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LETTER

Social immunity modulates competition between coinfecting pathogens

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Abstract

Coinfections with multiple pathogens can result in complex within-host dynamics affecting virulence and transmission. While multiple infections are intensively studied in solitary hosts, it is so far unresolved how social host interactions interfere with pathogen competition, and if this depends on coinfection diversity. We studied how the collective disease defences of ants – their social immunity – influence pathogen competition in coinfections of same or different fungal pathogen species. Social immunity reduced virulence for all pathogen combinations, but interfered with spore production only in different-species coinfections. Here, it decreased overall pathogen sporulation success while increasing co-sporulation on individual cadavers and maintaining a higher pathogen diversity at the community level. Mathematical modelling revealed that host sanitary care alone can modulate competitive outcomes between pathogens, giving advantage to fast-germinating, thus less grooming-sensitive ones. Host social interactions can hence modulate infection dynamics in coinfected group members, thereby altering pathogen communities at the host level and population level.

Keywords

Argentine ants, grooming, host–pathogen interactions, immune-mediated competition, infectious disease, *Metarhizium* fungus, multiple infections, pathogen competition, pathogen diversity, social insects.

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INTRODUCTION

Infectious diseases are common and their pathology, epidemiology and evolution result from a complex interplay between hosts and pathogens. It is known that these within- and between-species interactions greatly affect host health and pathogen evolution (Frank 1996; Stein 2011; Alizon *et al.* 2013; Ferro *et al.* 2019; Papkou *et al.* 2019), yet their dynamics in often highly diverse ecological communities is far from understood (Pedersen & Fenton 2007; Johnson *et al.* 2015). This high pathogen diversity results in many host individuals being coinfected with several pathogen strains or species (Read & Taylor 2001; Balmer & Tanner 2011). Compared to single-pathogen infections, such coinfections often show altered virulence and transmission dynamics (Alizon *et al.* 2013; Bose *et al.* 2016; Tollenaere *et al.* 2016). Direct pathogen–pathogen interactions (Unterweger *et al.* 2014) and indirect competition via the host's immune system (Lysenko *et al.* 2005) or resource competition (Chouvenc *et al.* 2012; Wale *et al.* 2017) have been shown to affect the outcome of coinfections. The strength and type of interactions will depend on how similar the coinfecting pathogens are. Compared to coinfections with different pathogen species, strains of the same species are expected to compete less and may even cooperate (Buckling & Brockhurst 2008). Furthermore, the antigenic similarity between the pathogens (Cox 2001; Read & Taylor 2001) will dictate whether the host's immune response acts synergistically against the coinfecting pathogens, or will be less effective against one of them, introducing a host-mediated bias in pathogen competition (*immune-mediated competition*; Lysenko *et al.* 2005; Raberg *et al.* 2006; Ulrich & Schmid-Hempel 2012).

Research on multiple infections and the mechanisms of pathogen interactions within the host has been almost exclusively limited to solitary hosts, which need to cope with their infections on their own. In contrast, members of social groups often fight disease together by joint hygiene or sanitary care, performed as part of parental care or among adults. For example, primates groom each other to remove lice or ticks (Gomes *et al.* 2009), earwigs protect one another from fungal disease by grooming (Boos *et al.* 2014), and burying beetles preserve the mouse carcasses that serve as a long-term food resource for their offspring from decay by antimicrobials (Cotter & Kilner 2010). The social insects – social bees, wasps, ants and termites – have evolved a large arsenal of sophisticated disease defences that protect their colonies from disease (Schmid-Hempel 1998; Cremer *et al.* 2007; Wilson-Rich *et al.* 2009; Evans & Spivak 2010). This *social immunity* (Cremer *et al.* 2007; Cremer 2019b) is achieved by collective nest hygiene (Diez *et al.* 2012; Pull *et al.* 2018a), sanitary care (Rosengaus *et al.* 1998; Hughes *et al.* 2002; Davis *et al.* 2018), infection management (Pull *et al.* 2018b) and changes in the social interaction networks upon pathogen exposure (Stroeymeyt *et al.* 2018). Social insects thereby effectively interfere with the course of disease, reducing the risk of infection and further transmission.

How successful a pathogen is in infecting the host and producing transmission stages will thus strongly depend on the presence and type of coinfecting pathogens in the host, and whether and how the host interacts with conspecifics. Recent

theoretical and experimental research has made significant progress in understanding, on the one hand, some of the consequences of coinfections on virulence and pathogen epidemiology in individual hosts (Thomas *et al*. 2003; de Roode *et al*. 2005; Choisy & de Roode 2010; Cressler *et al*. 2014; Susi *et al*. 2015; Ramiro *et al*. 2016; Duncan *et al*. 2018); and on the other, how social immunity interferes with the course of infection in individual hosts and pathogen spread through the colony (Stroeymeyt *et al*. 2018; Pull *et al*. 2018b). However, how the social interactions between hosts could affect pathogen competition in infected individuals and how this may affect the overall pathogen community is largely unexplored.

We study ants as a model system to test for the effect of collective disease defences – their social immunity – on pathogen competition and success during coinfections. As pathogens, we use the entomopathogenic fungus *Metarhizium*, which is a generalist pathogen of arthropods, infecting ants and many solitary insects (Pull *et al*. 2013; Angelone & Bidochka 2018). *Metarhizium* fungi are obligate killers that actively penetrate the host's cuticle, kill the host and grow out millions of new infectious stages ('conidiospores', hereafter 'spores') (Gillespie *et al*. 2000). Transmission to new hosts occurs via direct contact to sporulating cadavers (Hughes *et al*. 2002) or via spores shed into the environment (Gillespie *et al*. 2000).

To reflect natural pathogen diversity (Keller *et al*. 2003), we coinfected individual ants with two fungal strains from the same pathogen community isolated from the field (Steinwender *et al*. 2014). Coinfections comprised either two strains of the same *Metarhizium* species, or of two different *Metarhizium* species. Each coinfection combination was tested for pathogen success in two different host social contexts: (1) individually exposed ants and (2) exposed ants grouped with nestmates. Exposed ants are known to remove infectious particles from their body surface by grooming, but are additionally groomed by their nestmates (allogrooming), which represents one of the most common sanitary care behaviours in social insects, effectively reducing the risk of disease after exposure (Reber *et al*. 2011; Theis *et al*. 2015).

Aim of our study was to test how pathogen coinfection diversity and host social context may interact to affect pathogen virulence, competition success and transmission potential. We determined virulence as the rate of host killing and assessed the outcome of within-host pathogen interactions and transmission potential of both coinfecting strains by their spore production. To this end, we determined both, the spore quantity and diversity per cadaver, and the resulting pathogen diversity at the community level.

METHODS

Experimental procedures

We used the Argentine ant, *Linepithema humile* (Mayr, 1868), as host and three strains each of two species of *Metarhizium* fungi (Bischoff *et al*. 2009), *M. robertsii* and *M. brunneum* as pathogens (Table S1).

Host killing rate and spore production. We exposed individual Argentine ant workers to a 50:50 mix of two pathogens, in all possible combinations of the six strains (Table S1), leading to either same-species co-exposures (*M. robertsii–M. robertsii*, R1-R2, R1-R3, R2-R3, and *M. brunneum–M. brunneum,* B1-B2, B1-B3, B2-B3), or different-species co-exposures (*M. robertsii–M. brunneum,* R1-B1, R1-B2, R1-B3, R2-B1, R2-B2, R2-B3, R3-B1, R3-B2, R3-B3). Ants were either kept individually (n = 30/combination) or in groups with two unexposed workers (n = 50/combination). In our experimental system, death typically occurs in 4–8 days. To assure complete sporulation (taking up to 5 days after death), spores from ants dying within 8 days after exposure were collected at day 13. Sporulating cadavers (individual: n = 134, group: n = 133) were used to determine mortality (Fig. 1a), spore number (Fig. 1b) and spore identity (Fig. 2). Spore packages were collected from the cadavers, their DNA extracted, quantified and converted to spore numbers using a standard curve. Strains were identified using microsatellite markers (Enkerli *et al*. 2005; Oulevey *et al*. 2009).

Spore removal by grooming. We performed a grooming experiment to determine (1) the efficiency of sanitary care in spore removal and (2) whether grooming biases pathogen species on the cuticle. We exposed workers to a 50 : 50 pathogen mix (R2-B3) as above and analysed the spore number per strain remaining on the ant after 2 h of grooming in the group (self- and allogrooming, n = 22), compared to individual ants (self-grooming only, n = 21), and to the applied dose measured immediately after exposure (n = 21; Fig. 3). For pathogen quantification via real-time PCR, we developed primers and probes for each species for the cuticle-degrading protease PR1 (St. Leger *et al*. 1992).

Pathogen germination speed. We determined the duration of spore germination by incubating all six strains (n = 14 replicates/strain) in liquid fungal growth medium in a microplate reader (Synergy H1^{TM} Hybrid Multi-Mode, BioTek) over 72 h with measurements of optical density every 20 min (modified from Milutinović *et al*. 2015). Time to germination was obtained as a breakpoint (in hours) at which the growth curve changes from the stationary to the exponential phase (Fig. 4).

Data analysis

All statistical analyses were performed in R version 3.5.1 (R Core Team 2018).

Host killing rate and spore production. We tested for the significance of our two main effects, pathogen 'coinfection diversity' (same species vs. different species) and host 'social context' (individual vs. group), as well as their interaction. We included 'pathogen combination' as random effect for all models (generalised linear mixed model with binomial error terms and logit-link function, GLMM: Figs 1a and 2a,b and linear mixed model, LMM: Fig. 1b), using the *lme4* package (Bates *et al*. 2015). For the analysis of strain-specific outgrowth (Fig. 2b), we further included 'experimental dish' in which the ants were kept as a random effect, and 'focus strain' (main effect) and its competitor as 'non-focus strain' (random effect) designated for each of the strains within a pathogen combination, to evaluate outgrowth success shifts within combinations and to infer a success hierarchy of the six strains (based on model intercepts, Table S2, Fig. S4). We

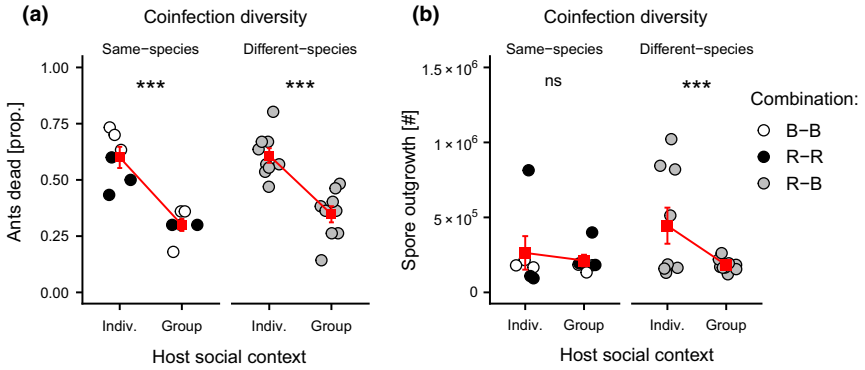

Figure 1 Effect of social immunity on virulence and transmission stage production depending on coinfection diversity. (a) Pathogen-induced host mortality was strongly affected by host social context, being reduced in the presence of nestmates performing sanitary care. This effect is independent of coinfection diversity (two strains from the same species of either *Metarhizium brunneum*, B-B [white], *Metarhizium robertsii*, R-R [black], or the two different pathogen species, R-B [grey]). (b) The number of outgrowing spores produced per cadaver was not affected by host social context for same-species coinfections yet was significantly reduced in grouped ants for different-species coinfections. Data points show means of each pathogen combination (see Figs S1 and S2 for details). Red squares depict the means per experimental group with standard error of the mean (SEM) as error bars. Benjamini–Hochberg corrected *P* values: *** denotes $P < 0.001$, n.s. denotes not significant.

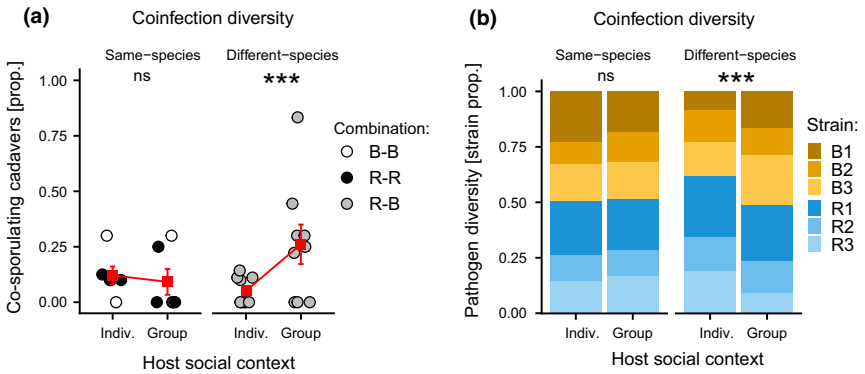

Figure 2 The effect of social immunity on cadaver- and community-level pathogen diversity. (a) The proportion of cadavers showing co-sporulation of both pathogens was increased in the group context compared to individual ants, yet only for coinfections with different pathogen species. Data points show means of each pathogen combination (see Figs S2 and S3 for detailed results per pathogen combination). Same colour code as in Fig. 1; red squares depict the means and error bars SEMs per experimental group. (b) The contribution of all six pathogen strains to the pathogen community (sporulation success of all cadavers) was affected by host social context, but only for different-species coinfections, where the group context introduced a greater balance between the two pathogen species. Strain proportions are calculated from the number of cadavers showing spore outgrowth of the respective strains (co-sporulating cadavers counted for both strains) and are depicted by individual colour (*Metarhizium brunneum* in shades of yellow, *Metarhizium robertsii* in shades of blue; see Figs S2 and S3 for details). Benjamini–Hochberg corrected *P* values: *** denotes $P < 0.001$, n.s. denotes not significant.

tested model assumptions (*influence.ME* package; Nieuwenhuis *et al.* 2012). As the untransformed data for spore number (Fig. 1b) did not meet model assumptions, we obtained normality by transforming the data (Box-Cox normalisation, *bestNormalize* package; Peterson 2017) (figure displays untransformed values). For all models, we first tested the significance of the overall model including the main effects and their interaction compared to a null model (intercept only) using likelihood ratio (LR) tests (Bolker *et al.* 2009). As all models were significant ($P \leq 0.003$), we tested the significance of the interaction. We then performed separate models for the same-species and different-species coinfections to test the effect of host social context, and corrected with the Benjamini–Hochberg procedure (Benjamini & Hochberg 1995) to protect against a false discovery rate (FDR) of 5%. We report corrected *P* values.

Spore removal by grooming. We compared the quantity of spores retrieved from the ants' body surface (1) directly after exposure (baseline) and after 2 h of (2) self-grooming (individual context) or (3) both self- and allogrooming (group context), by a Kruskal–Wallis test, followed by posthoc Benjamini–Hochberg corrected pairwise Mann–Whitney U tests (Fig. 3a; displaying proportion removal by grooming compared to the exposure dose). To test for a potential shift in the composition of pathogen species, we determined the proportion of *M. brunneum* compared to *M. robertsii* in all three groups and used a Kruskal–Wallis test (Fig. 3b) to test for differences between treatments.

Pathogen germination. Time to germination was determined by analysing strain growth curves using broken-line regression analysis and identification of the first breakpoint (change from stationary to exponential growth) using the *segmented*

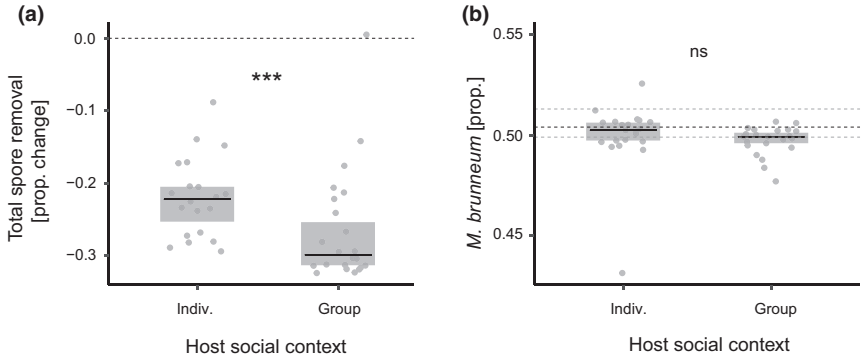

Figure 3 Pathogen removal by ant grooming behaviour. (a) Grooming reduced the total number of spores on the ant's cuticle after a 2-h grooming period by 22% in the individual ants and by 30% in the presence of allogrooming nestmates. The zero line depicts the baseline exposure dose (median 1.57×10^5 spores, interquartile range [IR]: 1.38×10^5–1.78×10^5). Data points give the proportional reduction from this baseline per ant (median number of spores retrieved from the ant in the individual context: 1.23×10^5 spores, IR: 1.20×10^5–1.25×10^5 and group context: 1.10×10^5 spores, IR: 1.08×10^5–1.19×10^5). The black lines indicate the median for each host social context and the boxes show 95% confidence intervals (CI). Benjamini–Hochberg corrected P value: *** denotes $P < 0.001$. (b) Host social context did not change the relative contribution of *Metarhizium brunneum* to *Metarhizium robertsii* from the baseline exposure with a 50 : 50 suspension (median proportion *M. brunneum* directly after exposure shown as black dotted line, IR as grey dotted lines, revealing non-significant difference from 50 : 50). Data points show the proportion of *M. brunneum* per ant, black lines the median for each social context and boxes the 95% CI. N.s. denotes a non-significant overall model.

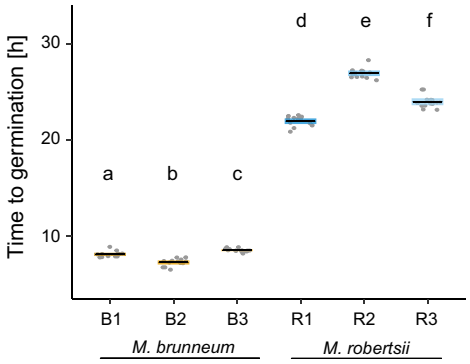

Figure 4 Germination speed of the fungal strains. Each strain had a characteristic time to germination, yet all *Metarhizium brunneum* strains germinated significantly faster in fungal growth medium than all *Metarhizium robertsii* strains. Data points show replicates, with the median germination duration per strain depicted as black lines and boxes representing 95% CI (same colour scheme as in Fig. 2b). Significance groups revealing differences between strains (Benjamini–Hochberg corrected P values of all pairwise posthoc comparisons $P < 0.001$) are denoted by different letters (a–f).

package (Muggeo 2003, 2008). Germination times were analysed using a Kruskal–Wallis test, followed by all pairwise Mann–Whitney U tests for posthoc comparisons (Benjamini–Hochberg corrected).

Model

We formulated a stochastic competition model (Huang *et al.* 2015) to test whether the differences in germination speed between *M. robertsii* (*R*) and *M. brunneum* (*B*; Fig. 4) were sufficient to explain the relative outgrowth shift observed in groups (Fig. 2), given the differential grooming efficiencies of self-grooming only vs. self- and allogrooming (Fig. 3). The model distinguishes three qualitatively different periods. (1) Initially, spores from both species are removed from the cuticle at a constant rate α per hour, which is equal for both, *M. robertsii* ($R \xrightarrow{\alpha} 0$) and *M. brunneum* ($B \xrightarrow{\alpha} 0$; Fig. 3b) but smaller for individuals than groups, with $\alpha_I < \alpha_G$ (Fig. 3a). (2) Once the germination time t_B of *M. brunneum* has been reached, its spores enter the host and reproduce, that is, grow within the host's body. During this period, spores of *M. brunneum* reproduce at a rate b_B (represented as $B \xrightarrow{b_B} 2B$), and die at rate d_B ($B \xrightarrow{d_B} 0$). Moreover, we include the higher-order relationship $B + B \xrightarrow{a_{BB}} B$ to describe within-species competition, reflecting limited host resources. In the meanwhile, spores of *M. robertsii* continue to be removed from the cuticle at rate α. (3) Once the germination time t_R of *M. robertsii* has been reached at time $t_R > t_B$, both pathogens compete within the host. Similar to *M. brunneum*, spores of *M. robertsii* reproduce at rate b_R, die at rate d_R, and they are subject to within-species competition at rate a_{RR}. In addition, there is now also between-species competition, represented by the relationships $R + B \xrightarrow{a_{RB}} B$ and $R + B \xrightarrow{a_{BR}} R$. Depending on how the within-species rates a_{RR} and a_{BB} compare to the between-species rates a_{RB} and a_{BR}, the two species can either stably coexist (leading to mixed outgrowth in a cadaver), or one species drives the other one to extinction (Hofbauer & Sigmund 1998). We fitted these competition parameters to match the experimentally observed spore proportions after completed sporulation (Fig. 2), and used them equally for ants kept as individuals and in groups to determine whether the observed differences in the parasites' germination time alone can explain any observed differences in spore outgrowth between host social contexts. We simulated the above-described process using the Gillespie algorithm (Gillespie 1976) to allow for stochastic effects (Gokhale *et al.* 2013; Constable *et al.* 2016). We include stochasticity to capture the experimental observation that identically exposed ants may result in different spore

outgrowth, due to random fluctuations during spore removal and competition.

See supplement for more details on experimental procedures, data analysis and model.

RESULTS

Social immunity reduces pathogen virulence independent of coinfection diversity

The presence of nestmate ants reduced the pathogen-induced mortality in the exposed ants from 60% when kept individually to 32% when kept in groups, independent of coinfection diversity (Fig. 1a, Fig. S1, GLMM; $\text{interaction}_{\text{coinfection diversity*social context}}$: $\chi^2 = 0.467$, d.f. = 1, $P = 0.4944$; effect of social context in same-species coinfection: $\chi^2 = 41.843$, d.f. = 1, $P < 0.001$; different-species coinfection: $\chi^2 = 47.838$, d.f. = 1, $P < 0.001$). Such improvement of survival after exposure to pathogenic fungi is a well-documented result of social immunity, as sanitary care by grooming removes fungal spores from the body surface (Rosengaus *et al*. 1998; Hughes *et al*. 2002; Liu *et al*. 2019), especially in the areas that are out of reach to the exposed individuals themselves. Here we show that this effect is also found for coinfections, independent of whether these consist of the same or different pathogen species. The presence of nestmates in this critical phase, before cuticle penetration and when spores are not yet firmly attached, therefore strongly reduces the effective exposure dose. This is highly relevant as the disease outcome of *Metarhizium* is dose-dependent (Hughes *et al*. 2002; Konrad *et al*. 2012).

Social immunity reduces the production of transmission stages in different-species coinfections

We measured how many spores grew out of cadavers that died from successful *Metarhizium* infection, and found that host social context affected spore number depending on coinfection diversity (Fig. 1b; LMM; $\text{interaction}_{\text{coinfection diversity*social context}}$: $\chi^2 = 6.537$, d.f. = 1, $P = 0.011$). This significant interaction resulted from several strain combinations, mostly in different-species coinfections, showing about 5-fold to 10-fold higher spore production in the individual vs. group context. Hence, spore production was higher when ants were alone, but only when coinfections consisted of different pathogen species (effect of social context in same-species coinfection: $\chi^2 = 0.04$, d.f. = 1, $P = 0.844$; different-species coinfection: $\chi^2 = 17.69$, d.f. = 1, $P < 0.001$). These results support experimental studies showing increased investment into transmission stages in coinfections (Abu-Raddad *et al*. 2006; Lass *et al*. 2013), a result we could only retrieve in the individual context (Fig. 1b, Fig. S2).

Social immunity increases the likelihood of future coinfections with different pathogen species

Of all sporulating cadavers, the majority (*c*. 85%) showed pure outgrowth of only a single pathogen species. Mixed outgrowth of the two coinfecting pathogens from the same cadaver was overall rare, and depended on coinfection diversity influenced by host social context (Fig. 2a, Fig. S2: GLMM; $\text{interaction}_{\text{coinfection diversity*social context}}$: $\chi^2 = 6.452$, d.f. = 1, $P = 0.011$). Social immunity altered the proportion of mixed outgrowth only for different-species coinfections (effect of social context in same-species coinfection: $\chi^2 = 0.107$, d.f. = 1, $P = 0.743$; different-species coinfection: $\chi^2 = 13.023$, d.f. = 1, $P < 0.001$). In groups, more than 30% of all cadavers showed outgrowth of both strains after different-species coinfections, in contrast to only 5% in individual ants (Fig. 2a). Therefore, social immunity over-proportionally favoured cadavers with co-sporulation of two pathogen species, increasing the likelihood of continued coinfections in the next hosts.

Social immunity maintains a diverse pathogen community otherwise lost in different-species coinfections

To determine the relative contribution of each of the six strains to the transmission pool for the next generation, we compared the number of cadavers showing outgrowth of each strain (co-sporulating cadavers counted for both strains) between our four experimental groups. Overall, *M. robertsii* and *M. brunneum* contributed equally to the next generation of spores (*c*. 50 : 50) in all experimental groups, except for the cadavers of different-species coinfections emerging from individual ants (Fig. 2b). Here, *M. robertsii* had a competitive advantage with the proportion of cadavers sporulating with *M. robertsii* amounting to 62% while the two species showed approximately equal contributions (49% *M. robertsii*) in the group context. Thereby, social immunity affected the population-wide pathogen community in different-species coinfections, but not in same-species coinfections (Fig. 2b; GLMM; trending-significant $\text{interaction}_{\text{focus strain*coinfection diversity*social context}}$: $\chi^2 = 10.209$, d.f. = 5, $P = 0.0695$; effect of social context in same-species coinfection: $\chi^2 = 14.796$, d.f. = 11, $P = 0.230$; different-species coinfection: $\chi^2 = 46.080$, d.f. = 11, $P < 0.001$). Within species, some strains were more successful than others (ranging from 8 to 27% of total contribution) and they were also differently affected by social immunity. Whereas *M. robertsii* strain R1 successfully produced the highest share of cadavers independent of host social context (Figs S3 and S4), other strains, for example, *M. brunneum* B1 and B3 gained advantage in the group vs. individual context. Overall, *M. robertsii* showed higher competitive abilities than *M. brunneum* in individual hosts, being over-represented in the spore community. However, social immunity interfered with the dominance of *M. robertsii* by creating conditions that strengthen *M. brunneum*, thereby maintaining higher pathogen diversity in the community.

Ant sanitary care unselectively reduces cuticle spore load

The gained advantage of *M. brunneum* under social immunity may result from ant behaviour and/or fungal growth patterns. To evaluate whether the ants remove *M. robertsii* over-proportionally, thereby directly biasing the spore composition on the ants' cuticles by selective grooming, we exposed ants to a 50:50 mix of *M. robertsii* and *M. brunneum* (R2-B3; see supplement) and kept them for 2 h in the individual or group context. In this early period after exposure, preceding spore

germination (see below), *Metarhizium* spores only adhere lightly by hydrophobic bonds (Gillespie *et al.* 2000) and can be effectively removed by grooming (self-grooming only in the individual context vs. self- and allogrooming in the group context) (Vestergaard *et al.* 1999; Thomas & Read 2007; Walker & Hughes 2009). As expected (Hughes *et al.* 2002; Reber *et al.* 2011; Okuno *et al.* 2012), individual self-grooming alone clearly reduced the cuticle spore load of the ants compared to the applied load, and sanitary care by nestmates lead to an even stronger reduction (Fig. 3a; Kruskal–Wallis test $\chi^2 = 35.942$, d.f. = 2, $P < 0.001$; all pairwise posthoc comparisons $P < 0.002$). Yet, we found no significant change in the relative proportions of *M. brunneum* to *M. robertsii* on the ants' cuticle in either social context (Fig. 3b; Kruskal–Wallis test $\chi^2 = 4.703$, d.f. = 2, $P = 0.096$). This suggests that the increased success of *M. brunneum* when competing with *M. robertsii* in grouped vs. individual ants (Fig. 2b) cannot be explained by spore proportion bias on the ants' cuticles caused by selective grooming, assuming that the first 2 h are representative for all grooming.

Faster germination gives social immunity less time to act on *M. brunneum*

Even if performed unbiased, the spore removal efficiency of grooming will depend on the adherence strength of the spores, which increases with spore germination. Once the fungus has penetrated the host body, grooming is no longer efficient (Walker & Hughes 2009).

Depending on their germination speed, the different fungi may, hence, show different time windows during which grooming is efficient and they are susceptible to sanitary care. Determination of the *in vitro* germination periods of our six strains indeed revealed that all strains of *M. brunneum* germinated significantly faster than any strain of *M. robertsii* (Fig. 4; Kruskal–Wallis test $\chi^2 = 79.736$, d.f. = 5, $P < 0.001$; all pairwise posthoc comparisons $P < 0.001$). The variation in germination onset within species was small for *M. brunneum* strains (std. dev.: 0.6 h, max. difference 1.2 h), but amounted to up to 4.7 h for *M. robertsii,* with strain R1 being the fastest *M. robertsii* strain (std. dev.: 2.1 h). Overall, all *M. brunneum* strains germinated in approximately 1/3 of the time of *M. robertsii* (median *M. brunneum*: 8.1 h, *M. robertsii*: 23.9 h). These data suggest that, on the ants' cuticle, *M. brunneum* will be exposed to grooming for a shorter time, as it enters the body faster.

Model dynamics reveal a competitive advantage for fast-germinating pathogens under social immunity

Our experiments showed that social immunity did not only reduce overall (absolute) pathogen success (Fig. 1), but moreover shifted the balance between pathogen species (relative success; Fig. 2), despite the fact that we could not observe any selectively performed sanitary care (Fig. 3). We devised a model to determine whether the observed differential growth characteristics of the two pathogen species (Fig. 4) could suffice to explain the modulation of competitive success between the two pathogen species by social immunity. In particular, we were interested whether the combination of (1) larger spore removal efficiency in the group- vs. individual social context (Fig. 3b) and (2) the faster germination speed, that is, shorter time window for grooming of *M. brunneum* (Fig. 4) could explain why social immunity improves the competitive advantage of *M. brunneum*. Supporting our experimental data (Fig. 2b), our model resulted in higher outgrowth of *M. robertsii* in individual ants (Fig. 5a) while the two pathogens showed a balanced outgrowth in grouped ants (Fig. 5b). The model further revealed that both the higher grooming efficiency in the group and the difference in germination time between the coinfecting pathogens affect the outcome of the pathogen competition over a wide parameter range (Fig. 5c,d) and hence a wider range of biological scenarios than could be tested in our experiment. Importantly, no other parameters than the grooming efficiency differed between ants kept individually or in the group. In particular, the competition parameters of the model were set equal for both social contexts. Thus, our simulations confirm that the pure fact that *M. robertsii* spores have a longer germination time and are exposed to allogrooming for longer can explain the observed outgrowth patterns and the modulatory effect of social immunity on the outcome of pathogen competition.

DISCUSSION

Our work revealed that social host interactions can have a major influence on pathogen communities. Using multiple pathogen combinations, we identified robust and general patterns of how social immunity influences pathogen competition when ants are coinfected with different fungal pathogen species, where we found it to (1) reduce spore production in combinations that otherwise showed high sporulation success in individual hosts (Fig. 1b), (2) increase the proportion of cadavers with simultaneous outgrowth of both pathogen species (Fig. 2a) and (3) induce a shift in the relative success of coinfecting pathogens (Fig. 2b, Fig. S3). Thereby, social immunity maintained a higher pathogen diversity at both, the cadaver level and the community level.

As expected by the reduction of exposure dose on the cuticle (Fig. 3) and dose-dependency of *Metarhizium* virulence (Hughes *et al.* 2002), social immunity greatly improved host survival (Rosengaus *et al.* 1998; Theis *et al.* 2015), independent of coinfection diversity (Fig. 1a). In our obligate-killer system (Gillespie *et al.* 2000), coinfection diversity did not affect virulence (Fig. 1a) while it altered the production of transmission stages. We found that individual hosts that were coinfected by several different-species combinations showed increased spore production (Fig. 1b, Fig. S2), which is in line with previous work reporting increased transmission stage production under pathogen competition (Moss *et al.* 1995; Abu-Raddad *et al.* 2006; Lass *et al.* 2013; Louhi *et al.* 2015; Susi *et al.* 2015). This suggests different within-host dynamics depending on the within-host diversity of pathogens in our system, which could result from a less efficient immune response when fighting multiple enemies (de Roode *et al.* 2003), or from increased pathogen growth and competition for the same host resources after host death (López-Villavicencio *et al.* 2011). Social immunity interfered with this

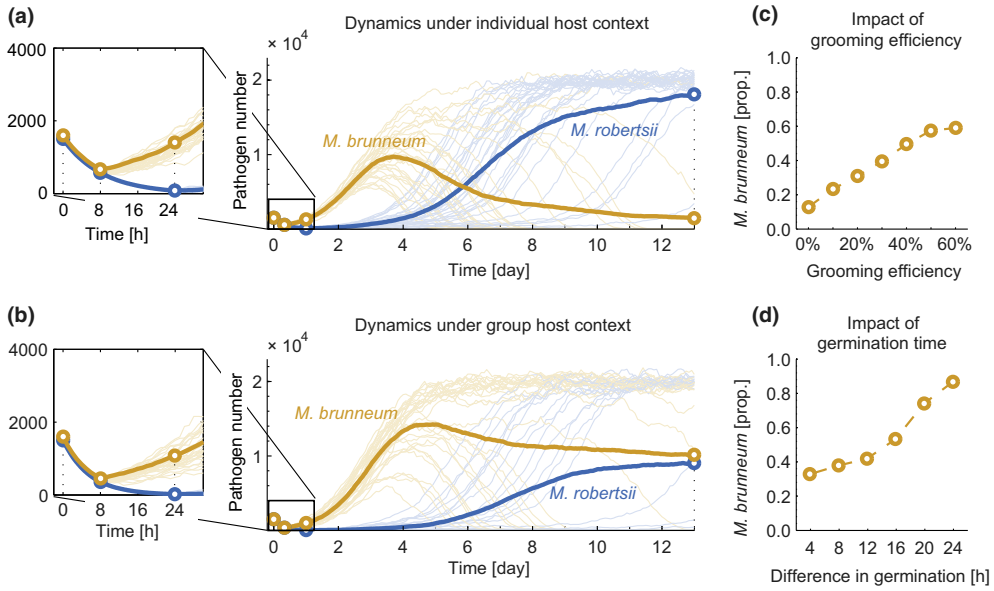

Figure 5 Modelling pathogen competition dynamics under varying host social contexts. Based on a stochastic model, we have repeatedly simulated the competition between *Metarhizium brunneum* (yellow) and *Metarhizium robertsii* (blue) for the individual (a) and group (b, c, d) context. (a, b) 30 representative sample paths (in light colours) as well as the resulting average values (in bold colours). In the first phase (0–8 h), grooming equally removes spores of both species from the ant's cuticle until *M. brunneum* spores have germinated and entered the body. In the second phase (8–24 h), *M. brunneum* already establishes an infection in the host, and interacts with the host immune system, while *M. robertsii* is still being groomed off by the ants on the cuticle. In the third phase (> 24 h), *M. robertsii* also enters the host (yet in lower numbers due to longer exposure to grooming), where the two fungi interact both with one another and the host immune system, grow and produce final spore outgrowth (day 13). Plots provide the timeline of pathogen success over the period of 13 days, with inserts showing details of the first two phases (model parameters: experimentally derived parameter values: $\alpha_I = 0.1221$/h, $\alpha_G = 0.1779$/h, $t_B = 8.1$h, $t_R = 23.9$ h; fitted parameter values: $d_R = d_B = 1.20$, $b_R = b_B = 1.26$, $a_{RR} = a_{BB} = 3 \times 10^{-6}$, $a_{RB} = 3.03 \times 10^{-6}$ and $a_{BR} = 3 \times 10^{-5}$). (c, d) Effect of different parameter values on the final proportion of *M. brunneum* (on total spore production) in the group context (c) with varying spore removal efficiency by the additional allogrooming by nestmates as compared to self-grooming only, and (d) with varying difference in germination speed between the two coinfecting pathogens. *M. brunneum* gains competitive advantage when social grooming is highly effective (c) and when there is a considerable lag in the two germination times (d). Each data point is based on 500 simulations. For (c), we used the same removal rate as in (a), $\alpha_I = 0.217$/h, and we have continually increased α_G starting from α_I. For (d), we fixed the germination time of *M. robertsii* to $t_R = 23.9$ h, and have continuously decreased the germination time t_B.

increased spore production (Fig. 1b). We suggest this to be a consequence of additional nestmate allogrooming, which reduces the effective spore dose on the cuticle stronger than self-grooming alone (Fig. 3a). Reduced *Metarhizium* exposure doses lead to reduced spore production in ants (Hughes *et al.* 2004).

Not only did nestmates reduce the overall spore amount on the cuticle, this behaviour also changed the competitive success of the two pathogen species relative to one another. *M. robertsii*, which dominated the spore outgrowth in individual hosts, lost its dominance when nestmates provided sanitary care (Fig. 2b). We could not find any indication that this was caused by the ants actively biasing spore composition on the cuticle by selectively removing more *M. robertsii* from the exposed individual (Fig. 3). However, our model revealed that even in the absence of selective grooming, the slower germination of *M. robertsii* (Fig. 4) is sufficient to explain its lower success in groups. Slower germination prolonged the time window of being exposed to the ants' social grooming, causing over-proportional spore removal in *M. robertsii* (Fig. 5). Lower dose can cause even highly competitive genotypes to lose their advantage (Ben-Ami & Routtu 2013; Ummidi & Vadlamani 2016), possibly because they are more easily overcome by the immune system or competitors when in small numbers. Testing our model for a wide range of parameter values revealed that the predictions are robust to different allogrooming efficiencies (Fig. 5c), which can change, for example, with the number of grooming nestmates (Hughes *et al.* 2002). Also, our findings may be similarly applicable to a variety of other pathogen combinations with differing germination time (Fig. 5d).

Faster germination benefited *M. brunneum* by reducing its sensitivity to grooming, but it did not provide a higher within-host competitive ability in individually kept ants, as found in other systems where the first-established pathogens gain competitive advantage over competitors that enter the host later (de Roode *et al.* 2005; Ben-Ami *et al.* 2008; Hoverman *et al.* 2013; Natsopoulou *et al.* 2014). This hints to a possible trade-off in our system between germination speed and competitiveness, both against other pathogens, or the host immune system (Mukherjee & Vilcinskas 2018). Prior residency may induce an over-proportional cost to *M. brunneum* as the first pathogen needs to fight the host alone, for example, by releasing factors modulating host immunity or toxins to kill the host (Gillespie *et al.* 2000; Beys-da-Silva *et al.* 2014; Mukherjee & Vilcinskas 2018), which may benefit the later-

incoming *M. robertsii* (Hughes & Boomsma 2004; Chouvenc *et al.* 2012).

We found that the presence of grooming nestmates induced more co-sporulation in cadavers after different-species coinfections, but not after same-species coinfections (Fig. 2a). The increased grooming efficiency in social groups, coupled with differences in pathogen germination speed (Fig. 4), could have prevented competitive exclusion of one of the strains, which is otherwise common for *Metarhizium* coinfections (Figs S2 and S3; Pauli *et al.* 2018). Cadavers producing spores of both species will lead to a higher probability that hosts will get coinfected by a single cadaver in the next round of transmission. Social immunity therefore increases pathogen diversity at both the cadaver level and at the community level so that the likelihood of continued coinfections with different pathogen species will remain high for future hosts.

Understanding the underlying mechanistic details of pathogen competition and the ecological context of disease spread is crucial for understanding processes that shape pathogen communities (Pedersen & Fenton 2007; Johnson *et al.* 2015). Altered pathogen communities could feedback to affect host–pathogen interactions and their evolution (Boots *et al.* 2009; Alizon *et al.* 2013). This could furthermore depend on the composition of the host community, that is, the number of solitary and social species present and their territorial and ecological overlap (Cremer *et al.* 2018; Cremer 2019a).

CONCLUSION

We find that social host interactions have the potential to change the dynamics of pathogen competition in social hosts compared to when hosts are fighting disease on their own. This phenomenon parallels the immune-mediated competition described for solitary hosts where host immunity mediates indirect advantage to one of the genotypes (Lysenko *et al.* 2005; Raberg *et al.* 2006) and could be described as '*socially mediated competition*'. Social interactions may affect disease outcomes in a variety of group-living species, and should be considered when modelling or experimentally testing epidemiological dynamics, especially for commercially important host species like the honeybee (Brosi *et al.* 2017), or ecologically relevant species, like invasive species (Ugelvig & Cremer 2012; Cremer 2019a).

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ETHICS STATEMENT

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AUTHORS' CONTRIBUTIONS

The experiments were conceived by SC, MS and BM and performed by MS, AVG, BM and EN. Data analysis was performed by BM and MS, the model developed by CH with input from BM and SC, and the manuscript written by SC, BM and CH. The manuscript was approved by all authors.

DATA AVAILABILITY STATEMENT

All data are accessible on Dryad: https://doi.org/10.5061/dryad.crjdfn318.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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