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Opposing effects of allogrooming on disease transmission in ant societies

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To prevent epidemics, insect societies have evolved collective disease defences that are highly effective at curing exposed individuals and limiting disease transmission to healthy group members. Grooming is an important sanitary behaviour—either performed towards oneself (self-grooming) or towards others (allogrooming)—to remove infectious agents from the body surface of exposed individuals, but at the risk of disease contraction by the groomer. We use garden ants (*Lasius neglectus*) and the fungal pathogen *Metarhizium* as a model system to study how pathogen presence affects self-grooming and allogrooming between exposed and healthy individuals. We develop an epidemiological SIS model to explore how experimentally observed grooming patterns affect disease spread within the colony, thereby providing a direct link between the expression and direction of sanitary behaviours, and their effects on colony-level epidemiology. We find that fungus-exposed ants increase self-grooming, while simultaneously decreasing allogrooming. This behavioural modulation seems universally adaptive and is predicted to contain disease spread in a great variety of host–pathogen systems. In contrast, allogrooming directed towards pathogen-exposed individuals might both increase and decrease disease risk. Our model reveals that the effect of allogrooming depends on the balance between pathogen infectiousness and efficiency of social host defences, which are likely to vary across host–pathogen systems.

1. Introduction

Disease dynamics in societies are affected by both within-host processes (immunity) and between-host interactions (parasite transmission). Despite their joint importance for understanding disease dynamics, immunity and parasite transmission are often studied separately. In addition, the role of individual behaviour, which affects contact rates and the probability of exposure, is often unknown. Behavioural changes of infectious individuals—and those they interact with—can influence both the individual course of disease and the transmission to others. Animal behaviour therefore forms a crucial link between individual immunity and parasite transmission [1]. Studying behaviour is thus of particular relevance when trying to understand disease dynamics in societies where individuals engage in intensive social interactions and have evolved collective anti-parasite defences ('social immunity' [2]), which complement the individual immunity of each group member.

Collective defences include sanitary behaviours that often involve the use of antimicrobials (either obtained from the environment [3,4], or self-produced [5,6] or produced by a symbiotic partner [7,8]). Very important sanitary behaviours are cleaning/grooming, whereby potentially infectious particles are removed from the body surface [9,10]. Familiar examples include washing and delousing, both of which can be directed towards the performing individual (self-grooming) or towards its group members (allogrooming). In addition to hygienic measures, the social interaction networks of groups play an important role for disease dynamics, as they predict disease transmission routes within societies [11]. The

fact that social interactions are often inherently heterogeneous across individuals, owing to societies being composed of different age or task groups, can lead to 'organizational immunity' ([12–14], reviewed in [15]). Organizational immunity provides prophylactic protection to particularly valuable group members, such as the offspring or reproductive individuals, by means of reduced exposure risk. Additional modulation of these social interaction networks upon pathogen exposure can further strengthen this effect [2,12,15–18].

Changes in the behaviour of infectious individuals, or their group members, are likely to be reflected in their social interaction network (though experimental confirmation is scarce, see [15]), as some connections may be intensified and others eliminated, for example through caretaking [19–21] or isolation [22–25] of diseased individuals. Such behavioural modulations, in addition to the direction in which behaviours are performed, can strongly impact the routes of disease spread in the group, by affecting the risk of pathogen exposure and transmission of the disease. Some individuals may thus become the source and others the sink for pathogen transmission, depending on the frequency and direction of their interactions [26]. Moreover, in the analysis of directed behaviours, it is important to compare the ratios between performed and received behaviours, but it is also important to determine how any changes in the ratios occur when comparing pathogen-free and pathogen-exposed conditions. If, for example, two individuals A and B obtained equal grooming in pathogen-free conditions, but A receives more than B under pathogen exposure, it is important to determine whether the higher grooming of A versus B is a result of increased grooming of A, or, conversely, a relative reduction in grooming of B. Two distinct behaviours can thus result in a similar ratio, but are important to distinguish as they can have very different implications on disease dynamics.

In this work, we therefore aimed to bring both levels—individual behaviour and collective disease dynamics—together by integrating individual behaviours and interaction patterns between exposed and healthy group members into epidemiological modelling. Although behavioural responses to pathogen presence are assumed to have crucial effects on disease dynamics in all societies, most complex societies cannot easily be manipulated experimentally, making such studies very rare [1,27]. Insects societies, however, are very suitable for investigating individual behaviour and disease dynamics, as they can be kept in small to intermediately sized groups in controlled laboratory settings, enabling behavioural observation of all individuals, while allowing experimental manipulation of particular parameters [28,29]. Despite obvious differences to other societies (e.g. reproductive division of labour), they may still provide us important insights into how general factors affect disease spread within a society. Thus, recently, a few studies have developed epidemiological models to investigate how disease transmission in insect societies can be affected by (i) social organization and interaction networks [17], (ii) mobility and density of infectious individuals and nest architecture [30], (iii) individual and social immunity and nest hygiene [31] and (iv) immunization of nest-mates through social contact to exposed individuals [32]. However, these studies did not base their models on experimentally observed interaction rates under pathogen presence.

Using societies of the invasive garden ant, *Lasius neglectus*, we determined the behavioural changes of individuals exposed to the fungal pathogen *Metarhizium*, and their healthy nest-mates. We focused on individually and mutually expressed sanitary behaviours, that is, self- and allogrooming, given that previous work revealed these to be the most likely routes to propagate—but also contain—the fungal pathogen in this well-established host–pathogen study system [32]. Our experiments provide a social interaction network based on grooming behaviours, and, by taking into account the direction of these behaviours between partners, we could create a directed grooming network.

We also developed an epidemiological model to determine whether the observed grooming behaviours are adaptive, i.e. that they lead to a predicted decrease in disease spread within the colony, and also to disentangle the effects of changing allogrooming direction. The parameters used in the model were derived from the experimentally obtained grooming and mortality data from our particular host–pathogen system. In a second step, we applied parameter settings to study a wider range of host–pathogen systems, with either higher pathogen transmissibility/infectiousness, or more efficient social host defences. To this end, we simulated the spread of disease depending on different grooming rates and different probabilities of grooming leading to either (i) healing of the exposed individual, (ii) infection of the previously healthy nest-mates, or a combination of both. Moreover, by developing a deterministic, large-scale model, we were able to extrapolate our findings from a small-scale experimental set-up to larger, more natural society sizes.

2. Material and methods

(a) Experiment

We studied changes in behaviour and survival of the invasive garden ant, *Lasius neglectus*, after exposure to the entomopathogenic fungus *Metarhizium brunneum* (strain Ma275, KVL 03-143, formerly named *M. anisopliae*, but now recognized as a sister species [33]). Twenty-four nests of *L. neglectus* were collected from four populations (Jena, Germany; Volterra, Italy; Bellaterra and Seva, Spain; six nests per population; as detailed in [25]). From each nest, we set up two subnests (in individual Petri dishes of Ø 9 cm and a 2 × 1 cm brood chamber indentation in the plaster ground), each containing three larvae and six individually colour-marked workers to allow scoring of individual behaviour and survival throughout the experiment. The two subnests were randomly assigned to either a 'sham control' treatment, where one worker received 0.03 µl of a control solution (0.05% Triton X), or a 'fungus exposure' treatment, where the treated worker received the same amount of a fungal conidiospore suspension (freshly harvested Ma275 conidiospores from 6.5% sabaroud dextrose agar plates grown at 23°C with 99% germination rate, at concentration 1×10^9 ml^{-1} in 0.05% Triton X; as detailed in [25]), resulting in 24 replicates each of the sham control and fungus exposure treatment. The five nest-mates ($n = 120$ for each treatment, total $n = 240$) remained untreated (figure 1*a*). We performed behavioural observations in the first 5 days after treatment, and followed the mortality of ants over 12 days. The experimental data were obtained by (i) reanalysing an already published dataset [25], as detailed below and (ii) using additional data from the same experiment (L.V.U. and S.C. 2006, unpublished data). For additional details, please see Ugelvig & Cremer [25].

(i) Self- and allogrooming rates

Individual (self-) and social (allo-) grooming behaviours were recorded during observational scan sampling (five scan samples

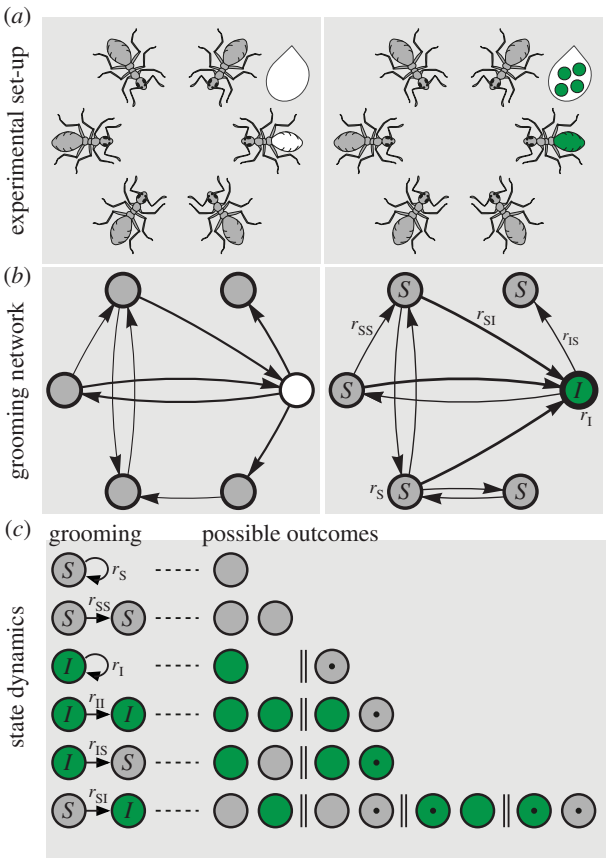

Figure 1. Experimental set-up, observed grooming networks and model state dynamics. (*a*) The experimental set-up comprised nests of five untreated ants (grey), plus one ant treated with either a sham control solution (white) or fungal conidiospore suspension (green). (*b*) An exemplified grooming network, constructed for each treatment group, indicates the average rates of grooming behaviours and maximum number of interacting ants per replicate. Ants are illustrated as nodes (same colour scheme as in panel *a*), with untreated ants denoted susceptible, *S*, and treated ants infectious, *I*. Grooming rates are split into individual self- (r_S and r_I) and direction-resolved allogrooming (r_{SS}, r_{IS} and r_{SI}) and indicated by the line weight of circle perimeters and arrows, respectively. See figure 2 for significance of up- and downregulation of grooming rates after pathogen exposure compared with the sham control. (*c*) Ants in the SIS model have one of two states: susceptible (*S*, grey) or infectious (*I*, green). State changes (black dots inside circles) from $S \rightarrow I$ and $I \rightarrow S$ can occur via self-grooming (circular arrows) or via allogrooming during social contact (straight arrows), with state change propensities depending on the observed grooming rates and the infection and healing probabilities (α and β) as detailed in the model. Experimental colonies initially only contained a single *I* individual, but disease contraction may increase this number over time, resulting in allogrooming rates among infectious individuals (r_{II}) being integrated into the model, whereas interactions involving no possibility of state change (r_S and r_{SS}) are not considered.

per day on each of the 5 days post-treatment; duration of each scan was one to several seconds; time in-between scans $\geq$ 20–30 min to assure independent behavioural recordings as individual grooming bouts rarely exceed 1 min). Grooming rates were calculated as proportion of total observations during which the particular grooming behaviour occurred. In these experiments, 22% (66/288) of the ants could not be observed for the full 25 scans, owing to mortality before day five, as well as 2% of the ants escaping [25], leading to a mean of 23.3 ($\pm$0.8 s.e.m.) scans per individual ant. In contrast to [25], we here calculated directed allogrooming rates, separated by the direction in which they were performed, i.e. both from the treated individual towards its nest-mates, and from the nest-mates towards the treated individual (figure 1*b*).

In accordance with the model (see §2.2), treated ants are termed infectious (*I*), and their nest-mates susceptible (*S*) individuals (figure 1*b*). For the sake of simplicity, we keep this terminology equal for the fungus exposure and the sham control group, but indicate clearly which experimental group we refer to. In both groups, we experimentally determined the self-grooming rates of treated workers (r_I) and their nest-mates (r_S), and the allogrooming rates among untreated nest-mates (r_{SS}), from treated workers to their nest-mates (r_{IS}), and from nest-mates to the treated workers (r_{SI}; figure 1*b*).

(ii) Mortality of ants

To determine the mortality of ants, we monitored daily the survival of fungus-exposed *I* workers over 12 days following exposure that were either reared in isolation (1*I* alone; $n = 82$), or together with five untreated nest-mates (1*I* + 5*S*; $n = 24$). All dead ants were surface-sterilized [34], kept under humid conditions at 23°C for three weeks, and checked for fungal outgrowth [25]. Only ants that died from a confirmed *Metarhizium* infection were included in the calculations of mortality rate.

(iii) Data analysis

To visualize the outcome of our behavioural observations, we exemplify an artificial average grooming network for both the sham control and the fungus exposure treatment. For each type of grooming, we (i) determined the maximum number of individuals interacting within any replicate, and took this as the number of arrows to be displayed, and (ii) calculated the mean grooming rates across replicates, and displayed this as the relative weight of circle perimeters (for self-grooming) and arrows (for allogrooming). Note that self-grooming occurs at much higher rates than allogrooming, for which reason depicted self-grooming rates were divided by five to be in the same order as allogrooming rates, yet directly comparable between treatments (figure 1*b*).

Grooming rates were calculated from the observational data as grooming events per observation, thus yielding a single value per replicate for both the treated ants and their nest-mates ($n = 24$ each for both sham control and fungus exposure). The latter was done by calculating the mean for the five untreated nest-mate ants per replicate, thereby preventing pseudoreplication. We found no significant effect of population on grooming rates, neither for the sham control (Kruskal–Wallis test, $\chi^2 = 2.750$, d.f. = 3, $p = 0.432$), nor the fungus exposure treatment ($\chi^2 = 3.267$, d.f. = 3, $p = 0.352$). For this reason, we did not take population into account in the later statistical analyses. We also found no significant effect of time when separating the observed self- and directed allogrooming events into the five observation days (electronic supplementary material, figure S1), neither for the sham control (Kruskal–Wallis tests, all d.f. = 4; self-grooming of treated individuals: $\chi^2 = 2.024$, $p = 0.731$, and nest-mates: $\chi^2 = 1.532$, $p = 0.821$; allogrooming by treated individual: $\chi^2 = 1.672$, $p = 0.796$, and nest-mates: $\chi^2 = 6.659$, $p = 0.155$) nor for the fungus exposure treatment (self-grooming of treated individuals: $\chi^2 = 7.178$, $p = 0.127$ and nest-mates: $\chi^2 = 3.394$, $p = 0.494$; allogrooming by treated individual: $\chi^2 = 7.613$, $p = 0.107$ and nest-mates: $\chi^2 = 3.878$, $p = 0.423$). We therefore calculated a constant grooming rate (grooming events/observations) for each grooming behaviour.

To test whether presence of a fungal pathogen affected self- (r_S, r_I) or allogrooming (r_{SS}, r_{IS} and r_{SI}) rates, we standardized grooming rates from the fungus exposure group by subtracting the average of the respective grooming rates from the sham control (figure 2). We then applied *t*-tests to compare the

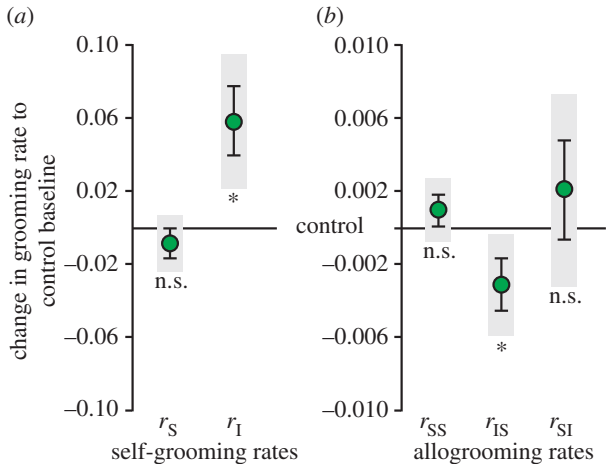

Figure 2. Observed pathogen-induced grooming rate changes. Change in observed (*a*) self-grooming and (*b*) directional allogrooming rates (grooming events per observations) after fungal exposure (green) compared with the sham control treatment (zero baseline). Fungus-exposed individuals significantly increased self-grooming (r_I) and decreased allogrooming of their nest-mates (r_{IS}), compared with individuals treated with the sham control solution. Plots represent mean $\pm$ s.e.m. and 95% CIs (grey shading), $n = 24$; asterisks indicate a significant difference from the sham control baseline at the 0.05 level; n.s., non-significant. Note that the variation in the rates r_S and r_{SS} across replicates appears smaller than rates involving the treated individual, as these are based on the means of the five nest-mates per replicate.

standardized grooming rates to the sham control baseline (=0), after having normalized the data by $\sqrt{(X+1)}$ transformation (in the case of r_S, r_I), or confirmed robustness of the results, despite deviations from normality, using a sign test (in the case of r_{SS}, r_{IS} and r_{SI}). Note that the variation in the rates r_S and r_{SS} across replicates appears smaller than rates involving the treated individual, as these are based on the mean of the five nest-mates per replicate. All statistical analyses were performed in R v. 3.1.2 [35].

(b) Model

We modelled disease spread within ant colonies using a compartmentalized SIS model, in which ants were categorized into two different states: ants susceptible to an infection are denoted by S, and ants exposed to the fungal pathogen, meaning that they are infectious to others and can die of the infection, are denoted by I (figure 1*b*). We use a basic model similar to Konrad *et al.* [32], but here focus on the contraction of disease, i.e. neither addressing the effect of immunization (as in [32]) nor the effect of pathogen spread *per se* (as in [36]). We thereby model disease spread in the colony as the fraction of infectious versus susceptible ants, which represents the final outcome of the pathogen interacting with the combined individual and collective, behavioural and physiological anti-pathogen defences of the hosts. Importantly, we here extend the model by including (i) the effect of self-grooming and (ii) the direction of allogrooming on the propensity of state changes, allowing the allogrooming rates (i.e. grooming events per time) from $I \to S$ and $S \to I$ to differ from each other. We derive the propensities of state changes ($I \to S$ or $S \to I$) in an ant colony after fungal exposure of a treated ant, either by self-grooming alone, or during social interactions between infectious and susceptible individuals (as shown in figure 1*c*). These propensities depend on both the grooming rates and the probabilities of state change per time spent grooming.

We include the self-grooming rate r_I of I individuals, the allogrooming rates r_{IS} (performed by I towards S) and r_{SI} (performed by S towards I), as well as the experimentally undetermined allogrooming rate between infectious workers (r_{II}). The latter rate applies after cross-infection has changed the state of a second or third individual in the colony to I, and is thus relevant for longer-term simulation of disease dynamics. Note that only interactions relevant for disease transmission are considered, whereas self-grooming of susceptible ants or interactions between two susceptible ants (at rates r_S and r_{SS}, respectively) are not included, as such behaviours cannot lead to state changes by either infection or healing (figure 1*c*).

The probabilities of state changes are defined as the 'infection probability' α (change from S to I) and 'healing probability' β (change from I to S). To keep the model general, we allow for healing probability β to be potentially different if grooming is performed by the individuals themselves or by their nest-mates, by including a separate healing probability by self-grooming β_s and by allogrooming β_a. As an example, when an S and I individual meet, the propensity for the infectious individual I to change state to S through allogrooming from the susceptible individual, without the latter getting infected ($(S,I) \to (S,S)$), is thus the product of the allogrooming rate from S to I, r_{SI}, the abundance of susceptible and infectious individuals present, $S{\cdot}I$, the healing probability by allogrooming β_a, and the probability that the grooming, susceptible ant does not get infected during the grooming process, which is $1 - \alpha$.

Using $r'_{ij} := r_{ij} \cdot N$ to account for the total number of individuals (N) in the colony (only relevant for self-grooming), we arrive at the following state transitions:

state change	propensity	description
$I \to S$	$r_I \beta_S I$	I gets healed via self-grooming
$(I,I) \to (I,S)$	$r'_{II} \beta_a I(I-1)/N$	I grooms I and heals it
$(I,S) \to (I,I)$	$r'_{IS} \alpha IS/N$	I grooms S and infects it
$(S,I) \to (S,S)$	$r'_{SI}(1-\alpha)\beta_a SI/N$	S grooms I and heals it
$(S,I) \to (I,S)$	$r'_{SI} \alpha \beta_a SI/N$	S grooms I and heals it, but gets infected
$(S,I) \to (I,I)$	$r'_{SI} \alpha(1-\beta_a)SI/N$	S grooms I and gets infected

(i) Stochastic small-scale model

We developed a stochastic model, exactly matching our experimental set-up of small colonies of six ants (starting with a single I and five S individuals) to infer the infection probability α, and healing probability by social immunity β_a, for our host–pathogen system.

Infection probability α. Any initially susceptible ant S has the propensity p_1 to become infected

$$p_1 := r'_{SI} \alpha \cdot \frac{I}{N} + r'_{IS} \alpha \cdot \frac{I}{N} = (r'_{SI} + r'_{IS}) \alpha \cdot \frac{I}{N} \qquad (2.1)$$

i.e. either during grooming of an infectious ant at rate r_{SI}, or by being groomed by an infectious ant at rate r_{IS}, assuming that grooming is the only source of infection in the ant colony. As conidiospores of *Metarhizium* attach strongly to the cuticle of their hosts within 48 h, and are only transferable before that [37,38], we set the time window for infection to 48 h. We further assume no 'secondary healing effect' for ants becoming infected (i.e. no $S \to I$ can revert back to S) in this period, so that the fraction of infectious ants is $1 - \exp(-p_1 \cdot 48\text{ h})$ following the theory

of Poisson processes. Susceptible nest-mates becoming infectious were estimated from nest-mates dying of the fungus. Studies show that susceptible ants—as well as termites—rarely contract the disease and die after contact with *Metarhizium*-exposed nest-mates [21,32,39,40]. For *L. neglectus*, this fraction was as low as 2% (3/150 [32]) at the present pathogen dose, which is equivalent to a lethal dose of approximately 60% when applied to *L. neglectus* ants in the absence of social interactions ($\text{mortality}_{\text{isolated ants}}$). We thus find $\text{mortality}_{\text{isolated ants}} \cdot (1 - \exp(-p_1 \cdot 48\text{ h})) = 0.02$, which gives us p_1 and allows us to solve for α in equation (2.1).

Healing probability β. Any initially infectious ant I has the propensity p_2 to become susceptible with

$$p_2 := r_I\beta_S + \frac{r'_{SI}\beta_a S}{N}, \tag{2.2}$$

either by self-grooming at rate r_I, or by being groomed by another ant. As our experimental groups consisted of only one infectious ant I with five susceptible nest-mates S, we need to consider this to happen at rate r_{SI} in first-order approximation. As fungal conidiospores can only be removed within the first 48 h, we again consider this to be the relevant time window for our estimation. Thus, we assume a fraction of $\exp(-p_2 \cdot 48\text{ h})$ individuals still remaining infectious after 48 h.

(ii) Deterministic large-scale model

To generalize to larger colony sizes than those in our experimental set-up, we approximated the small-scale model by a large-scale model based on differential equations, which allowed us to determine an analytical condition for the disease to go extinct. We approximate the discrete number of ants by continuous values S and I, between 0 and the total number of ants, now assuming (i) a large number of ants in the colony that (ii) interact randomly according to grooming rates (specified in figure 1*b*). Natural colony sizes of ants vary from dozens to several millions [41], with invasive supercolonies of *L. neglectus* being in the high end of the range [42]; hence, the first assumption is valid for our system and across other species of ants. The assumption of free mixing is a simplification when compared with natural conditions, where spatial and behavioural colony structures imply some colony compartmentalization [29]. However, such structure is predicted to be reduced in invasive ants such as *L. neglectus*, which are characterized by their open colony structure allowing individuals to mix freely [43–45].

Taking the limit $\Delta t \to 0$ and integrating all state changes, we arrive at the following stochastic differential equation for the number of infectious ants:

$$\mathrm{d}I = \mu(I)\mathrm{d}t + \sigma(I)\mathrm{d}W,$$

with Wiener process W. Here, the drift term $\mu(I)$ summarizes the deterministic part of $+1$ and -1 jumps from the Markov jump process describing changes in I:

$$\mu(I) = -r_I\beta_s I - \frac{r'_{II}\beta_a I(I-1)}{N} + \frac{r'_{IS}\alpha I(N-I)}{N} - \frac{r'_{SI}(1-\alpha)\beta_a I(N-I)}{N} + \frac{r'_{SI}\alpha(1-\beta_a)I(N-I)}{N}.$$

Moreover, we have replaced the number of susceptible ants S by $N - I$, where N denotes the total number of ants. The noise part is modelled by the diffusion term $\sigma(I)$, which is equal to the rooted correlation thereof, so

$$\sigma(I)^2 = r_I\beta_s I + \frac{r'_{II}\beta_a I(I-1)}{N} + \frac{r'_{IS}\alpha I(N-I)}{N} + \frac{r'_{SI}(1-\alpha)\beta_a I(N-I)}{N} + \frac{r'_{SI}\alpha(1-\beta_a)I(N-I)}{N}.$$

Technically, we have approximated the discrete model of a Markov jump process by a diffusion process. To take the limit of $N \to \infty$, we replace the actual number of infectious ants I by the continuous percentage $i := I/N$ of the total number of ants ($i \in [0,1]$). This again is a diffusion, which can be determined from above by dividing by N to satisfy

$$\mathrm{d}i = \mu(i)\mathrm{d}t + \frac{1}{\sqrt{N}}\sigma(i)\mathrm{d}W,$$

with drift

$$\mu(i) = -r_I\beta_s i - r'_{II}\beta_a i^2 + r'_{IS}\alpha i(1-i) - r'_{SI}(1-\alpha)\beta_a i(1-i) + r'_{SI}\alpha(1-\beta_a)i(1-i),$$

and squared diffusion

$$\sigma(i)^2 = r_I\beta_s i + r'_{II}\beta_a i^2 + r'_{IS}\alpha i(1-i) + r'_{SI}(1-\alpha)\beta_a i(1-i) + r'_{SI}\alpha(1-\beta_a)i(1-i)$$

where we have neglected terms in $1/N \approx 0$.

For sufficiently large N, we can neglect the diffusion term because it scales with $1/\sqrt{N}$ (electronic supplementary material, figure S2). Thus, to understand the disease dynamics for sufficiently large N, it suffices to study the deterministic part of the stochastic differential equation. It is given by the ordinary differential equation (ODE)

$$\frac{\mathrm{d}i}{\mathrm{d}t} = (\gamma - r_I\beta_s)i - (\gamma + r'_{II}\beta_a)i^2$$

which describes the time evolution of the mean percentage i of infectious ants with

$$\gamma := r'_{IS}\alpha + r'_{SI}(\alpha - \beta_a)$$

For small i, the quadratic term in the former equation can be neglected, and the condition for the disease to go extinct is simply $\gamma \le r_I\beta_s$, that is,

$$r'_{IS}\alpha + r'_{SI}(\alpha - \beta_a) \le r_I\beta_s.$$

Moreover, for small i, the number of infectious individuals behaves according to $\mathrm{d}i/\mathrm{d}t = (\gamma - r_I\beta_s)i + O(i^2)$, in other words $i(t) \approx C\exp((\gamma - r_I\beta_s)t)$. This implies that if the disease goes extinct, the speed of the process is governed by degradation with rate $r_I\beta_s - r'_{IS}\alpha - r'_{SI}(\alpha - \beta_a)$.

We use this model to derive the condition where the disease will go extinct and to perform a sensitivity analysis for r_I, r_{IS} and r_{SI}, by simulating the range of α/β_a from highly infectious pathogens ($\alpha > \beta_a$), to systems where the social immunity of the host is highly efficient ($\alpha < \beta_a$).

3. Results and discussion

(a) Grooming rates

(i) Self-grooming

We found no significant change in the self-grooming rates of nest-mates of fungus-exposed ants when compared with the sham control baseline (r_S: t-test; $t_{23} = -1.321$, $p = 0.200$). However, self-grooming of the fungus-exposed ants themselves was significantly increased compared with the sham control (r_I: $t_{23} = -3.007$, $p = 0.006$; figure 2*a*). The latter is in line with other studies generally finding that ants increase self-grooming in response to the exposure of external pathogens like entomopathogenic fungi [24,46–49] (but see [50]). Interestingly, elevated self-grooming is not found in termites upon fungal exposure [21,51,52], perhaps owing to less flexibility caused by morphological constraints (absence of a wasp waist) [21]. Self-grooming functions to remove infectious agents from the body surface, and elevated self-grooming by

healthy nest-mates after contact with an exposed individual might thus be expected, but has not been found [38].

(ii) Allogrooming

In our experiment, fungus exposure of one ant did not lead to significant changes in allogrooming rates among its nest-mates compared with the sham control baseline (r_{SS}: $t_{23} = 0.998$, $p = 0.329$). The fungus-exposed ant, on the other hand, significantly reduced its grooming activity towards its nest-mates (r_{IS}: $t_{23} = -2.453$, $p = 0.022$), whereas allogrooming performed by the nest-mates towards the fungus-exposed ant was not significantly changed compared with the sham control baseline (r_{SI}: $t_{23} = 0.647$, $p = 0.524$; figure 2*b*). Studies of allogrooming behaviour in social insects have typically focused on the positive effect it has on the survival of pathogen-exposed individuals, and therefore tend to only report the allogrooming those particular individuals receive. Termites seem to consistently upregulate allogrooming towards fungus-exposed individuals [21,53,54], and it appears that allogrooming is much more important in reducing pathogen load, and thus infection risk, when compared with self-grooming [51,52]. This is less clear in ants, where some studies report increased allogrooming of infectious individuals by their nest-mates [24,38,40,47,49], and others do not [48,50]. When elevated responses are reported they often occur immediately after exposure, although responses at later stages where the fungus is no longer infectious have also been found [38], along with upregulation of allogrooming towards individuals injected with internal immune elicitors [55]. Interestingly, but not surprisingly, the latter has not been shown to elevate self-grooming rates [55].

To the best of our knowledge, only one other study experimentally determined allogrooming rates from fungus-exposed individuals towards their nest-mates, although this route is also important for spread of the pathogen in the colony. Similar to our study, Bos *et al.* [24] reported a reduction—in their study non-significant—of allogrooming by infectious individuals to their nest-mates, suggesting 'self-removal' of the infectious individual [24,25,56] (but see [57] for disease-independent self-removal). Moreover, the fact that nest-mates did not change their mutual allogrooming rates in our experiment indicates that there is no upregulation of allogrooming among untreated colony members when co-inhabiting the nest with an exposed individual. Yet, previous contact with infectious individuals has been reported to increase allogrooming responses towards incoming nest-mates, independent of their health status [38,48].

(b) Infection and healing probabilities

We employ the stochastic small-scale model to determine the ratio of the infection and healing probability by social immunity α/β_a, for the studied *Lasius–Metarhizium* system based on the mortality rate obtained in the experiment. To this end, we use the experimentally derived mortality rates to first determine the infection and healing propensities p_1 and p_2 (defined in Material and methods), and use equations (2.1) and (2.2) to solve for the infection and healing probabilities α and β_a.

(i) Infection probability α

We found the mortality of fungus-exposed I ants dying from a *Metarhizium* infection when reared in isolation to be 57% (47/82). We can therefore solve for the infection propensity p_1 via $0.57 \cdot (1 - \exp(-p_1 \cdot 48\text{ h})) = 0.02$, and infer the infection probability α from equation (2.1) (see Material and methods for derivation):

$$\alpha = p_1 \frac{N}{(r'_{SI} + r'_{IS}) \cdot I} = \frac{-\log(1 - 0.02/0.57)}{48\text{ h}} \cdot \frac{N}{(r'_{SI} + r'_{IS}) \cdot I}$$
$$= 0.046 \pm 0.027\ \text{h}^{-1}\ (\text{mean} \pm \text{s.d.})$$

(ii) Healing probability β

Metarhizium-induced mortality of fungus-exposed I ants in groups with five susceptible nest-mates was 42% (10/24) in our experiment. Hence, we can relate the fraction of ants remaining infectious after 48 h and the mortality of infectious isolated ants via $0.57 \cdot \exp(-p_2 \cdot 48\text{ h}) = 0.42$. Assuming an equal healing probability for self- and allogrooming, $\beta_s = \beta_a$, we find the healing probability by social immunity, β_a, using equation (2.2) to be (see Material and methods for derivation):

$$\beta_a = \frac{p_2}{r_I + r'_{SI} S/N} = 0.044 \pm 0.031\ \text{h}^{-1} (\text{mean} \pm \text{s.d.})$$

In our experimental system, the infection probability α and the healing probability by social immunity β_a—both characterized by considerable variation—are not significantly different from each other (distribution test; β_a being from outside the distribution of α, $p = 0.423$; α being from outside the distribution of β_a, $p = 0.579$; for details, see electronic supplementary material, figure S3). This renders the infection/healing ratio (α/β_a) equal to 1.045, not significantly different from 1.0.

To test whether the observed changes in the grooming network (figures 1*b* and 2) represent an adaptive behavioural modulation in response to fungal exposure of the treated ant, we used our epidemiological model to reveal how disease dynamics in the colony change as a function of the observed grooming rates and infection and healing probabilities.

(c) Condition for the disease to go extinct

We used our large-scale deterministic model to determine the condition for the disease to go extinct to be (see Material and methods for derivation)

$$r'_{IS}\alpha + r'_{SI}(\alpha - \beta_a) \leq r_I \beta_s.$$

It thus follows that the infection will go extinct faster if (i) the self-grooming efficiency, β_s, or the self-grooming rate of the infectious individual, r_I, increases, and (ii) the infection probability α or the rate whereby the infectious ants groom the susceptibles, r_{IS}, decreases. For the grooming of susceptibles towards infectious ants, r_{SI}, we find that the results depend on the relative effect of pathogen infectiousness and efficiency of social immunity. Increased r_{SI} is harmful (disease goes extinct slower) for host–pathogen systems with high pathogen infectiousness ($\alpha > \beta_a$), and beneficial (disease goes extinct faster) for systems in which social host defences are efficient against the pathogen ($\alpha < \beta_a$). Note that the condition for the disease to go extinct is independent of the self-grooming of susceptible nest-mates, r_S. A change in self-grooming of susceptible nest-mates living with a fungus-exposed individual is therefore not predicted, which is consistent with our experimental observations (figure 2*a*) and published work [38].

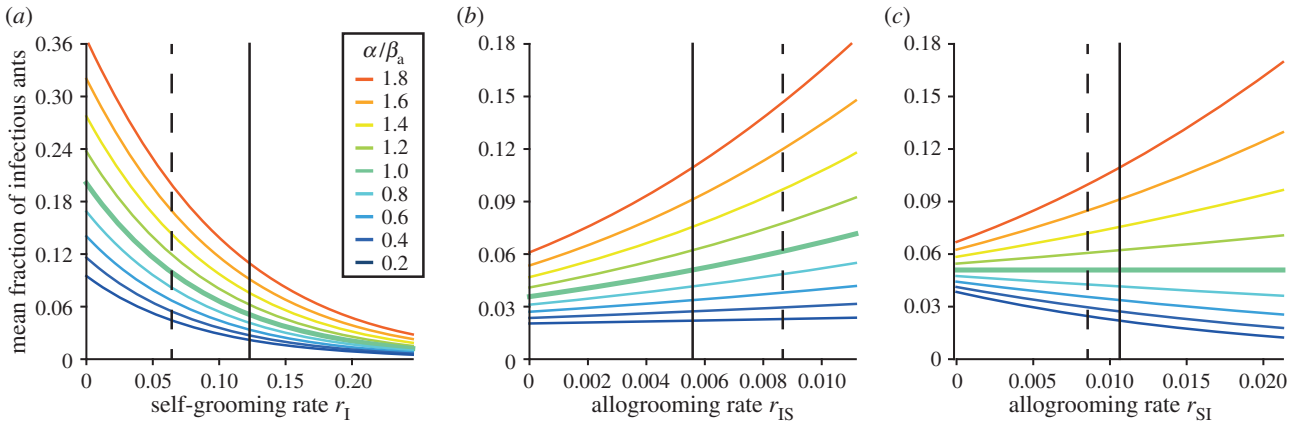

Figure 3. Predicted effects of varying grooming rates and infection versus healing probabilities on disease spread. Simulation results based on the epidemiological SIS model for the mean fraction of infectious ants as a function of grooming rates (*a*) r_I, (*b*) r_{IS} and (*c*) r_{SI} with varying infection probability α and healing probability via allogrooming β_a. The experimentally obtained α/β_a ratio of 1.045—approximated by the 1.0 line—is highlighted (thick green line), and the observed mean grooming rates for the fungus exposure (solid vertical line) and sham control (dashed vertical line) groups are shown in the simulation plots.

In order to understand how the modulation of grooming rates and infection/healing probabilities affect disease dynamics, we carried out a sensitivity analysis and relate this to our experimental results.

(d) Simulation of the mean fraction of infectious ants

Using the experimentally determined rates and estimated probabilities in the range of estimated values, we simulated the mean fraction of infectious ants per colony in the SIS model using the initial condition of one infectious I and five susceptible S ants. To generalize to other host–pathogen systems with different infection/healing probability ratios (figure 3), we varied grooming rates r_I, r_{IS} and r_{SI} and infection probabilities for α/β_a ratios ranging between 1.8 and 0.2, where (i) values more than 1 represent high pathogen infectiousness ($\alpha > \beta_a$), (ii) 1 denotes the case of equal probabilities of infection and healing ($\alpha = \beta_a$) and (iii) values less than 1 characterize efficient social immunity ($\alpha < \beta_a$). If the observed shifts in transmission-relevant grooming rates after fungal exposure in our experiment (figure 2) are adaptive, they should relate to model predictions with a lower mean fraction of infectious ants in the colony.

We observed a significant increase in the self-grooming rate of the treated ant r_I, as a result of fungus exposure (figure 2*a*). Our model simulation predicts this to decrease the fraction of infectious ants in the colony, both in our ant–fungus system ($\alpha/\beta_a = 1.045$) but also generally in other host–pathogen systems, independent of whether $\alpha > \beta_a$ or $\alpha < \beta_a$ (figure 3*a*). The observed upregulation of self-grooming in fungus-exposed individuals might therefore be an adaptive behaviour to reduce disease spread in many host–pathogen systems.

For the allogrooming between infectious and susceptible group members, we first consider allogrooming directed from the infectious ants to its susceptible nest-mates, r_{IS}. The infectious ants showed a significant decrease of grooming towards their nest-mates (figure 2*b*), which is predicted to lower the number of infectious colony members. Again, this is generally true for all ratios of α/β_a, i.e. including that of our system (figure 3*b*). The observation that infectious individuals decreased allogrooming of their nest-mates does not seem to represent a trade-off caused by their increased self-grooming. Fungus-exposed individuals self-groomed approximately 12.3% of their total time observed (compared with 6.4% in the sham control), whereas allogrooming performance changed from 4.3% in the sham control to 2.8% of total observed time after fungus exposure. Overall, fungus-exposed ants showed a non-significant trend to increase their overall grooming (Wilcoxon test, $W = 217$, $p = 0.143$; electronic supplementary material, figure S1). We therefore conclude that the observed reduction in r_{IS} is not caused by time-limitation, but rather represents an adaptive behavioural change, resulting in lower disease spread in the colony.

Allogrooming in the opposite direction, that is, performed by the susceptible towards the treated ants, r_{SI}, was not significantly altered after fungal exposure (figure 2*b*). Such absence of an observed alteration of r_{SI} fits model predictions at $\alpha/\beta_a = 1.045$ (approximated by the 1.0 line, figure 3*c*), as the slope of the curve is zero, and thus behavioural changes would not result in a change of the mean fraction of infectious colony members. The case of allogrooming from susceptible to infectious individuals is particularly interesting, as the model shows that increased allogrooming in the direction from S to I could have both beneficial and detrimental effects on the society.

Only in host–pathogen systems where social immunity is highly efficient against a particular pathogen ($\alpha < \beta_a$) can intensified grooming of infectious individuals by their nest-mates lead to an overall decrease in the fraction of infectious ants in the colony. Contrarily, in all systems where pathogen infectiousness is high ($\alpha > \beta_a$), such intensified grooming would promote disease spread within the colony, as susceptible individuals have a high risk of contracting the disease whilst taking care of their infectious nest-mates (figure 3*c*).

(e) General and comparative perspectives

Owing to its dual role in containing and transmitting eventual diseases, allogrooming is caught in a trade-off between protection and exposure. This dichotomous effect also became apparent in a model developed by Fefferman *et al.* [31] to understand disease dynamics in a termite–fungus system. They found allogrooming to be beneficial for colony survival when pathogen exposure was periodic, as it controlled exposure risk, but costly under constant pathogen exposure, where it rather promoted pathogen transmission throughout the colony [31]. Directed grooming has also been found important for disease dynamics in non-insect

societies, e.g. in groups of meerkats and brown spider monkeys, where transmission of tuberculosis and gastrointestinal helminths, respectively, was correlated with grooming interactions, with individuals performing grooming being at higher risk of infection than individuals receiving grooming [19,58]. Interestingly, these examples point to grooming as an important mechanism of transmission of also internal pathogens, in addition to external ones like the infectious stages of fungi [19,32,58–60]. Despite these potential costs of social allogrooming, the behaviour is widespread among both vertebrate and invertebrate animal groups, and there is compelling evidence that it is beneficial in reducing pathogen loads [21,40,61–64].

The described dichotomous effect of allogrooming may also explain the diversity of outcomes described in empirical studies of ants, including both increased or unchanged allogrooming from nest-mates towards pathogen-exposed individuals [24,38,40,47–50]. Such diversity in allogrooming is expected owing to the diversity of host–pathogen systems studied, as both the infectiousness of pathogens and the expressed collective defences may reach a different balance in each system. This can be due to species- or strain-derived differences in pathogenicity [54,65,66], dosage dependence of infection risk after exposure [21,40], or different expression or efficiency of host sanitary actions [51,52,54,66]. Note that a reduction in allogrooming from nest-mates to exposed individuals has not yet been reported in any previous study. Assuming that this is not an effect of publication bias, it might be due to the fact that ant societies either rarely face highly infectious pathogens or that their social defences, particularly allogrooming, are extremely efficient in reducing disease spread in the colony. The latter is supported by the vast literature on social immunity (reviewed in [2,67,68]) and the fact that allogrooming is largely universal among eusocial insects (ants [5,24,25,32,38,40,47–50]; bees [67–70]; wasps [71]; termites [21,51,52,54,72]) and in other complex societies (e.g. primates and meerkats [10,19]).

4. Conclusion

In an experimental ant–fungus system (*Lasius neglectus* ants and *Metarhizium brunneum* fungus), we observed significant changes in grooming patterns upon fungus exposure, as exposed ants (i) increased individual self-grooming rates, and (ii) reduced allogrooming of nest-mates. An epidemiological model revealed that these behavioural changes are predicted to reduce disease transmission within the colony. This prediction may also be valid in other host–pathogen systems with varying ratios of pathogen infectiousness and efficiency of collective host defences, and it can be generalized from our small experimental group sizes to larger, more natural colony sizes. In line with the obtained model predictions, most empirical studies on ants reported increased self-grooming upon pathogen exposure [24,38,40,47,49], while our empirically observed, and theoretically predicted, reduction in allogrooming from the exposed individual to its nest-mates awaits confirmation by other studies.

To more fully understand the evolution of allogrooming and its costs and benefits, it would be insightful to study directed allogrooming patterns in relation to the particular infection versus healing probabilities, found in different host–pathogen systems. However, an exact match of model predictions and observed behaviour would require that the insects can very accurately assess pathogen infectiousness and transmission in order for them to perform the optimal strategy. However, the expected continuous arms race between hosts and their (multiple) pathogens makes this trait hard to evolve, particularly because the cost of making a wrong assessment may potentially be very large (figure 3*c*). We may thus expect that selection should be weak on behaviours with potentially opposing effects on disease transmission (particularly if accurate assessment of the optimal strategy is low), but should strongly favour both a pathogen-induced increase in self-grooming and a reduction of allogrooming performed by infectious individuals to their healthy, susceptible nest-mates, as these are consistently beneficial for containing disease (i.e. independent of an exact assessment). Here, we may only expect deviations from theory if expression of such behaviours may be restricted or have little effect, as seems to be the case of self-grooming in termites [21,51,52]. Yet, testing these predictions is complicated by the fact that pathogen transmission not always reflects disease transmission. Some low levels of pathogen spread in societies can be beneficial, given that they can induce immune-stimulating low-level infections of previously healthy group members, via social immunization [32].

Data accessibility. All experimental datasets supporting this article have been made publicly available in the data repository Dryad: doi:10.5061/dryad.dj2bf.

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Authors' contributions. S.C. and F.J.T. conceived the study. The experiment was designed and performed by L.V.U. and S.C., and analysed by L.V.U. and C.M. The models were developed and parameters estimated by F.J.T. Figures were created by L.V.U. and F.J.T. The manuscript was written by S.C. and L.V.U. as principal writers with substantial contributions from F.J.T. and C.M. All authors approved the final version of the manuscript.

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