteria via direct interaction was influenced by the boldness of both the exposed and susceptible individuals. In the direct contact experiment, we detected the

presence of CNK02 on the cuticles of 36/66 susceptible spiders (55%) that were allowed to interact with exposed spiders (Fig. 3). We detected inter-individual transmission of cuticular bacteria in 20/29 (69%) of the cases where the exposed individual was bolder, compared to 15/36 (42%) of the cases where the susceptible individual was bolder (Fig. 4a). We found evidence that inter-individual transmission was more likely to occur when exposed spiders had higher boldness than their paired susceptible individual (Nominal logistic regression: $\chi^2 = 7.58$, DF = 1, $p = 0.006$; Table 1; Fig. 4b). Further, an additional analysis verified that the absolute value of the difference in boldness between spiders did not predict the likelihood of transmission. Transmission was also more likely when susceptible individuals were in better body condition, that is, they weighed more than predicted based on their body size (Nominal logistic regression: $\chi^2 = 5.70$, df = 1, $p = 0.02$; Table 1; Fig. 4c). The aggressiveness of the exposed individual did not predict the likelihood of bacterial transmission (Nominal logistic regression: $p = 0.25$; Table 1), though there was a non-significant trend for a greater incidence of bacterial transmission to occur with more aggressive susceptible spiders (Nominal logistic regression: $p = 0.06$, Table 1). Aggressiveness was not correlated with boldness in this study (Nominal logistic regression: $\chi^2 = 5.39$, df = 8; $p = 0.72$), although a negative relationship between these traits has been described previously (Keiser *et al.* 2014b). Although not influenced by any independent variables (Nominal logistic regression, all $p > 0.07$; Table 1), we estimated a vast range from 25 to 16,100 CFUs/ml of CNK02 on the cuticles of susceptible spiders after having cohabitated with an exposed individual (26 samples, $\bar{x} = 3,513$ CFUs/ml, SD = 5161).

We detected CNK02 on the cuticles of susceptible spiders in only 5/33 cases (15%) when exposed spiders interacted with a susceptible spider's silk alone and never with the susceptible spider directly. We did, however, detect CNK02 on the silk with which exposed spiders

interacted in 12/33 cases (36%). Thus, 5/12 cases (42%) where the silk became contaminated with CNK02 resulted in exposure to the susceptible individual. Evidence of indirect transmission was not influenced by the traits of either the exposed or susceptible individual (Logistic regression: all $p > 0.10$; Table 1).

5.4 DISCUSSION

Inter-individual variation in behavior and contact networks of infected and susceptible individuals have vast consequences for many emerging diseases in wildlife (Jankowski *et al.* 2013) and humans (Lloyd-Smith *et al.* 2005). Here, we did not find support for our original hypothesis, but rather found that *S. dumicola* contact networks are behaviorally disassortative, and that the transmission of cuticular bacteria is more likely when exposed individuals were bolder than their susceptible colony-mates. Thus, under some conditions this system might be poised for rapid and widespread transmission of cuticular microbes, harmful or otherwise. Presuming that at least a subset of resident cuticular bacteria can be harmful under some circumstances, as is the case for this species (Keiser *et al.* In press), the observed social network pattern may help explain the high incidence of idiopathic colony extinction in *S. dumicola* and other species of social spiders (Aviles 1997; Henschel 1998; Pruitt & Goodnight 2014).

5.4.1 Network patterns and bacterial transmission

In our observed spider contact networks, behavioral disassortativity was more prevalent than expected at random. Many animal and human networks, however, are characterized by positive assortativity, (*i.e.*, homophily) (Newman 2002; Newman 2003; Lusseau & Newman 2004), where individuals preferentially interact with others like themselves. It has been suggested that infectious agents spread more slowly when hosts engage in disassortative networks (Newman 2002). For instance, simulated outbreaks of foot and mouth disease in livestock are shortened due to disassortative contacts (Kiss, Green & Kao 2006). Additionally, individuals often tend to avoid visibly-infected conspecifics (Zylberberg, Klasing & Hahn) whose recognition may be heightened via their altered behaviors, further reducing the likelihood of transmission. Whether, and to what degree, our observed social interaction patterns in *S. dumicola* would afford colonies reduced overall transmission among individuals (*i.e.*, a form of “social immunity”; Traniello, Rosengaus & Savoie 2002; Cremer, Armitage & Schmid-Hempel 2007; Hock & Fefferman 2012), or alternatively, enhanced susceptibility to transmission is yet unresolved; we discuss both possibilities below.

Our data suggest that the ability of the disassortative nature of these spiders’ interactions to prevent or facilitate the transmission of cuticular microbes would depend on the traits of the exposed and susceptible individuals. Transmission was more likely when there was a difference in the boldness of the exposed versus susceptible individual, but more so in one direction. When the exposed individual was bolder than the susceptible individual, transmission was more likely (69%). Conversely, when the susceptible individual was bolder than the exposed individual, the incidence of transmission was lower (42%). Thus, disassortative contacts networks in *S. dumicola* colonies could, depending on the situation considered, either intensify or reduce the

incidence of bacterial transmission throughout the colony. If the index case (aka “patient zero”) for a transmission event is a bold individual, this could beget rapid transmission to shyer colony members, which is the more common behavioral phenotype within these societies. However, if the index case is a shy individual, bacterial transmission to bolder individuals could be constrained. Although reduced, the observed incidence of transmission from shyer to bolder spiders was still considerable, and colony-wide bacterial transmission would not be completely quelled if the index case were a shy spider.

The aggregate effects that these interaction networks and patterns of transmission have on colony performance would thus depend on whether bold individuals or shy individuals are more likely to be the index case, and whether different behavioral phenotypes are differentially likely to encounter, and become colonized by, novel environmental microbes. In *S. dumicola*, the available data suggest that bold individuals more readily interact with the environment outside of the colony’s nest during foraging (Pruitt & Keiser 2014; Pruitt & Pinter-Wollman 2015b), suggesting that these individuals may be the pathway by which environmental microbes are subsequently transmitted to shy colony-mates.

5.4.2 Transmission through contact vs. shared silk

Notably, we found that the incidence of transmission was greater when individuals were allowed to interact directly compared to cases where individuals only interacted indirectly via shared experience with the same silk. This suggests an important role of body contact or affiliative behavioral interactions in the transmission of cuticular bacteria. A recent experiment using freshwater snails demonstrated that bodily contact is a major determinant of the dispersal

of a defensive symbiont from “donor” to “receiver” hosts, and, similar (in terms of trait disassortativity) to the data presented here, the degree of transmission was greater when donor hosts were larger than their unexposed receivers, with the opposite being true if receivers were larger than donors (Hopkins *et al.* 2015). This highlights the need to test the generality of trait disassortativity and transmission across many different host-symbiont systems.

5.4.3 Future directions

For studies of wildlife disease, variation in the behavioral traits of infected and susceptible individuals is rarely explored in conjunction, despite ample evidence of its importance from the biomedical and human epidemiological fields. More comprehensive experiments should test how syndromes of behavioral traits can combine to influence the likelihood of individuals’ acquisition, colonization, and transmission of microbes (i.e., “behavioral competence”; Barron *et al.* 2015). Future experiments could also incorporate how the composition of resident cuticular microbial communities can combine with behavioral traits to drive the likelihood of bacterial colonization and transmission (Harris *et al.* 2006; Cogen, Nizet & Gallo 2008; Harris *et al.* 2009). More specifically in regards to this system, it would be informative to identify the role that males play in social contact networks, including sexual interactions, and their influence on bacterial transmission. Although colony sex ratios are strongly female-biased [39], males could have a high impact on bacterial transmission if they are highly interactive and/or susceptible to exposure. Further, it would be informative to observe matched pairs of exposed and susceptible individuals and record the frequency and nature of their interactions to determine if bacteria are transmitted simply by bodily contact or if other affiliative/aggressive interactions are linked to transmission. Our data here verify that the behavioral traits of exposed and susceptible

individuals jointly influence the likelihood of inter-individual transmission of cuticular bacteria, and represent a fundamental step towards understanding how individual traits can explain larger-scale epidemiological processes. These data thus reinforce the growing sentiment that comprehensive models of epidemiological processes must account for behavioral variation at the level of the individual.

Table 5-1. (A,B) Two nominal logistic regressions predicting the presence or absence of CNK02 on the cuticles of susceptible individuals after interacting with exposed individuals directly or indirectly. (C) A GLMM predicting the estimated bacterial load collected

A. Transmission after direct interaction			
Effect	DF	χ^2	P-value
Difference in boldness between individuals	1	7.58	0.006**
Aggressiveness of exposed individual	7	9.00	0.25
Aggressiveness of susceptible individual	9	16.74	0.06
Body condition of susceptible individual	1	5.70	0.02*
Body condition of exposed individual	1	0.51	0.47
B. Transmission after indirect interaction			
Effect	DF	χ^2	P-value
Difference in boldness between individuals	1	0.53	0.46
Aggressiveness of exposed individual	6	10.58	0.10
Aggressiveness of susceptible individual	7	8.63	0.28
Body condition of susceptible individual	1	0.81	0.37
Body condition of exposed individual	1	0.39	0.53
C. Estimated bacterial load transmitted			
Effect	DF	χ^2	P-value
Difference in boldness between individuals	1	0.42	0.52
Aggressiveness of exposed individual	6	9.30	0.16
Aggressiveness of susceptible individual	5	6.95	0.22
Body condition of susceptible individual	1	0.37	0.54
Body condition of exposed individual	1	3.33	0.07

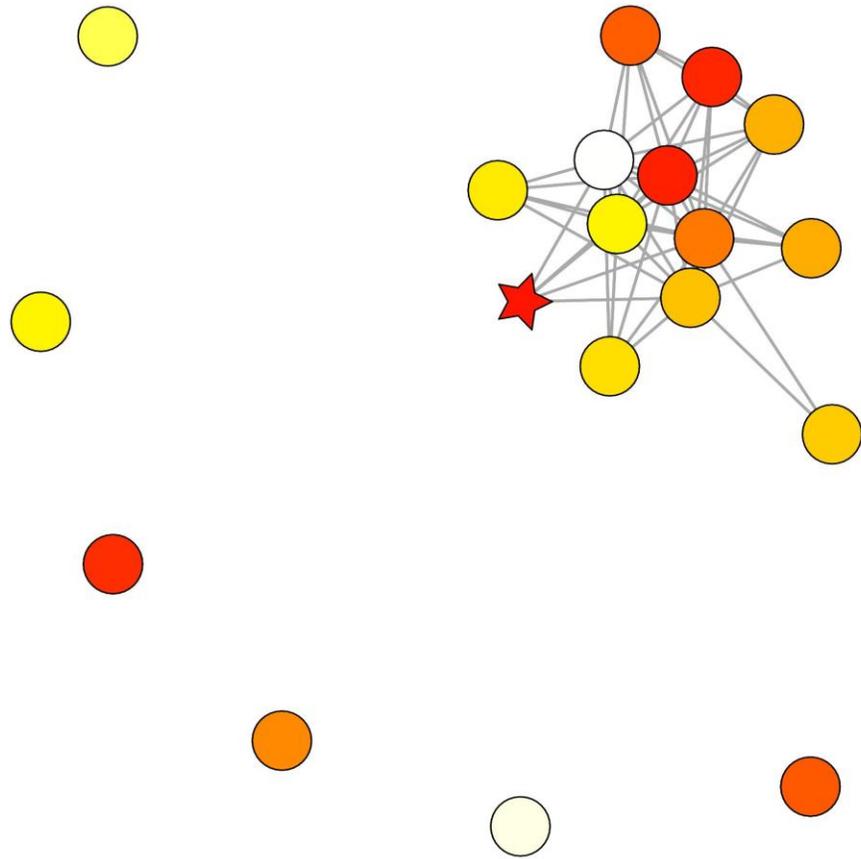


Figure 5-1. Interaction patterns among 19 spiders in an experimental colony of marked individuals.

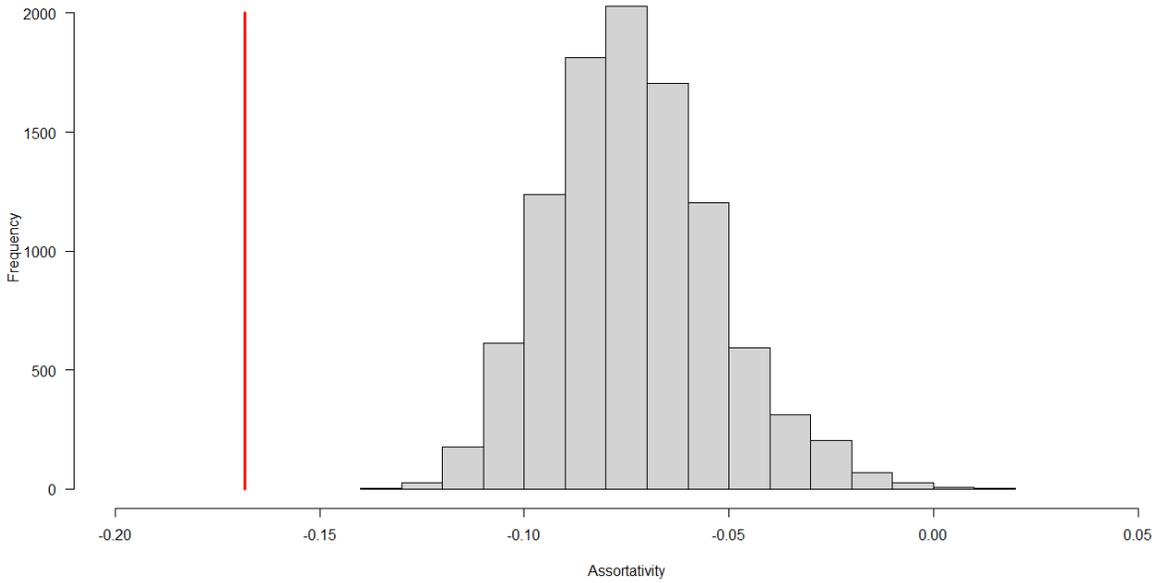


Figure 5-2. Average assortativity of all observed networks (red vertical line) is significantly smaller than the average assortativity of each of the 10,000 sets of randomized networks (histogram).

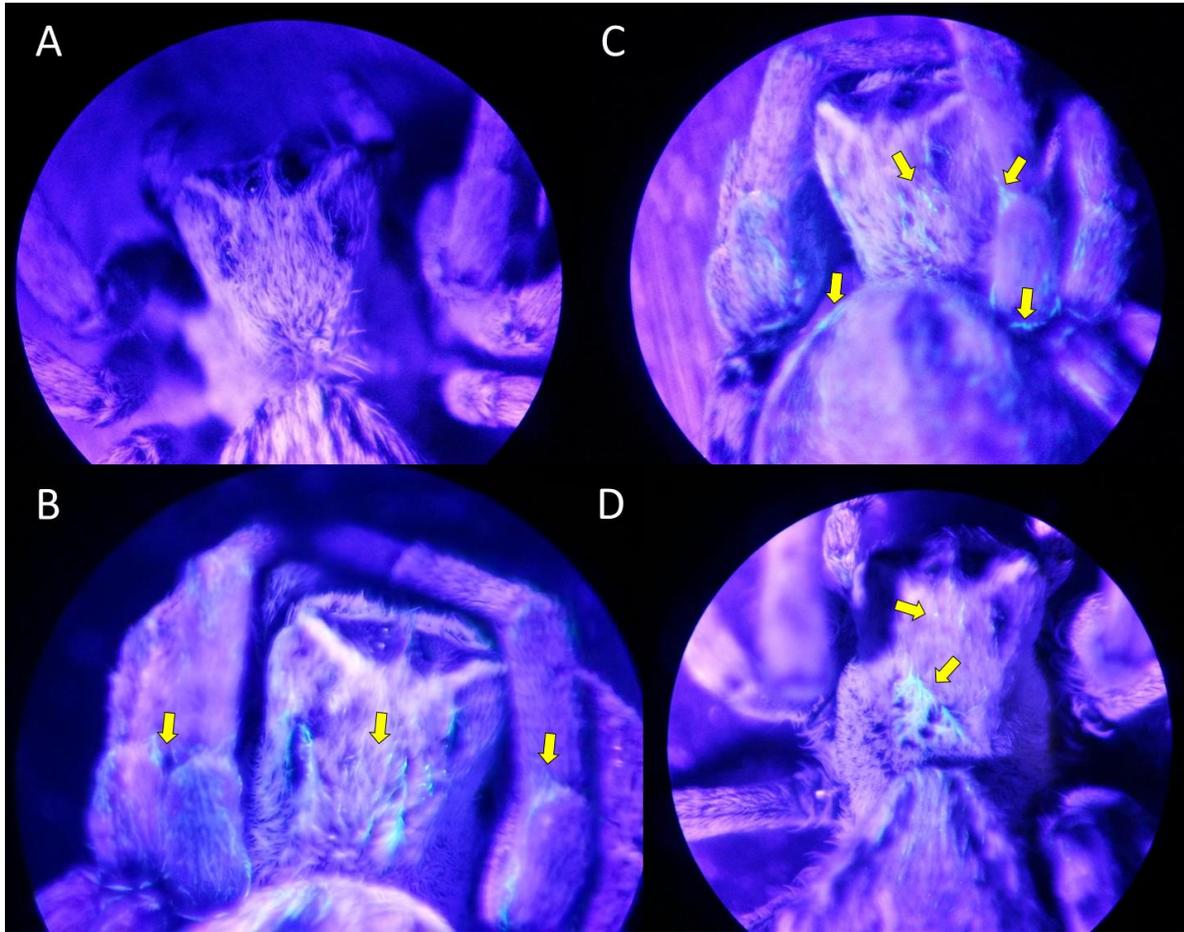


Figure 5-3. Spiders under long-wave UV light: (a) exposed to with sterile PBS (control). (b) 4h after exposure to CNK02. (c) 48h after exposure to CNK02. (d) An unexposed spider that has interacted with an exposed spider for 24h. Areas of green fluorescence suggest the presence of *Pantoea* +pGLO.

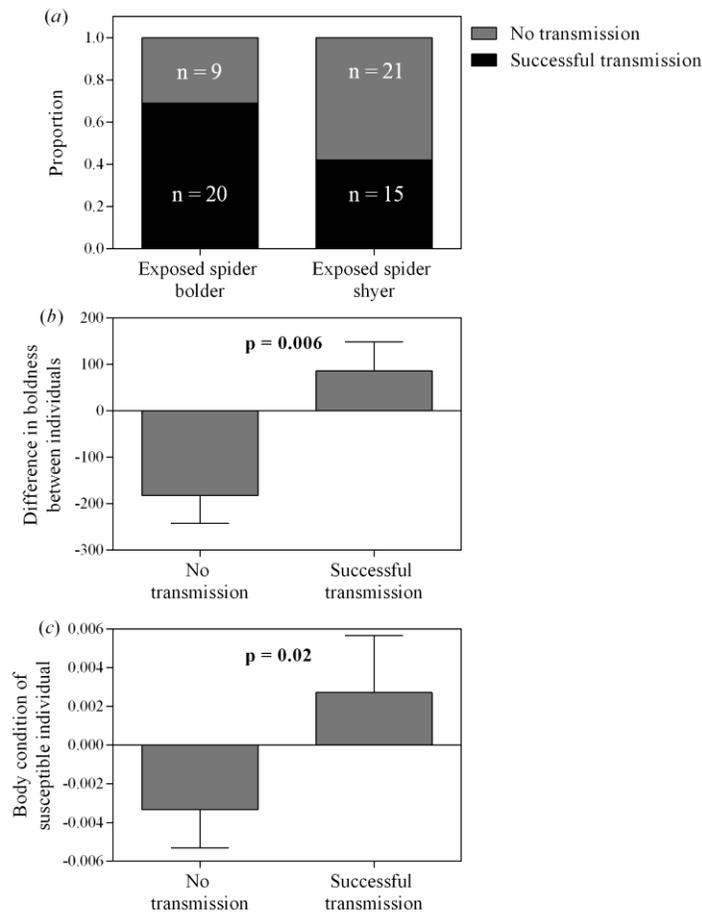


Figure 5-4. Direct transmission of cuticular bacteria is influenced by the phenotypes of both exposed and susceptible individuals. (a) a greater proportion of pairs in which the exposed individual was bolder resulted in successful transmission and (b) transmission was more likely when exposed individuals were bolder than their susceptible colony-mates (positive values denote a bolder exposed individual, while negative values denote a bolder susceptible). (c) Transmission was also more likely when susceptible individuals had a positive body condition, *i.e.* weighed more than predicted based on their body size.

BIBLIOGRAPHY

- Adelman, J.S., Moyers, S.C., Farine, D.R. & Hawley, D.M. (2015) Feeder use predicts both acquisition and transmission of a contagious pathogen in a North American songbird. *Proc. R. Soc. B*, pp. 20151429. The Royal Society.
- Alberts, S.C., Sapolsky, R.M. & Altmann, J. (1992) Behavioral, endocrine, and immunological correlates of immigration by an aggressive male into a natural primate group. *Hormones and Behavior*, **26**, 167-178.
- Aviles, L. (1997) 23• Causes and consequences of cooperation and permanent-sociality in spiders. *The evolution of social behavior in insects and arachnids*, 476.
- Avilés, L., Varas, C. & Dyreson, E. (1999) Does the African social spider *Stegodyphus dumicola* control the sex of individual offspring? *Behavioral Ecology and Sociobiology*, **46**, 237-243.
- Bansal, S., Grenfell, B.T. & Meyers, L.A. (2007) When individual behaviour matters: homogeneous and network models in epidemiology. *J R Soc Interface*, **4**, 879-891.
- Barber, I. & Dingemanse, N.J. (2010) Parasitism and the evolutionary ecology of animal personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 4077-4088.
- Barron, D.G., Gervasi, S.S., Pruitt, J.N. & Martin, L.B. (2015) Behavioral competence: how host behaviors can interact to influence parasite transmission risk. *Current Opinion in Behavioral Sciences*, **6**, 35-40.
- Begon, M., Bennett, M., Bowers, R.G., French, N.P., Hazel, S. & Turner, J. (2002) A clarification of transmission terms in host-microparasite models: numbers, densities and areas. *Epidemiology and Infection*, **129**, 147-153.
- Bilde, T., Coates, K., Birkhofer, K., Bird, T., Maklakov, A., Lubin, Y. & Aviles, L. (2007) Survival benefits select for group living in a social spider despite reproductive costs. *Journal of Evolutionary Biology*, **20**, 2412-2426.
- Blyton, M.D.J., Banks, S.C., Peakall, R., Lindenmayer, D.B. & Gordon, D.M. (2014) Not all types of host contacts are equal when it comes to *E. coli* transmission. *Ecology Letters*, **17**, 970-978.
- Boyer, N., Reale, D., Marmet, J., Pisanu, B. & Chapuis, J.L. (2010) Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. *Journal of Animal Ecology*, **79**, 538-547.
- Brent, Lauren J.N., Franks, Daniel W., Foster, Emma A., Balcomb, Kenneth C., Cant, Michael A. & Croft, Darren P. Ecological Knowledge, Leadership, and the Evolution of Menopause in Killer Whales. *Current Biology*.
- Brey, P.T., Lee, W.-J., Yamakawa, M., Koizumi, Y., Perrot, S., Francois, M. & Ashida, M. (1993) Role of the integument in insect immunity: epicuticular abrasion and induction of

- cecropin synthesis in cuticular epithelial cells. *Proceedings of the National Academy of Sciences*, **90**, 6275-6279.
- Brown, C. & Irving, E. (2014) Individual personality traits influence group exploration in a feral guppy population. *Behavioral Ecology*, **25**, 95-101.
- Bumann, D. & Krause, J. (1993) Front individuals lead in shoals of three-spined sticklebacks (*Gasterosteus aculeatus*) and juvenile roach (*Rutilus rutilus*). *Behaviour*, **125**, 189-198.
- Chang, A.T. & Sih, A. (2013) Multilevel selection and effects of keystone hyperaggressive males on mating success and behavior in stream water striders. *Behavioral Ecology*, **24**, 1166-1176.
- Cho, I. & Blaser, M.J. (2012) The human microbiome: at the interface of health and disease. *Nature Reviews Genetics*, **13**, 260-270.
- Clark, C.W. & Mangel, M. (1986) The evolutionary advantages of group foraging. *Theoretical Population Biology*, **30**, 45-75.
- Cogen, A., Nizet, V. & Gallo, R. (2008) Skin microbiota: a source of disease or defence? *British Journal of Dermatology*, **158**, 442-455.
- Cornman, R.S., Tarpay, D.R., Chen, Y., Jeffreys, L., Lopez, D., Pettis, J.S., Vanengelsdorp, D. & Evans, J.D. (2012) Pathogen webs in collapsing honey bee colonies. *PloS one*, **7**, e43562.
- Cote, J., Fogarty, S., Tymen, B., Sih, A. & Brodin, T. (2013) Personality-dependent dispersal cancelled under predation risk. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20132349.
- Cremer, S., Armitage, S.A. & Schmid-Hempel, P. (2007) Social immunity. *Current Biology*, **17**, R693-R702.
- Croft, D.P., Krause, J., Darden, S.K., Ramnarine, I.W., Faria, J.J. & James, R. (2009) Behavioural trait assortment in a social network: patterns and implications. *Behavioral Ecology and Sociobiology*, **63**, 1495-1503.
- DiRienzo, N., Niemelä, P.T., Skog, A., Vainikka, A. & Kortet, R. (2015) Juvenile pathogen exposure affects the presence of personality in adult field crickets. *Frontiers in Ecology and Evolution*, **3**, 36.
- Dizney, L. & Dearing, M.D. (2013) The role of behavioural heterogeneity on infection patterns: implications for pathogen transmission. *Animal Behaviour*, **86**, 911-916.
- Dornhaus, A. (2008) Specialization does not predict individual efficiency in an ant. *PLoS biology*, **6**, e285.
- Dornhaus, A., Powell, S. & Bengston, S. (2012) Group size and its effects on collective organization. *Annual review of entomology*, **57**, 123-141.
- Dwyer, G., Elkinton, J.S. & Buonaccorsi, J.P. (1997) Host heterogeneity in susceptibility and disease dynamics: tests of a mathematical model. *The American Naturalist*, **150**, 685-707.
- Dyer, J.R., Croft, D.P., Morrell, L.J. & Krause, J. (2009) Shoal composition determines foraging success in the guppy. *Behavioral Ecology*, **20**, 165-171.
- Ezenwa, V.O., Gerardo, N.M., Inouye, D.W., Medina, M. & Xavier, J.B. (2012) Animal behavior and the microbiome. *Science*, **338**, 198-199.
- Fefferman, N.H. & Ng, K.L. (2007) How disease models in static networks can fail to approximate disease in dynamic networks. *Physical Review E*, **76**.
- Fogarty, S., Cote, J. & Sih, A. (2011) Social personality polymorphism and the spread of invasive species: a model. *The American Naturalist*, **177**, 273-287.

- Gahr, P., DeVries, A.S., Wallace, G., Miller, C., Kenyon, C., Sweet, K., Martin, K., White, K., Bagstad, E. & Hooker, C. (2014) An outbreak of measles in an undervaccinated community. *Pediatrics*, **134**, e220-e228.
- Galvani, A.P. & May, R.M. (2005) Epidemiology: Dimensions of superspreading. *Nature*, **438**, 293-295.
- Gardy, J.L., Johnston, J.C., Sui, S.J.H., Cook, V.J., Shah, L., Brodtkin, E., Rempel, S., Moore, R., Zhao, Y. & Holt, R. (2011) Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. *New England Journal of Medicine*, **364**, 730-739.
- Gautrais, J., Theraulaz, G., Deneubourg, J.-L. & Anderson, C. (2002) Emergent polyethism as a consequence of increased colony size in insect societies. *Journal of Theoretical Biology*, **215**, 363-373.
- Gibbins, L. (1998) Mary Mallon: disease, denial, and detention. *Journal of Biological Education*, **32**, 127-132.
- Gopinath, S., Hotson, A., Johns, J., Nolan, G. & Monack, D. (2013) The Systemic Immune State of Super-shedder Mice Is Characterized by a Unique Neutrophil-dependent Blunting of T_H1 Responses. *PLoS Pathog*, **9**, e1003408.
- Gordon, D.M. (2013) The rewards of restraint in the collective regulation of foraging by harvester ant colonies. *Nature*, **498**, 91-93.
- Grinsted, L., Pruitt, J.N., Settepani, V. & Bilde, T. (2013) Individual personalities shape task differentiation in a social spider. *Proceedings of the Royal Society B: Biological Sciences*, **280**.
- Hampson, K., Dushoff, J., Cleaveland, S., Haydon, D.T., Kaare, M., Packer, C. & Dobson, A. (2009) Transmission dynamics and prospects for the elimination of canine rabies. *PLoS biology*, **7**, e1000053.
- Harcourt, J.L., Ang, T.Z., Sweetman, G., Johnstone, R.A. & Manica, A. (2009) Social feedback and the emergence of leaders and followers. *Current Biology*, **19**, 248-252.
- Harris, R.N., Brucker, R.M., Walke, J.B., Becker, M.H., Schwantes, C.R., Flaherty, D.C., Lam, B.A., Woodhams, D.C., Briggs, C.J. & Vredenburg, V.T. (2009) Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *The ISME journal*, **3**, 818-824.
- Harris, R.N., James, T.Y., Lauer, A., Simon, M.A. & Patel, A. (2006) Amphibian pathogen *Batrachochytrium dendrobatidis* is inhibited by the cutaneous bacteria of amphibian species. *EcoHealth*, **3**, 53-56.
- Harwood, G. & Avilés, L. (2013) Differences in group size and the extent of individual participation in group hunting may contribute to differential prey-size use among social spiders. *Biology letters*, **9**, 20130621.
- Hee, J.J., Holway, D.A., Suarez, A.V. & Case, T.J. (2000) Role of propagule size in the success of incipient colonies of the invasive Argentine ant. *Conservation Biology*, **14**, 559-563.
- Henschel, J., Lubin, Y. & Schneider, J. (1995) Sexual competition in an inbreeding social spider, *Stegodyphus dumicola* (Araneae: Eresidae). *Insectes sociaux*, **42**, 419-426.
- Henschel, J.R. (1998) Predation on social and solitary individuals of the spider *Stegodyphus dumicola* (Araneae, Eresidae). *Journal of Arachnology*, 61-69.
- Hock, K. & Fefferman, N.H. (2012) Social organization patterns can lower disease risk without associated disease avoidance or immunity. *Ecological Complexity*, **12**, 34-42.
- Holbrook, C.T., Barden, P.M. & Fewell, J.H. (2011) Division of labor increases with colony size in the harvester ant *Pogonomyrmex californicus*. *Behavioral Ecology*, **22**, 960-966.

- Hopkins, S.R., Boyle, L.J., Belden, L.K. & Wojdak, J.M. (2015) Dispersal of a defensive symbiont depends on contact between hosts, host health, and host size. *Oecologia*, 1-12.
- Horner, V., Proctor, D., Bonnie, K.E., Whiten, A. & de Waal, F.B. (2010) Prestige affects cultural learning in chimpanzees. *PloS one*, **5**, e10625.
- Ingram, K.K., Pilko, A., Heer, J. & Gordon, D.M. (2013) Colony life history and lifetime reproductive success of red harvester ant colonies. *Journal of Animal Ecology*, **82**, 540-550.
- Jakob, E.M., Marshall, S.D. & Uetz, G.W. (1996) Estimating fitness: a comparison of body condition indices. *Oikos*, 61-67.
- Jankowski, M.D., Williams, C.J., Fair, J.M., Owen, J.C. & Ren, X. (2013) Birds shed RNA-viruses according to the Pareto Principle. *PloS one*, **8**, e72611.
- Jeanson, R. & Fewell, J.H. (2008) Influence of the social context on division of labor in ant foundress associations. *Behavioral Ecology*, **19**, 567-574.
- Jeanson, R., Fewell, J.H., Gorelick, R. & Bertram, S.M. (2007) Emergence of increased division of labor as a function of group size. *Behavioral Ecology and Sociobiology*, **62**, 289-298.
- Jin, J., Su, Y., Tao, Y., Guo, S. & Yu, Z. (2013) Personality as a Predictor of General Health in Captive Golden Snub-Nosed Monkeys (*Rhinopithecus roxellana*). *American journal of primatology*, **75**, 524-533.
- Johannesen, J., Hennig, A., Dommermuth, B. & Schneider, J.M. (2002) Mitochondrial DNA distributions indicate colony propagation by single matri-lineages in the social spider *Stegodyphus dumicola* (Eresidae). *Biological Journal of the Linnean Society*, **76**, 591-600.
- Kao, R., Gravenor, M., Charleston, B., Hope, J., Martin, M. & Howard, C. (2007) *Mycobacterium bovis* shedding patterns from experimentally infected calves and the effect of concurrent infection with bovine viral diarrhoea virus. *Journal of the Royal Society Interface*, **4**, 545-551.
- Karau, S.J. & Williams, K.D. (1993) Social loafing: A meta-analytic review and theoretical integration. *Journal of personality and social psychology*, **65**, 681.
- Keiser, C.N., Jones, D.K., Modlmeier, A.P. & Pruitt, J.N. (2014a) Exploring the effects of individual traits and within-colony variation on task differentiation and collective behavior in a desert social spider. *Behavioral Ecology and Sociobiology*, **68**, 839-850.
- Keiser, C.N., Modlmeier, A.P., Singh, N., Jones, D.K. & Pruitt, J.N. (2014b) Exploring how a shift in the physical environment shapes individual and group behavior across two social contexts. *Ethology*, **120**, 825-833.
- Keiser, C.N., Modlmeier, A.P., Singh, N., Jones, D.K. & Pruitt, J.N. (2014c) Exploring How a Shift in the Physical Environment Shapes Individual and Group Behavior across Two Social Contexts. *Ethology*, n/a-n/a.
- Keiser, C.N. & Pruitt, J.N. (2014a) Personality composition is more important than group size in determining collective foraging behaviour in the wild. *Proceedings of the Royal Society B: Biological Sciences*, **281**.
- Keiser, C.N. & Pruitt, J.N. (2014b) Personality composition is more important than group size in determining collective foraging behaviour in the wild. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141424.
- Keiser, C.N., Shearer, T.A., DeMarco, A.E., Brittingham, H.A., Knutson, K.A., Kuo, C., Zhao, K. & Pruitt, J.N. (2016) Cuticular bacteria appear lethal to social spiders in mixed but not monoculture inoculations. *Current Zoology*.

- Keiser, C.N., Shearer, T.A., DeMarco, A.E., Brittingham, H.A., Knutson, K.A., Kuo, C., Zhao, K. & Pruitt, J.N. (In press) Cuticular bacteria appear lethal to social spiders in mixed but not monoculture inoculations. *Current Zoology*.
- Keiser, C.N., Wright, C.M. & Pruitt, J.N. (2015) Warring arthropod societies: Social spider colonies can delay annihilation by predatory ants via reduced apparency and increased group size. *Behavioural Processes*, **119**, 14-21.
- Kekäläinen, J., Lai, Y.-T., Vainikka, A., Sirkka, I. & Kortet, R. (2014) Do brain parasites alter host personality?—Experimental study in minnows. *Behavioral Ecology and Sociobiology*, **68**, 197-204.
- Kiss, I.Z., Green, D.M. & Kao, R.R. (2006) The network of sheep movements within Great Britain: network properties and their implications for infectious disease spread. *Journal of The Royal Society Interface*, **3**, 669-677.
- Klein, S.L. (2003) Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology & behavior*, **79**, 441-449.
- Knörnschild, M., Nagy, M., Metz, M., Mayer, F. & von Helversen, O. (2010) Complex vocal imitation during ontogeny in a bat. *Biology letters*, **6**, 156-159.
- Kortet, R., Hedrick, A.V. & Vainikka, A. (2010) Parasitism, predation and the evolution of animal personalities. *Ecology Letters*, **13**, 1449-1458.
- Krafft, B. & Cookson, L.J. (2012) The role of silk in the behaviour and sociality of spiders. *Psyche: A Journal of Entomology*, **2012**.
- Kralj-Fišer, S. & Schuett, W. (2014) Studying personality variation in invertebrates: why bother? *Animal Behaviour*, **91**, 41-52.
- Krause, J. & Ruxton, G.D. (2002) *Living in groups*. Oxford University Press.
- Kurvers, R.H., Prins, H.H., van Wieren, S.E., van Oers, K., Nolet, B.A. & Ydenberg, R.C. (2010) The effect of personality on social foraging: shy barnacle geese scrounge more. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 601-608.
- Laskowski, K.L. & Pruitt, J.N. (2014) Evidence of social niche construction: persistent and repeated social interactions generate stronger personalities in a social spider. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20133166.
- Lass, S., Hudson, P.J., Thakar, J., Saric, J., Harvill, E., Albert, R. & Perkins, S.E. (2013) Generating super-shedders: co-infection increases bacterial load and egg production of a gastrointestinal helminth. *Journal of The Royal Society Interface*, **10**, 20120588.
- Leticia Avilés & Paul Tufiño (1998) Colony Size and Individual Fitness in the Social Spider *Anelosimus eximius*. *The American Naturalist*, **152**, 403-418.
- Li, Y., Shen, Q., Zhao, C., Li, X. & Zhao, L. (1999) Transformation of biological control strain of *Pantoea agglomerans* by electroporation. *Wei sheng wu xue tong bao*, **27**, 245-249.
- Lloyd-Smith, J.O., Schreiber, S.J., Kopp, P.E. & Getz, W. (2005) Superspreading and the effect of individual variation on disease emergence. *Nature*, **438**, 355-359.
- Lopes, P.C., Adelman, J., Wingfield, J.C. & Bentley, G.E. (2012) Social context modulates sickness behavior. *Behavioral Ecology and Sociobiology*, **66**, 1421-1428.
- Lubin, Y. & Bilde, T. (2007a) The evolution of sociality in spiders. *Advances in the Study of Behavior*, **37**.
- Lubin, Y. & Bilde, T. (2007b) The evolution of sociality in spiders. *Advances in the Study of Behavior*, **37**, 83-145.

- Lusseau, D. & Newman, M.E. (2004) Identifying the role that animals play in their social networks. *Proceedings of the Royal Society of London B: Biological Sciences*, **271**, S477-S481.
- Matthews, L., McKendrick, I., Terner, H., Gunn, G., Synge, B. & Woolhouse, M. (2006) Super-shedding cattle and the transmission dynamics of *Escherichia coli* O157. *Epidemiology and Infection*, **134**, 131-142.
- McCallum, H., Barlow, N. & Hone, J. (2001) How should pathogen transmission be modelled? *Trends in Ecology & Evolution*, **16**, 295-300.
- McComb, K., Moss, C., Durant, S.M., Baker, L. & Sayialel, S. (2001) Matriarchs as repositories of social knowledge in African elephants. *Science*, **292**, 491-494.
- McComb, K., Shannon, G., Durant, S.M., Sayialel, K., Slotow, R., Poole, J. & Moss, C. (2011) Leadership in elephants: the adaptive value of age. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 3270-3276.
- McFall-Ngai, M., Hadfield, M.G., Bosch, T.C., Carey, H.V., Domazet-Lošo, T., Douglas, A.E., Dubilier, N., Eberl, G., Fukami, T. & Gilbert, S.F. (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, **110**, 3229-3236.
- Milgram, S., Bickman, L. & Berkowitz, L. (1969) Note on the drawing power of crowds of different size. *Journal of Personality and Social Psychology*, **13**, 79.
- Modlmeier, A.P., Keiser, C.N., Watters, J.V., Sih, A. & Pruitt, J.N. (2014a) The keystone individual concept: an ecological and evolutionary overview. *Animal Behaviour*, **89**, 53-62.
- Modlmeier, A.P., Laskowski, K.L., DeMarco, A.E., Coleman, A., Zhao, K., Brittingham, H.A., McDermott, D.R. & Pruitt, J.N. (2014b) Persistent social interactions beget more pronounced personalities in a desert-dwelling social spider. *Biology Letters*, **10**, 20140419.
- Modlmeier, A.P., Liebmann, J.E. & Foitzik, S. (2012) Diverse societies are more productive: a lesson from ants. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2142-2150.
- Nentwig, W. (1985) Social spiders catch larger prey: a study of *Anelosimus eximius* (Araneae: Theridiidae). *Behavioral Ecology and Sociobiology*, **17**, 79-85.
- Newman, M.E. (2002) Assortative mixing in networks. *Physical Review Letters*, **89**, 208701.
- Newman, M.E. (2003) Mixing patterns in networks. *Physical Review E*, **67**, 026126.
- Newton, I.L., Sheehan, K.B., Lee, F.J., Horton, M.A. & Hicks, R.D. (2013) Invertebrate systems for hypothesis-driven microbiome research. *Microbiome Science and Medicine*, **1**, 1-9.
- Paine, R.T. (1969) A Note on Trophic Complexity and Community Stability. *The American Naturalist*, **103**, 91-93.
- Perkins, S.E., Cagnacci, F., Stradiotto, A., Arnoldi, D. & Hudson, P.J. (2009) Comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. *Journal of Animal Ecology*, **78**, 1015-1022.
- Pike, T.W., Samanta, M., Lindström, J. & Royle, N.J. (2008) Behavioural phenotype affects social interactions in an animal network. *Proceedings of the Royal Society of London B: Biological Sciences*, **275**, 2515-2520.
- Pontier, D., Fromont, E., Courchamp, F., Artois, M. & Yoccoz, N.G. (1998) *Retroviruses and sexual size dimorphism in domestic cats (Felis catus L.)*.

- Poulin, R. (2013) Parasite manipulation of host personality and behavioural syndromes. *The Journal of Experimental Biology*, **216**, 18-26.
- Poulsen, M. & Sapountzis, P. (2012) Behind every great ant, there is a great gut. *Molecular Ecology*, **21**, 2054-2057.
- Powers, K.S. & Avilés, L. (2007) The role of prey size and abundance in the geographical distribution of spider sociality. *Journal of Animal Ecology*, **76**, 995-1003.
- Prop, J. & Deerenberg, C. (1991) Spring staging in brent geese *Branta bernicla*: feeding constraints and the impact of diet on the accumulation of body reserves. *Oecologia*, **87**, 19-28.
- Pruitt, J.N. (2012) Behavioural traits of colony founders affect the life history of their colonies. *Ecology Letters*.
- Pruitt, J.N. (2013) A real-time eco-evolutionary dead-end strategy is mediated by the traits of lineage progenitors and interactions with colony invaders. *Ecology Letters*.
- Pruitt, J.N. & Goodnight, C.J. (2014) Site-specific group selection drives locally adapted group compositions. *Nature*.
- Pruitt, J.N., Grinsted, L. & Settepani, V. (2013a) Linking levels of personality: personalities of the 'average' and 'most extreme' group members predict colony-level personality. *Animal Behaviour*.
- Pruitt, J.N., Grinsted, L. & Settepani, V. (2013b) Linking levels of personality: personalities of the 'average' and 'most extreme' group members predict colony-level personality. *Animal Behaviour*, **86**, 391-399.
- Pruitt, J.N. & Keiser, C.N. (2014) The personality types of key catalytic individuals shape colonies' collective behaviour and success. *Animal Behaviour*, **93**, 87-95.
- Pruitt, J.N. & Pinter-Wollman, N. (2015a) The legacy effects of keystone individuals on collective behavior scale to how long they remain within a group. *Proceedings of the Royal Society B-Biological Sciences*, *in press*.
- Pruitt, J.N. & Pinter-Wollman, N. (2015b) The legacy effects of keystone individuals on collective behaviour scale to how long they remain within a group. *Proc. R. Soc. B*, pp. 20151766. The Royal Society.
- Purcell, J. & Avilés, L. (2007) Smaller colonies and more solitary living mark higher elevation populations of a social spider. *Journal of Animal Ecology*, **76**, 590-597.
- R Core, T. (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Regoes, R.R., Hottinger, J.W., Sygnarski, L. & Ebert, D. (2003) The infection rate of *Daphnia magna* by *Pasteuria ramosa* conforms with the mass-action principle. *Epidemiology and Infection*, **131**, 957-966.
- Riechert, S.E. & Hedrick, A.V. (1993) A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta* (Araneae, Agelenidae). *Animal Behaviour*, **46**, 669-675.
- Rypstra, A.L. & Tirey, R.S. (1991) Prey size, prey perishability and group foraging in a social spider. *Oecologia*, **86**, 25-30.
- Sanders, J.G., Powell, S., Kronauer, D.J., Vasconcelos, H.L., Frederickson, M.E. & Pierce, N.E. (2014) Stability and phylogenetic correlation in gut microbiota: lessons from ants and apes. *Molecular Ecology*, **23**, 1268-1283.
- Sapolsky, R.M. (2005) The influence of social hierarchy on primate health. *Science*, **308**, 648-652.

- Sapolsky, R.M. & Share, L.J. (2004) A pacific culture among wild baboons: its emergence and transmission. *PLoS biology*, **2**, e106.
- Scheuring, I. & Yu, D.W. (2012) How to assemble a beneficial microbiome in three easy steps. *Ecology Letters*, **15**, 1300-1307.
- Schneider, J.M., Roos, J., Lubin, Y. & Henschel, J.R. (2001) Dispersal of *Stegodyphus dumicola* (Araneae, Eresidae): They do Balloon after All! *Journal of Arachnology*, **29**, 114-116.
- Seaman, B. & Briffa, M. (2015) Parasites and personality in periwinkles (*Littorina littorea*): Infection status is associated with mean-level boldness but not repeatability. *Behavioural Processes*, **115**, 132-134.
- Settepani, V., Grinsted, L., Granfeldt, J., Jensen, J.L. & Bilde, T. (2013) Task specialization in two social spiders, *Stegodyphus sarasinorum* (Eresidae) and *Anelosimus eximius* (Theridiidae). *Journal of Evolutionary Biology*, **26**, 51-62.
- Shen, Z., Ning, F., Zhou, W., He, X., Lin, C., Chin, D.P., Zhu, Z. & Schuchat, A. (2004) Superspreading SARS events, Beijing, 2003. *Emerging infectious diseases*, **10**, 256.
- Sih, A., Bell, A. & Johnson, J.C. (2004) Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, **19**, 372-378.
- Sih, A., Cote, J., Evans, M., Fogarty, S. & Pruitt, J. (2012a) Ecological implications of behavioural syndromes. *Ecology Letters*, **15**, 278-289.
- Sih, A., Cote, J., Evans, M., Fogarty, S. & Pruitt, J. (2012b) Ecological implications of behavioural syndromes. *Ecology Letters*.
- Sih, A., Hanser, S.F. & McHugh, K.A. (2009) Social network theory: new insights and issues for behavioral ecologists. *Behavioral Ecology and Sociobiology*, **63**, 975-988.
- Sih, A. & Watters, J.V. (2005) The mix matters: behavioural types and group dynamics in water striders. *Behaviour*, **142**, 9-10.
- Sloan Wilson, D., Clark, A.B., Coleman, K. & Dearstyne, T. (1994) Shyness and boldness in humans and other animals. *Trends in Ecology & Evolution*, **9**, 442-446.
- Smith, J.M. & Szathmary, E. (1997) *The major transitions in evolution*. Oxford University Press.
- Smith, M.L., Ostwald, M.M., Loftus, J.C. & Seeley, T.D. (2014) A critical number of workers in a honeybee colony triggers investment in reproduction. *Naturwissenschaften*, 1-8.
- Sumpter, D.J., Krause, J., James, R., Couzin, I.D. & Ward, A.J. (2008) Consensus decision making by fish. *Current Biology*, **18**, 1773-1777.
- Traniello, J.F., Rosengaus, R.B. & Savoie, K. (2002) The development of immunity in a social insect: evidence for the group facilitation of disease resistance. *Proceedings of the National Academy of Sciences*, **99**, 6838-6842.
- Vallet-Gely, I., Lemaitre, B. & Boccard, F. (2008) Bacterial strategies to overcome insect defences. *Nature Reviews Microbiology*, **6**, 302-313.
- Ward, P.I. & Enders, M.M. (1985) Conflict and cooperation in the group feeding of the social spider *Stegodyphus mimosarum*. *Behaviour*, **94**, 167-182.
- White, L.A., Forester, J.D. & Craft, M.E. (2015) Using contact networks to explore mechanisms of parasite transmission in wildlife. *Biological Reviews*.
- Woolhouse, M.E., Dye, C., Etard, J.-F., Smith, T., Charlwood, J., Garnett, G., Hagan, P., Hii, J., Ndhlovu, P. & Quinnell, R. (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences*, **94**, 338-342.
- Wright, C., Keiser, C. & Pruitt, J. (2016) Behavioral composition can facilitate collective defenses and alter colony-level behavioral plasticity in a social spider. *Animal Behaviour*.

- Wright, C.M., Holbrook, C.T. & Pruitt, J.N. (In press) Animal personality aligns task specialization and task efficiency in a spider society. *Proceedings of the National Academy of Sciences*.
- Wright, C.M., Keiser, C.N. & Pruitt, J.N. (2015) Personality and morphology shape task participation, collective foraging and escape behaviour in the social spider *Stegodyphus dumicola*. *Animal Behaviour*, **105**, 47-54.
- Yip, E.C., Powers, K.S. & Avilés, L. (2008) Cooperative capture of large prey solves scaling challenge faced by spider societies. *Proceedings of the National Academy of Sciences*, **105**, 11818-11822.
- Zilber-Rosenberg, I. & Rosenberg, E. (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS microbiology reviews*, **32**, 723-735.
- Zylberberg, M., Klasing, K.C. & Hahn, T.P. (2013) House finches (*Carpodacus mexicanus*) balance investment in behavioural and immunological defences against pathogens. *Biol Lett*, **9**, 20120856.