

Queen presence mediates the relationship between collective behaviour and disease susceptibility in ant colonies

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Abstract

1. The success of social living can be explained, in part, by a group's ability to execute collective behaviours unachievable by solitary individuals. However, groups vary in their ability to execute these complex behaviours, often because they vary in their phenotypic composition. Group membership changes over time due to mortality or emigration, potentially leaving groups vulnerable to ecological challenges in times of flux. In some societies, the loss of important individuals (e.g. leaders, elites and queens) may have an especially detrimental effect on groups' ability to deal with these challenges.
2. Here, we test whether the removal of queens in colonies of the acorn ant *Temnothorax curvispinosus* alters their ability to execute important collective behaviours and survive outbreaks of a generalist entomopathogen.
3. We employed a split-colony design where one half of a colony was maintained with its queen, while the other half was separated from the queen. We then tested these subcolonies' performance in a series of collective behaviour assays and finally exposed colonies to the entomopathogenic fungus *Metarhizium robertsii* by exposing two individuals from the colony and then sealing them back into the nest.
4. We found that queenright subcolonies outperformed their queenless counterparts in nearly all collective behaviours. Queenless subcolonies were also more vulnerable to mortality from disease. However, queenless groups that displayed more interactions with brood experienced greater survivorship, a trend not present in queenright subcolonies. Queenless subcolonies that engage in more brood interactions may have had more resources available to cope with two physiological challenges (ovarian development after queen loss and immune activation after pathogen exposure).
5. Our results indicate that queen presence can play an integral role in colony behaviour, survivorship and their relationship. They also suggest that interactions between workers and brood are integral to colonies survival. Overall, a social group's history of social reorganization may have strong consequences on their collective behaviours and their vulnerability to disease outbreaks.

KEYWORDS

ants, collective behaviour, entomopathogenic fungus, queen presence, *Temnothorax*

1 | INTRODUCTION

The complex cooperative behaviours of animal societies represent one of the most impressive phenomena in nature, in part because they can execute tasks not achievable by solitary individuals. Collective motion, cooperative defences against enemies and the construction of complex physical structures are only a few examples of the amazing feats of animal group behaviour. However, not all groups are equally adept at executing these feats, and these differences in collective behaviour can have far-reaching consequences. Some fish shoals explore their environment more widely compared to others, potentially driving species' invasion into new habitats (Cote, Fogarty, Brodin, Weinersmith, & Sih, 2010; Fogarty, Cote, & Sih, 2011). Some colonies of honeybees contain more active foragers and colony defenders, which is directly associated with increased colony productivity and survival (Wray, Mattila, & Seeley, 2011). This variation likely stems from differences in group composition, which ultimately determines a group's ability to execute collective tasks (Farine, Montiglio, & Spiegel, 2015; Jandt et al., 2014). Groups containing a favourable composition of different types of individuals, i.e. an adaptive mixture of behavioural phenotypes (Pruitt & Goodnight, 2014) or morphological castes (Schmid-Hempel, 1992), will excel at collective tasks and generally outperform groups with suboptimal compositions (Farine et al., 2015). However, groups also experience changes in composition via emigration, group fission/fusion or death, thus making phenotypic composition and organizational schemes a dynamic feature of social groups (Krause & Ruxton, 2002). The loss of important individuals can have particularly far-reaching effects on the social group (Borgatti, 2006; Krause, Croft, & James, 2007).

Societies which experience rapid alterations to their organizational system (e.g. loss of key group members) may be susceptible to challenges during times of flux (Sapolsky & Share, 2004), even if their social organization is resilient against that loss (Lusseau, 2003). Eusocial insect societies represent an interesting case where the queen, often the sole reproductive unit and thus a highly influential member of the colony, can be lost. In some ant societies, the loss of the queen results in the eventual death of the colony (Kronauer, 2009). In the time between queen loss and colony death, the social organization of the colony changes rapidly. Workers establish aggressive dominance hierarchies and high-ranking workers begin to produce their own eggs (Brunner & Heinze, 2009; Stroeymeyt, Brunner, & Heinze, 2007), although some ants will continue to tend larvae for some time before they begin to lay their own eggs (Villalta, Angulo, Devers, Cerdá, & Boulay, 2015). This aggressive reorganization is energetically costly and the resulting shift in colony task participation can reduce worker life span (Gobin, Heinze, Strätz, & Roces, 2003; Tsuji, Kikuta, & Kikuchi, 2012). Thus, the presence of the queen is critical to the maintenance of colonies' reproductive division of labour and the reduction of within-colony aggression (referred to as "social harmony": Konrad, Pamminger, & Foitzik, 2012). Indeed, a group of individuals whose behavioural tendencies are in flux via altered dominance hierarchies will likely be less cohesive or consistent in their group behaviours (Della-Rossa, Chadæuf, Boissy, & Dumont, 2013; Gottier, 1968). To our

knowledge, the degree to which the loss of the queen alters colonies' execution of multiple collective tasks is yet unknown. Furthermore, societies, just like individuals, can be confronted with multiple ecological challenges simultaneously. Thus, as groups reorganize their social structure to serve the demands of important collective behaviours like foraging or navigation, they may confront another challenge that require a wholly different set of demands (Parrish & Edelman-Keshet, 1999).

A classic potential challenge of social living is an increased transmission of infectious agents that can lead to disease outbreaks. For instance, colonially nesting cliff swallows garner enhanced foraging efficiency and growth at larger colony sizes (Brown, 1988), although these denser colonies also experience an increased incidence of ectoparasitism (Brown & Brown, 1986). Honeybee colonies rely heavily on division of labour, although infection with deformed wing virus hinders their ability to organize colony tasks (Natsopoulou, McMahon, & Paxton, 2016). Under the best conditions, groups are able to defend against challenges like parasite infection. However, groups already under the stress of the loss of an important individual may be more susceptible and at greater risk of mortality.

Here, we aim to test how changes in the social organization of *Temnothorax curvispinosus* ant colonies alter their ability to execute important collective behaviours and their susceptibility to outbreaks of a generalist fungal pathogen. Specifically, we ask (i) to what degree will the loss of the queen alter colonies' brood recovery, foraging, exploratory behaviour and interactions with brood? (ii) Will queenless colonies be more susceptible to a fungal pathogen? and finally, (iii) will colonies that exhibit certain collective behavioural tendencies (e.g. engage more in foraging, brood care, etc.), be more susceptible to disease, and is this mediated by queen presence?

2 | MATERIALS AND METHODS

2.1 | Ant collection and maintenance

Temnothorax curvispinosus inhabits preformed cavities in acorns or twigs on deciduous forest floors across eastern USA. Colonies are small, ranging from less than 10 workers to over 300 depending on the season, and upwards of 30% of colonies are found to be queenless (Headley, 1943). We collected mature queenright colonies of *T. curvispinosus* in June 2016 from two sites near Geneva State Park in Geneva, OH (original colony sizes: 10–62 workers, 41% queenless). Colonies were transported to the laboratory at Rice University where they were maintained in clear plastic round dishes (diameter = 11 cm, height = 4.5 cm) with a plastic inlay artificial nest (7.5 cm × 2.5 cm × 0.3 cm; see Figure S1). Colonies were given an ad libitum supply of water and frozen *Drosophila melanogaster*. Prior to the onset of experiments, colonies were starved for 2 weeks, but still provided water ad libitum (following methods in Lichtenstein, Pruitt, & Modlmeier, 2015). Throughout maintenance and experimentation, colonies were maintained in an environmental chamber at 24°C and 65% relative humidity, with a 12 hr:12 hr light:dark cycle.

2.2 | Collective behavioural assays

The onset of experiments began by using a split-colony design where each queenright colony with >25 workers was split into groups (referred to henceforth as subcolonies), one with 10 workers and their original queen ($n = 20$ subcolonies) and one with 11 workers and no queen ($n = 24$ subcolonies) (e.g. Stroeymeyt et al., 2007). Four source colonies contained enough workers to produce more than one queenless subcolony. When splitting colonies, workers were chosen haphazardly as to which group they were placed, and workers were selected alternating from the inside of the nest and the outside of the nest, in attempts to place an equal number of brood care workers and foragers in each new subcolony (Robinson, Feinerman, & Franks, 2009). Subcolonies were maintained in 5-ml plastic test tubes until the onset of behavioural assays. All observations were made blindly in regard to the manipulation of each subcolony (i.e. queen presence and fungal exposure).

2.2.1 | Brood recovery

In ant colonies, brood rescue efficiency (the time to discover and recover brood that are displaced from the nest) is an important collective trait that is positively associated with colony productivity, and may be altered via rapid changes to colonies' social organization (Blight, Villalta, Cerdá, & Boulay, 2016). Directly after splitting colonies, using a fine paintbrush, we moved five larvae from the original colony into a new housing container (20 cm × 15 cm), 3 cm away from a new, clean artificial nest and immediately released one subcolony 3 cm away from the nest, orthogonally from the brood pile (see Figure S2; following methods from Modlmeier, Keiser, Shearer, & Pruitt, 2014). Using a stopwatch, we measured the time until the first worker discovered the brood pile, the time until the first larva was taken into the new nest, and the time until all five larvae were transported into the new nest.

2.2.2 | Colony exploratory behaviour

To find resources like new nest sites and potentially novel food sources, ant colonies must explore the environment outside of their nests. Colonies vary in their degree of exploratory behaviour, which has been shown to be associated with colony success in a number of ecological contexts, and malleable to colony composition (Blight, Albet Díaz-Mariblanca, Cerdá, & Boulay, 2016; Lichtenstein et al., 2015; Modlmeier, Liebmann, & Foitzik, 2012). Five days after the conclusion of brood recovery assays, we tested subcolonies' exploratory behaviour by moving a colony's nest into a larger plastic arena (diameter = 13.5 cm) with four unique, fresh leaf clippings collected from trees on the Rice University campus in Houston, TX, USA (*Quercus falcata*, *Quercus phellos*, *Lagerstroemia* sp. and *Eucalyptus* sp.). Clippings were placed at fixed distances from the nest entrance in a standardized geometry (see Figure S2). We then recorded the latency for individuals to discover each leaf clipping, and counted the number of clippings that were discovered within 30 min. Each colony was tested once daily for 3 days, and the order of the clippings was rotated between each trial.

2.2.3 | Colony foraging behaviour

The speed and magnitude by which ant colonies discover and utilize food sources is a primary driver of overall colony success (Carroll & Janzen, 1973). Three days after the conclusion of exploratory assays, we tested subcolonies' foraging behaviour by moving a colony's nest into a larger plastic arena (diameter = 13.5 cm) with two food sources (three frozen and thawed fruit flies and a 100- μ l droplet of 20% sugar water) equidistant from the nest entrance (see Figure S2). If any ants were outside the nest, they were placed into the nest with forceps before the colony was moved into the Petri dish. Using a stopwatch, we measured the time until each food source was discovered, as well as the number of ants feeding at each food source at 5-min intervals for 30 min. Each colony was tested once daily for 3 days, and the order of the food sources was rotated between each trial.

2.2.4 | Brood care observations

Interactions between workers and brood are not only vital to overall productivity but also colony immune priming and collective antiseptic behaviour (Moret & Schmid-Hempel, 2001; Purcell & Chapuisat, 2014; Tragust, Mitteregger et al., 2013). Three days after the conclusion of colony foraging assays, we observed each subcolony once daily for 3 days and noted the proportion of workers that were in direct contact with the brood vs. performing other tasks (e.g. inactivity, exploring outside the nest, etc.). Interactions with brood were defined as being in contact with the brood pile and antennating/cleaning/feeding the larvae.

2.3 | Experimental pathogen exposure

We used the generalist entomopathogenic fungus *Metarhizium robertsii* (ARSF# 2576) as a model pathogen for this experiment, obtained from the USDA-ARS Collection of Entomopathogenic Fungi Cultures in Ithaca, New York, USA. Despite criticism (Loreto & Hughes, 2016a), the use of generalist pathogens as models of infectious disease agents in eusocial insects is common for its utility, tractability and reproducibility in the laboratory (e.g. Gao, Bidochka, & Thompson, 2012; Konrad, Vyleta et al., 2012; Loreto & Hughes, 2016b). This strain of *M. robertsii* is virulent in *T. curvispinosus*, with a median time to death of 4 days after exposure (see Figure S3). After the completion of colony behaviour assays, two individuals from each experimental subcolony were exposed to an infectious fungal conidiospore suspension. The conidiospore suspension was produced by pouring a 0.05% solution of Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) onto the surface of a sporulating *M. robertsii* culture grown on Sabouraud dextrose agar, agitating the conidia with an inoculating loop, and collecting the suspension with a micropipette. Cultures were grown for 3 weeks before collecting conidia. The conidiospore concentration (7.26×10^7 conidiospores/ml) was quantified with a Bright-Line Phase Hemacytometer (Hausser Scientific Partnership). We dipped two workers from each colony individually for 3 s into the conidiospore suspension, and then placed them directly onto sterile filter paper to

dry. Exposed ants were maintained in pairs in 5-ml plastic test tubes for 24 hr before being placed back into their subcolonies ($n = 35$ subcolonies; 15 queenright and 20 queenless), after which we plugged the entrance of the nest with a piece of moistened cotton and wetted daily to provide the ants with water and maintain moisture levels required for fungal growth. Ants in control colonies were exposed to a suspension of non-infectious, autoclave-treated conidiospores at the same conidiospore concentration ($n = 9$ colonies; five queenright and four queenless). We counted the number of dead ants present in each subcolony daily for 20 days. For a further 10 days, we checked whether each subcolony had collapsed (i.e. all individuals had died). Thirty days represents a time-frame long enough to encompass both short-term transmission events and long-term colony-wide consequences of disease outbreaks in ants (Jaccoud, Hughes, & Jackson, 1999; Loreto & Hughes, 2016b). Once daily in the morning for the first 5 days after exposure, we also counted the number of individuals that were interacting with brood (described above). We confirmed the infection status of all deceased ants in this study by surface sterilizing individuals and placing them on wetted sterile filter paper to verify fungal growth through the cuticle (following protocols in Lacey, 1997).

2.4 | Statistical analyses

We used generalized linear mixed models (GLMM) to predict colonies' execution of each collective behaviour. Poisson probability distributions were used for count data (number of ants feeding, number of ants interacting with brood) and exponential probability distributions were used for continuous data (latency to discover brood, food and stimuli). Each model contained queen presence as an independent variable and trial number as a random effect. For all analyses, experimental colony ID nested in source colony ID was included as a random effect, because of the paired-design of our experimental replicates. We analysed the number of dead ants observed within colonies over 20 days following infectious ant introduction using a GLMM with a binomial distribution and an auto-regressed covariance matrix. We included queen presence, fungal exposure, time, queen presence \times fungal exposure and the interaction terms with time as independent variables. Subcolony ID nested in source colony ID and subcolony ID \times time were included as random effects. At the group level, we analysed the time until collapse (when all individuals in the colony had died) using a Wilcoxon signed-rank survival analysis (Custodio Martinez, 2007). We analysed the relationship between colony interactions with brood (before and after exposure to infectious ants) and the number of dead ants within colonies after 20 days using general linear mixed model (identity link function) containing queen presence, average proportion of colony interacting with brood, and their interaction term as independent variables. We used the *q* value package in R (R Core Team 2014) to test for false discovery rate from multiple testing (Storey, 2002), where *p* values that are smaller than their associated *q* value remain significant (Table S6). GLMM and repeated measures MANOVA were performed in JMP Pro 12.1 (©2015 SAS Institute, Inc., Cary, NC, USA), whereas survival analyses were performed in Graphpad Prism 7.0 (©2016 GraphPad Software, Inc., La Jolla, CA, USA).

3 | RESULTS

3.1 | Colony collective behaviours

The presence or absence of the queen within subcolonies altered the execution of nearly all collective behaviours. Queenright subcolonies were three times faster at discovering their brood after nest disturbance (GLMM: $\chi^2 = 11.52$, $p = .0007$, $Q = 0.002$; Table 1, Figure 1a), though they did not differ from queenless groups in their latency to move the discovered brood into a new nest site (GLMM: $\chi^2 = 0.76$, $p = .38$, $Q = 0.38$; Table 1). The presence of a queen did not alter the latency for subcolonies to discover novel stimuli (GLMM: $\chi^2 = 0.95$, $p = .33$, $Q = 0.37$) or food sources (GLMM: $\chi^2 = 2.65$, $p = .10$; $Q = 0.14$; Table 1; Figure 1b). However, once food items were discovered, we observed 60% more ants feeding at food sources in queenright subcolonies compared to queenless subcolonies (GLMM: $\chi^2 = 14.12$, $p = .0002$; $Q = 0.0007$; Table 1; Figure 1c). Finally, groups with their queen present had 60% more individuals interacting with the brood during focal observations (GLMM: $\chi^2 = 27.42$, $p < .0001$, $Q = 0.0004$; Table 1; Figure 1d).

3.2 | Group mortality after pathogen exposure

Subcolonies experienced over 2.4 times greater mortality 20 days after exposure to infectious workers compared to control colonies (GLMM: $\chi^2 = 344.14$, $df = 1$, $p < .0001$; $Q = 0.0004$; Table 2; Figure 2a). At the height of differences, mortality was nine times greater in infected colonies. About 76% of corpses (166/219) collected from infected colonies showed evidence of *M. robertsii* fungal growth, whereas 2% of corpses (1/46) from control colonies appeared to die from fungal infection. The infected corpse collected from a control colony may have become infected from cross-contamination, or *M. robertsii* may have been misidentified. The average mortality experienced by infected queenless colonies was 75% greater than that experienced by infected queenright colonies ($\chi^2 = 6.18$, $df = 1$, $p = .01$; $Q = 0.022$; Table 2; Figure 2a). At the group level, infected queenless subcolonies collapsed more quickly (i.e. all individuals in the colony had died) compared to queenright-infected colonies and control colonies (Wilcoxon survival analysis:

TABLE 1 Results of generalized linear mixed models predicting the effects of queen presence on the execution of five collective behaviours. Significant effects are noted with an asterisk

Dependent variable	df	χ^2	p-value
Brood recovery	1	11.52	.0007*
Transport of brood into new nest site	1	0.76	.38
Latency to discover novel stimuli	1	0.95	.33
Latency to discover food	1	2.65	.10
Number of workers collecting food	1	14.12	.0002*
Number of workers interacting with brood	1	27.42	<.0001*

FIGURE 1 Queen presence was associated with the execution of many, but not all, collective behaviours in *Temnothorax curvispinosus* colonies. Queenright subcolonies were (a) faster in discovering misplaced brood, (b) though queenless and queenright subcolonies did not differ in their latency to discover novel stimuli or food resources. (c) Queenright subcolonies had more workers engaged in food collection (d) and engaged in more interactions with brood before exposure to pathogens. Points represent mean values and error bars represent standard error of the mean

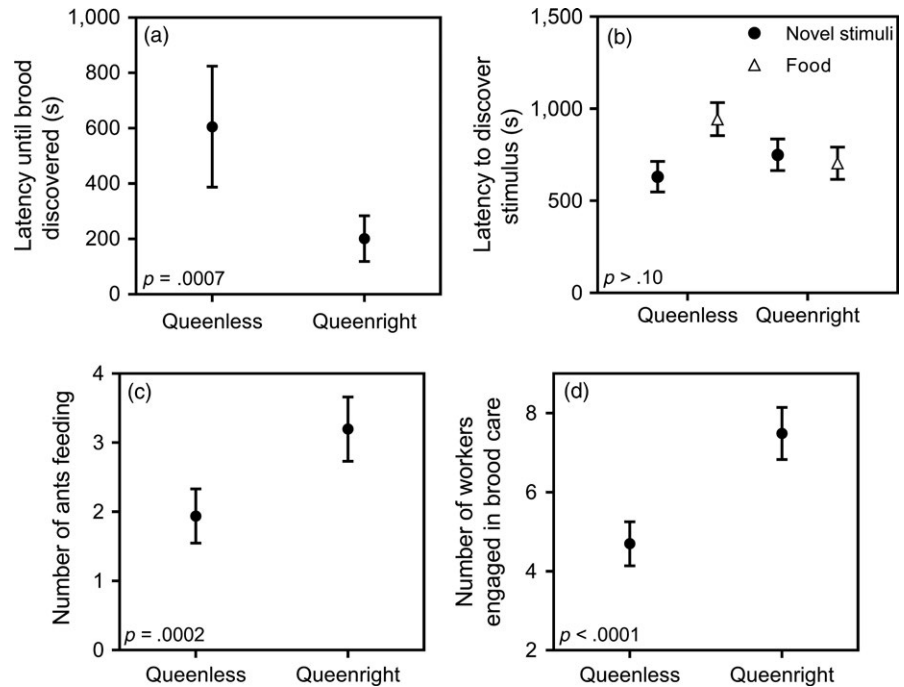


TABLE 2 Results of a generalized linear mixed model predicting the number of dead ants observed within colonies for the first 20 days after exposure. Significant effects are noted with an asterisk

Independent variable	df	χ^2	p-value
Queen presence	1	0.76	.38
Fungal exposure	1	344.14	<.0001*
Time	1	419.12	<.0001*
Queen presence × Fungal exposure	1	6.18	.01*
Time × Queen presence	1	4.29	.04*
Time × Fungal exposure	1	29.66	<.0001*
Time × Fungal exposure × Queen presence	1	1.02	.31

$\chi^2 = 8.18$, $df = 3$, $p = .04$; $Q = 0.068$; Figure 2b). Queenless subcolonies that engaged in more interactions with brood before exposure to infected ants experienced reduced mortality after exposure, a trend not observed in queenright subcolonies (queen presence × interaction with brood interaction term: GLMM: $F_{1,30} = 9.35$, $p = .005$; $R^2 = 0.48$; $Q = 0.01$; Figure 3a). This trend persisted when considering colonies' interactions with brood after infection, although the relationship was non-significant (GLMM: $F_{1,30} = 3.30$, $p = .08$, $R^2 = 0.35$; $Q = 0.12$; Figure 3b). The relationship between interactions with brood and mortality was not observed in control colonies ($p = .27$).

4 | DISCUSSION

The ability of many social groups to execute collective behaviours and their resilience against ecological challenges may be vulnerable to the removal of important individuals in the group. Here, we investigated the

degree to which the loss of the queen in ant colonies would alter their ability to perform colony tasks and their ability to withstand disease-associated mortality. We found, in general, that queenright subcolonies outperformed their queenless counterparts in nearly all collective behaviours, and were less susceptible to collapse (i.e. all individuals had died). However, queenless groups that engaged in more interactions with brood were found to survive longer, a trend we did not observe in queenright subcolonies. Taken together, queen presence plays an integral role in not only the execution of colony behaviours and resilience against disease but also mediates the relationship between these collective outcomes.

4.1 | Collective behaviours after queen loss

The presence of key individuals is fundamental in the execution of collective behaviours in social groups across diverse taxa (i.e. "keystone individuals," reviewed in: Modlmeier, Keiser, Watters, Sih, & Pruitt, 2014). Targeted removal experiments have identified that the loss of these important individuals can diminish groups' ability to accomplish collective tasks. Here, we found that queenright subcolonies outperformed their queenless counterparts in nearly all tested collective behaviours. As upwards of 30% of *T. curvispinosus* colonies are found to be queenless in nature (Headley, 1943; Stuart, 1985), these colonies will likely vary in their role in their population and even community. We speculate that queenless subcolonies executed collective behaviours more slowly or with fewer participants compared to queenright subcolonies because of recent shifting in group dominance hierarchies. Group fission causes rapid alterations to dominance hierarchies in diverse animal groups from ants to elephants (Brunner & Heinze, 2009; de Silva, Schmid, & Wittemyer, 2016). A shifting dominance structure in animal aggregations can alter the behavioural tendencies of individuals within the group (Rudin, Tomkins, & Simmons, 2016) and hinder the cohesion or consistency of group behaviours (Della-Rossa

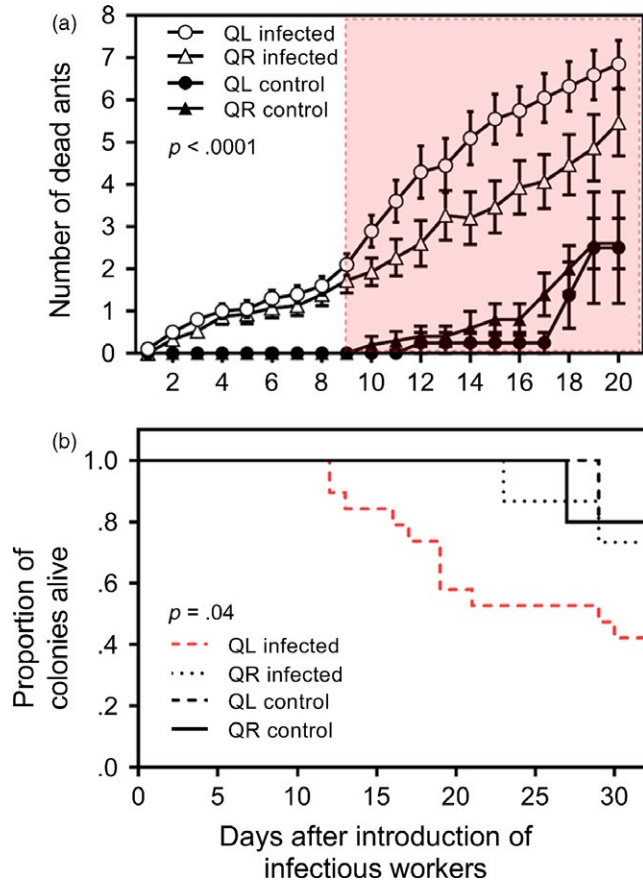


FIGURE 2 (a) Infected groups experience greater worker mortality compared to controls, with queenless (QL)-infected subcolonies experiencing the greatest mortality in early stages of pathogen transmission compared to queenright (QR) subcolonies. As two individuals were experimentally exposed to fungus in infected colonies, the red box highlights the time points in which mortality began arising from newly infected individuals (i.e. via pathogen transmission). This time point also represents double the median time to death for individuals infected experimentally with *Metarhizium robertsii* (see Figure S3). Points represent mean values and error bars represent standard error of the mean. (b) Infected colonies without a queen collapsed more quickly, that is, all individuals in the colony had died. Control colonies and queenright-infected colonies experienced similar survival

et al., 2013; Gottier, 1968). Furthermore, group cohesion may have been weakened after queen loss because of reduced nest-mate recognition, a phenomenon observed in some ants (Vander Meer, Preston, & Hefetz, 2008), but not others (Crosland, 1990; Lahav, Soroker, Vander Meer, & Hefetz, 1998; van Zweden, Dreier, & d'Etterre, 2009). Queens also exert reproductive control over colonies via behavioural and pheromonal mechanisms, and the absence of these cues undoubtedly plays a role in the underpinnings of the differences in collective behaviours observed here (Holman, Jørgensen, Nielsen, & d'Etterre, 2010; Vander Meer, 1995; Vander Meer & Alonso, 1998).

Here, we used group-wide measures of collective behaviours and ignored the degree to which tasks were divided among individuals. However, while some collective tasks are distributed among

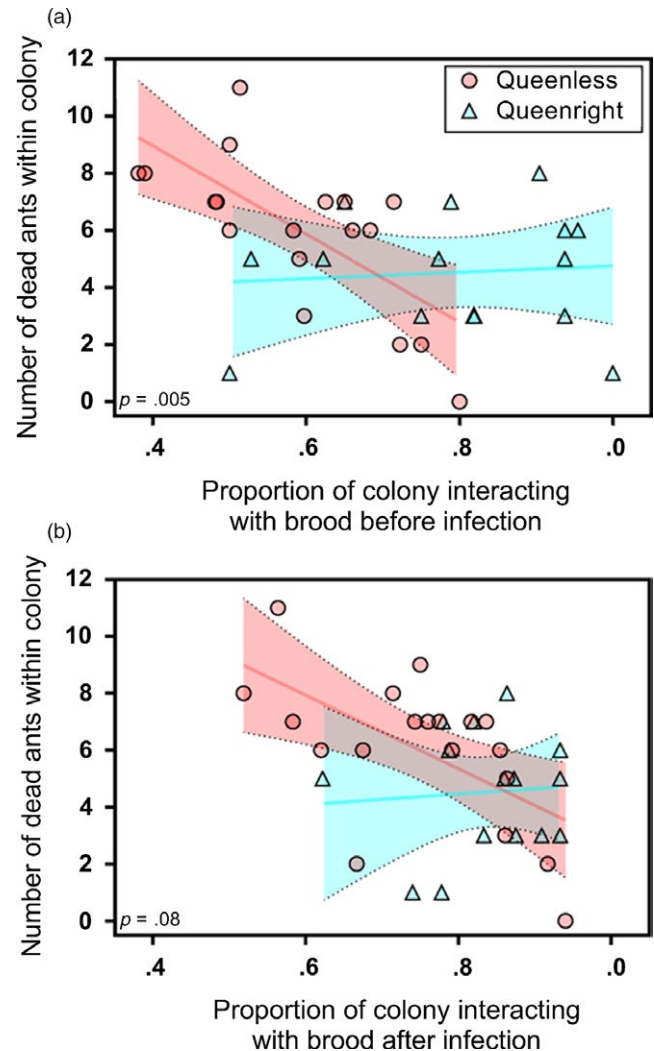


FIGURE 3 (a) Queenless subcolonies that engaged in more interactions with brood before exposure to infected ants experienced reduced mortality. (b) This trend persisted when considering colonies' interactions with brood after infection, although the relationship was non-significant. These trends were not present in queenright subcolonies (data not shown). Solid lines represent best fit lines and shaded regions within dotted lines represent 95% CI

entire groups (Bonabeau, Theraulaz, Deneubourg, Aron, & Camazine, 1997), others can be completed solely by one or a few key individuals (Modlmeier, Keiser, Watters et al., 2014; Pinter-Wollman, Hubler, Holley, Franks, & Dornhaus, 2012). Future studies on the loss of important individuals (e.g. elites, leaders, queens, etc.) should identify specifically how task participation is rearranged after a change in dominance hierarchy, and why this leads to suboptimal execution of collective behaviours. More specific to this system, where queenless colonies may actually be a subunit of a larger polydomous network of colonies (Alloway, Buschinger, Talbot, Stuart, & Thomas, 1982; Stuart, 1985; Stuart, Gresham-Bissett, & Alloway, 1993), future research should identify how the movement of workers between nests alters dominance hierarchies within queenless nests and potentially shifts collective behaviour as a result (Robinson, 2014).

4.2 | Disease susceptibility after queen loss

During the stressful period following the loss of a key individual, groups may be more susceptible to challenges like disease risk. Social stresses resulting from changes to group composition have been linked with decreased immunocompetence and increased disease risk in a diversity of mammals (Bartolomucci, 2007; de Groot, Ruis, Scholten, Koolhaas, & Boersma, 2001; Tamashiro, Nguyen, & Sakai, 2005). Here, we found that queenless subcolonies were more vulnerable to colony mortality after pathogen exposure. Interestingly, queenless groups that engaged in more interactions with their brood experienced reduced overall mortality (i.e. values closer to that of queenright groups). As colonies were deprived of food resources during this portion of the experiment, their interactions with brood (especially trophallaxis) may have increased relative to baseline interaction rates (Cassill & Tschinkel, 1995). Although we do not yet have direct evidence, we suggest the following interpretation: colonies that have their queen removed experience shifts in within-colony resource allocation (Linksvayer, 2008) and begin to activate their ovaries (Brunner & Heinze, 2009; Brunner, Kroiss, Trindl, & Heinze, 2011; Peeters, 1991). These stresses are exacerbated by pathogen exposure, which stimulate workers' costly innate immunity cascades (Schluns & Crozier, 2009). In insects, including eusocial insects with reproductive division of labour, there is a well-documented trade-off between reproduction and immunity (Calleri, Rosengaus, & Traniello, 2007; Pamminger, Treanor, & Hughes, 2016; Rolff & Siva-Jothy, 2002), and thus queenless workers that had already invested in ovarian development likely had less resources available for immune activation. When interacting with brood, workers not only feed and clean larvae but they also obtain resources from the brood (e.g. Herbers, 1983), which are used as vessels to store and digest certain resources that are regurgitated back to adult workers (i.e. "larval reciprocity"; Hölldobler & Wilson, 1990). This suggests that, perhaps, queenless ant colonies can withstand exposure to pathogens if they behave more like their queenright counterparts: engaging in more interactions with the brood, thereby storing more resources to help cope with future physiological challenges.

Trophallaxis among workers and brood within a colony may also spread effectors of antimicrobial activity and potentially prime the innate immune system of non-infected workers against infection (Hamilton, Lejeune, & Rosengaus, 2011; Moret & Schmid-Hempel, 2001; Purcell & Chapuisat, 2014). Furthermore, previous experiences in brood care improves workers' ability to remove conidiospores from brood surfaces (Westhus et al., 2014). This is especially important in ants where larvae do not develop a pupal cocoon, and thus are more susceptible to pathogen infection (Tragust, Ugelvig, Chapuisat, Heinze, & Cremer, 2013). In the garden ant *Lasius neglectus*, workers infected with a fungal pathogen are less likely to engage in brood care, although their uninfected colony-mates increase their investment in brood care after interacting with an infected worker (Ugelvig & Cremer, 2007). Thus, worker-brood interactions appear to be central to ants' individual and collective defence against infectious disease outbreaks. In

other animal societies, interactions among individuals were originally thought to simply increase parasite transmission; but current investigations have also found benefits to parasite defences via within-group affiliative interactions (e.g. allogrooming, social immunity: Meunier, 2015; Ezenwa, Ghai, McKay, & Williams, 2016).

5 | CONCLUSIONS

These data add to the tremendous evidence that group living is not only associated with an increased costs of infectious disease outbreaks but also that societies garner benefits associated with parasite defences (Ezenwa et al., 2016). In general, the benefits of social immunity and antiseptic behaviours garnered by eusocial insect colonies reduce pathogen load within nests and safeguard workers from pathogen encounter, infection and transmission (Cremer, Armitage, & Schmid-Hempel, 2007; Diez, Urbain, Lejeune, & Detrain, 2015; Karlik, Epps, Dunn, & Penick, 2016; Loreto & Hughes, 2016a; Schär, Larsen, Meyling, & Nash, 2015; Ugelvig & Cremer, 2007). However, even the benefits of social immunity cannot always protect subdivided or otherwise altered colonies from total group death (Loreto & Hughes, 2016b). Thus, a more nuanced relationship between colonies' collective behavioural tendencies and their history of social reorganization may better explain variation among colonies in their susceptibility to infectious disease outbreaks. Furthermore, these data suggest that queenless ant colonies in nature likely vary in their collective behaviours and survivorship, and their role in the ecosystem may vary compared to their queenright counterparts. Beyond eusocial insects, societies' responses to multiple stressors (i.e. disturbance to social organization and exposure to pathogens) have become a focus of some recent investigations into human socioecology, finding that social reorganization can inflate disease risk after periods of societal disturbance (Rael, Peterson, Gherzi, Childs, & Blum, 2016). Thus, we are hopeful that future studies may identify parallels between animal and human societies to generate general theories of social organization and disease ecology.

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AUTHORS' CONTRIBUTIONS

C.N.K. and S.V. designed the experiment; C.N.K., I.O.B., and E.S. executed the experiment; C.N.K. performed statistical analyses and prepared the manuscript with assistance from V.H.W.R. and J.B.S.

DATA ACCESSIBILITY

Data associated with this manuscript are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.25sk1> (Keiser et al., 2017).

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