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ORIGINAL ARTICLE

Disease prevention and resistance in social insects: modeling the survival consequences of immunity, hygienic behavior, and colony organization

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Abstract Understanding the origin of disease resistance in social insects is difficult due to the lack of well-established phylogenies of presocial and eusocial species and the absence of extant basal and intermediate forms. Moreover, comprehensive accounts of infection-control traits in social insect lineages are not available. Therefore, to explore the evolution of pathogen control in social insects we used cellular automata models to analyze the efficacy of immunity and nest hygiene, which we assumed were basal traits, and allogrooming, which likely followed the transition to eusociality, and their interactions with colony demography and patterns of worker spatial distribution. Models showed that nest hygiene provided an immediate survival benefit and that immunity lowered overall disease susceptibility under both constant and periodic exposure scenarios. Allogrooming increased survivorship in chronically challenged colonies but also increased pathogen transmission rates under conditions of periodic exposure. Colonies having demographies biased towards young or old individuals had slightly higher mortality than those with heterogeneous demographies. The distribution of older individuals relative to the nest center had no significant effect on susceptibility and provided only a minor survival advantage. Models indicated that nest hygiene and immunity function on different temporal scales and can interact with demography to lower disease risks. Our results suggest how infection control systems in social insects could have been built upon the inducible immune defenses and nest hygienic behaviors of solitary and presocial ancestors and served as important preadaptations to manage disease exposure and transmission in colonies of eusocial species.

Keywords Epidemiology · Termites · *Zootermopsis* · Ants · Host/Pathogen evolution

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Introduction

Theoretical and empirical studies addressing the relationship of sociality and disease transmission suggest that group living and social contact may not only increase the risk of pathogen and parasite exposure (Brown and Brown 1986; Nunn et al. 2000) but also enhance infection control (Loehle 1995; Rosengaus et al. 1998; Sanchez-Villagra et al. 1998; Schmid-Hempel 1998; Wilson and Reeson 1998; Rosengaus and Traniello 2001; Traniello et al. 2002; Wilson et al. 2002; Wilson and Cotter 2004). Social insects pose a particularly interesting problem for the study of the

disease-related benefits and costs of group living and evolution of disease control because they often live in densely populated colonies in which individuals frequently interact within a confined nest environment that favors microbial growth and hence may elevate infection risks. Yet, in spite of the potentially intense selection pressures posed by parasites and pathogens, social insects are extraordinarily diverse, abundant, and have significant ecological impact (Hölldobler and Wilson 1990; Abe et al. 2000). This success begs the question of how they evolved to resist disease as they transitioned from solitary life through presocial forms to eusociality and subsequently diversified.

To address this question, it is reasonable to assume that mechanisms of disease resistance in the solitary and presocial ancestors of social insect taxa were conserved and/or functioned as preadaptations for newly evolved individual and social strategies of infection control in nascent eusocial species. Given the distribution of immune function across phyla (Du Pasquier and Flajnik 1999), among insects (Hultmark 1993) and within the clades that contain the social Hymenoptera and the termites, we hypothesized that immunity is a basal trait. Although we have little insight into the nature of disease prevention and resistance in the ancestors of eusocial insects, when and why various methods of prophylaxis first occurred, and how such traits may have been expressed in group-living descendants, it is likely that disease-resistance traits such as immunity were conserved over the evolutionary progression from solitary life to eusociality. From what is known about how solitary and presocial forms cope with disease, the occurrence of nest hygienic behavior in presocial species suggests that such behaviors are also basal in eusocial insect biology (Field and Brace 2004). In addition, there is evidence of antimicrobial protection in presocial species (Bienvenu et al. 1968; Cane et al. 1983; Strohm and Linsenmaier 2001; Kaltenpoth et al. 2005). Such basal traits could then be modified and expressed in the context of a colony's population and be influenced by its social phenotype.

In most eusocial insects, understanding the evolution of infection control is compromised by an inability to analyze the historical progression of pathogen and parasite resistance in solitary and subsocial ancestors. Although there is some information suggesting how infection risk can influence the evolution of behavioral and physiological prophylaxis within a clade (Fernandez-Marin et al. 2003–2005), phylogenetic intermediates bridging solitary, presocial, and eusocial species that might show gradual transitions in individual immune function and group-level immunocompetence generally do not exist, thus limiting comparative analysis (Thorne and Traniello 2003). In any case, interpreting results of such analyses is rendered difficult by changes in nesting and foraging ecology that accompanied social evolution and altered the selective regimes influencing immunocompetence during adaptive radiation. Although it has been possible to trace the phylogenetic roots of immune response in some vertebrate groups (e.g., Pancer et al. 2004), little is understood of the evolution of immunity and other methods of disease resistance and prevention in social insects. Although immune response has been studied in the context of social evolution with respect to reproductive ability (Moret and Schmid-Hempel 2004) and recent molecular comparisons have begun to illuminate immune system diversity (Bulmer and Crozier 2004, 2006), the immunology, hygienic behavior, and other infection-control mechanisms of related presocial and subsocial species are largely unstudied. Moreover, there are no explicit considerations of infection risk, disease resistance and social organization among related solitary, presocial, and eusocial taxa.

In spite of these limitations, it is possible to analyze the evolution of disease prevention and resistance in social insects through modeling. Prior modeling has shown that nest structure, worker activity, and worker density can influence rates of pathogen transmission (Pie et al. 2004), as can division of labor, colony demography, and worker interactions (Naug and Camazine 2002). In this study we developed a cellular automata model that employs simple probabilistic rules to govern the movement of individuals within a colony and modeled infection risk and resistance based on nestmate developmental stage and individual health (c.f., Rhodes and Anderson 1996). We manipulated the presence and absence of nest hygiene, individual immunity, allogrooming, and social interactions that contribute to both exposure and resistance in colonies facing different pathogen challenges. By varying demographic distributions, we comparatively analyzed the impact of the behavioral and physiological defenses that likely characterized solitary and presocial ancestors, transitional forms and incipiently eusocial species to make historical inferences about the efficacy of these mechanisms. While previous empirical studies have described the contribution of a given mechanism to survival as one component of a suite of infection control methods, our modeling allowed us to assess the relative protective benefits of traits that may act individually or in concert to examine the evolution of disease prevention and resistance in social insects.

Materials and methods

We first developed an agent-based cellular automata model. An agent-based model follows the interactions of individual "agents", in our case "workers", over time as they interact. A cellular automata model divides a spatial representation

into discrete areas within which independent computations occur over time. In our models, the "cells" were discrete areas of the nest through which "workers" could move and interact. Our baseline model included the following defenses against disease: nest hygienic behavior, allogrooming, primary-exposure-based immunity, socially triggered immunity, colony demography, and the spatial distribution of workers within the nest. We then altered single aspects of the baseline model (the computational equivalent of a set of genetic "knock out" experiments) to study the significance of each mechanism, and hence its potential efficacy during the course of social evolution. These behavioral and physiological defense mechanisms and the alterations involved in removing a given mechanism from the baseline model are summarized in Table 1. By constructing cellular automata models incorporating only those aspects of colony organization and individual physiology and behavior being directly studied, our outcomes may be applied to any system possessing these mechanisms as independent attributes.

Our models incorporated the following aspects of pathogen exposure and defense: a) primary pathogen exposure (direct contact with disease agents distributed in the nest); b) secondary pathogen exposure (the exposure of individuals through social interactions with infected nestmates); c) primary immunity (individual physiological immunocompetence resulting from an exposure to a nonlethal concentration of a pathogen); and d) social interaction (the allogrooming of microbes from the integument that can lead to a reduction in pathogen load for both exposed and infected individuals). Social interaction can also involve the transmission of an inoculating dose, thus providing a socially mediated immunization of nestmates (Rosengaus and Traniello 1997; Rosengaus et al. 1999; Nelson et al. 2001; Traniello et al. 2002). Therefore, social interaction represents both a mechanism of secondary exposure (b, above) and an independent defense mechanism. Each of these aspects of exposure and defense were incorporated into the model via probabilities and threshold values acting on the likelihood of exposed individuals becoming infected (see Tables 2 and 3). Primary pathogen exposure was either constantly present or periodically recurrent. Workers entering cells holding infectious agents became diseased through primary exposure. In both exposure scenarios the introduction of infection into a cell can be interpreted either as an external source invading the entire nest or a single point of entry via a vehicle of exposure (a foraging worker or an infective cadaver).

To emulate a natural nest environment, the model placed new eggs and early stage immatures (mostly immobile) in a circular area (radius=three cells) at the center of the nest. The model placed mature individuals at random throughout the nest, though older individuals had a higher probability of being located farther from the center. Each later stage larva (instar ≥3) was allowed, but not required, to move one cell at random in any direction in each iteration of the model. The basic parameters of our model (Table 2) were informed by research on the social immunology of termites (Rosengaus et al. 1998; Rosengaus and Traniello 1991, 2001; Traniello et al. 2002) and the behavior and pathobiology of presocial and eusocial hymenopteran species (Field and Brace 2004; Hölldobler and Wilson 1990; Kaltenpoth et al. 2005). Intuitive definitions of all variables are given in Table 2.

In the baseline model, all workers were initially defined as immunologically naïve. During each subsequent iteration, workers became diseased or immune according to the application of rules and definitions given in Appendix 1 and Table 3. In each model, initial health values for each worker were assigned at random between 1 and 100 before the first iteration to represent an average cumulative health measure accounting for previous injury, stress, diet, and

Table 1 Models built to examine the impact of disease resistance mechanisms by altering the baseline model

Model modification studied	Difference from baseline model
Adult-biased demography	70% of 'worker' at the outset of the first iteration were adults (developmental stages ≥3)
Early-instar-biased demography	70% of 'workers' at the outset of the first iteration were in developmental stages 1 and 2
Random spatial assignment of individuals	Each worker is assigned to a random position in the nest, regardless of developmental stage
No nest hygiene	$Rthr_s$=0 for all stages
No allogrooming	Stage-dependent thresholds ID_s and II_s are set to 0 for all s
No nest hygiene and no allogrooming	$Rthr_s$=0 for all stages and Stage-dependent thresholds ID_s and II_s are set to 0 for all s
No immunity	Inoculated workers who did not become diseased reverted to naïve status
Maintenance of immunity	60% of population immune before the presence of disease
	35% of population immune before the presence of disease
	20% of population immune before the presence of disease
	15% of population immune before the presence of disease
	10% of population immune before the presence of disease

Table 2 Definitions of variables, parameters and the values used in the baseline model

Variable	Definition		
Worker properties			
W_j	Worker j		
$Wcell_{j,k}$	The position in the nest of worker j		
S	Developmental stage		
$Ws_{j,k}$	The developmental stage of worker j at iteration k		
$Wa_{j,k}$	The length of time worker j has been in the current developmental stage at iteration k		
$Wh_{j,k}$	The health value of worker j at iteration k		
$Wstat_{j,k}$	The infection status of worker j at iteration k		
N	Naïve		
In	Inoculated		
Im	Immune		
Dis	Diseased		
$Wdis_{j,k}$	Whether or not worker j is diseased at iteration k		
$Wdead_{j,k}$	Whether or not worker j is dead at iteration k		
$Win_{j,k}$	Whether or not worker j is inoculated at iteration k		
Sets of workers			
j_k* for cell $[r, \theta]$	The set of all workers in cell $[r, \theta]$ at iteration k		
j_d_k* for cell $[r, \theta]$	The set of all diseased workers in cell $[r, \theta]$ at iteration k		
j_i_k* for cell $[r, \theta]$	The set of all immune workers in cell $[r, \theta]$ at iteration k		
Nest properties			
$[r, \theta]$	Position in the nest		
$[r, \theta]\_p_k$	If cell $[r, \theta]$ contains primary pathogen exposure at iteration k		
$[r,\theta]\_s_{s,k}$	Whether cell $[r,\theta]$ contains enough diseased workers to automatically cause disease in workers of stage s at iteration k		
$[r,\theta]\_i_{s,k}$	Whether the threshold $Ithr_s$ has been satisfied for workers in stage s, in cell $[r,\theta]$, at iteration k		
Parameters			
	Stage	Value	
$Dthr_s$	1–2	40[a]	The percentage of diseased workers required in a cell for a naïve worker of stage s to become automatically diseased
	3–7	50	
ID_s	1	80[a]	The probability of a naïve worker in stage s becoming inoculated from secondary exposure in a single iteration
	2	70[a]	
	3–6	70	
	7	65	
$Ithr_s$	1–7	20	The percentage of immune workers required in a cell for a naïve worker of stage s to become automatically inoculated
II_s	1–2	20[a]	The probability of a naïve worker in stage s becoming inoculated from exposure to an immune worker in a single iteration
	3–4	20	
	5–7	30	
$Rthr_s$	1	40[a]	The threshold health value for removal from nest at stage s
	2	35[a]	
	3	30	
	4	15	
	5	10	
	6	10	
	7	5	
SL_s	1	28 days	The length of developmental stage s
	2	34 days	
	3	41 days	
	4	49 days	

Table 2 (continued)

Variable	Definition	
	5	55 days
	6	269 days
	7	–

[a] Values are estimated rather than empirically measured; all the other values are reported in Rosengaus and Traniello 2001.

senescence at the outset of the experiment. For simplicity, only disease caused a decrease in health. Health was decreased by an arbitrary amount (10 points) after inoculation from any source. Inoculation was therefore considered a uniform phenomenon regardless of its mechanism, representing a presumed cost of the induction of immune response. Once infection was established, health was decreased by one unit per iteration. "Death" occurred when health fell below a stage-dependent threshold value, which represented removal by any mechanism of nest hygiene, or when the health value became equal to 0, representing death from disease. Thus, a worker died 5 days (on average) after becoming diseased based on the pathobiology of termites and several disease agents (Rosengaus and Traniello 1997; Rosengaus et al. 1999).

In the baseline model, the demography of the initial population of workers was evenly distributed among all developmental stages. The model was run for 3,600 iterations (1 year) and replicated ten times. Figures illustrate overall results for each model. The values used for the duration of each developmental stage and other system-specific parameters (such as age-related susceptibility; see

Table 3 The mathematical model

Iteration	Disease transmission
First	Workers that began the iteration as either naïve or inoculated and are present in cells with primary exposure to the fungus became diseased. If $Wstat_{j,k\text{-}1}=n$ or in, and $[r,\theta]\_p_k=1$, then $Wstat_{j,k}=dis$ and $Wdis_{j,k}=1$
Second	Naïve workers became diseased if the percentage of diseased workers occupying the same cell was greater than a stage-dependent threshold $Dthr_s$. We defined $[r,\theta]\_s_{s,k}=1$ if $\left(\sum_{j\_d_k^*} 1/\sum_{j_k^*} 1\right) > Dthr_s$ for j_k^*={all workers occupying $[r,\theta]$ during iteration k} and $j\_d_k^*$={all diseased workers occupying $[r,\theta]$ during iteration k}. For each worker $j \in j_k^*$, if $Wstat_{j,k-1}=n$ and $Ws_{j,k}=s$ and $[r,\theta]\_s_{s,k}=1$, then $Wstat_{j,k}=dis$ and $Wdis_{j,k}=1$
Third	For every diseased worker in a cell, each naïve worker had a particular stage-dependent probability ID_s of exposure to infective agents, based on the assumption that individuals allogroomed and therefore transferred infective agents during social contact. Workers that received this inoculum became inoculated. Workers were restricted from moving for two iterations following inoculation and their health value was decreased by 10. For every worker f in $[r,\theta]$ with $Wstat_{f,k\ -1}=n$ and $Ws_{f,k}=s$ and $[r,\theta]\_s_{s,k}=0$, then for each $j \in j\_d_k^*$, with probability ID_i, $Ws_{f,k}=in$ and $Wh_{f,k}=Wh_{f,k\text{-}1}-10$
Fourth	Naïve workers became inoculated with probability II_s if the number of immune workers co-occupying the same cell was greater than the stage-dependent threshold $Ithr_s$. This was also based on the assumption of allogrooming inducing socially transmitted immunity (perhaps by providing an inoculum). We defined $[r,\theta]\_i_{s,k}=1$ if $\left(\sum_{j\_i_k^*} 1/\sum_{j_k^*} 1\right) > Ithr_s$ for j_k^*={all workers occupying $[r,\theta]$ during iteration k} and $j\_i_k^*$={all immune workers occupying $[r, \theta]$ during iteration k}. For each worker $j \in j_k^*$, if $Wstat_{j,k\ -1}=n$ and $Ws_{j,k}=s$ and $[r,\theta]\_i_{s,k}=1$, then $Wstat_{j,k}=in$ and $Wh_{f,k}=Wh_{f,k\ -1}-10$
Fifth	Inoculated workers occupying a cell with diseased nestmates, or occupying cells with primary exposure to fungus, became diseased with a 90% probability (Rosengaus and Traniello 2001). Therefore, if either $\sum_{j\_d_k^*} 1 \geq 1$ for $j\_d_k^*$={all diseased workers occupying $[r,\theta]$ during iteration k} or if $[r,\theta]\_p_k=1$, then with 90% probability, if $Wstat_{j,k\text{-}1}=in$, $Wstat_{j,k}=dis$ and $Wdis_{j,k}=1$
Sixth	Inoculated workers occupying a cell with no disease present became immune with a 70% probability (Rosengaus and Traniello 2001). Thus, if $\sum_{j\_d_k^*} 1 = 0$ for $j\_d_k^*$={all diseased workers occupying $[r,\theta]$ during iteration k} and $[r,\theta]\_p_k=0$, then with 70% probability, if $Wstat_{j,k\text{-}1}=in$, $Wstat_{j,k}=im$. The duration of immunity was specified as 300 iterations (30 days), after which the worker became naïve again

Rules governing the transmission of disease among workers were applied cell by cell to workers occupying each cell in the order presented. The model was implemented in the C programming language.

Table 2) were suggested by Rosengaus and Traniello (2001). We progressively decreased susceptibility from egg to adulthood. Further details of the model implementation are given in Appendix 1.

Due to the highly stochastic nature of our models, we employed an iterated Monte Carlo method to statistically evaluate results (McCarthy and Thompson 2001). Two survival curves were considered to be significantly different if one curve was consistently higher than the other over the interval reported in more than 95% of pairwise comparisons. This definition of significance is independent of the level of protection, which is reported as the average percentage of difference in survival between two curves.

Results

Nest hygiene and pathogen transmission

Nest hygiene afforded survival benefits that were significantly greater than in the baseline model under constant and periodic disease exposure scenarios (Fig. 1a,b). Additionally, individual and social immunity each had significant protective benefits (Fig. 1c,d). Comparisons of the benefits of nest hygiene and immunity revealed significant differences in the relative protective effect of each defense and its temporal scale of action. Under constant exposure conditions, immunity consistently provided a significant survival advantage that was never outweighed by the benefit of nest hygiene, although nest hygiene alone offered significant protection (~10% overall colony survival; Fig. 1e). However, early during periodic exposure, the removal of young immatures had a significantly greater colony-level survival benefit than immunity, though immunity conferred significantly greater protection after the second cycle of exposure (Fig. 1f).

Allogrooming and colony survival

Allogrooming transmitted pathogens and reduced the pathogen level of exposed individuals and thus mediated social immunity, affecting colony survival differently in each exposure scenario (Fig. 2a,b). Surprisingly, under constant exposure, the absence of allogrooming significantly increased colony survival compared to the baseline model (Fig. 2a). In this scenario, the reduction in the transmission of disease achieved by limiting social contact is sufficient to make it advantageous to avoid behaviors such as allogrooming. Under periodic exposure, however, allogrooming increased colony survival (Fig. 2b). As expected, nest hygienic behavior was of significantly greater benefit in the presence of allogrooming, acting to help control the associated increase in exposure risk (Fig. 2c,d). The benefit of allogrooming was significantly less than that of immunity or nest hygiene under either disease presence scenario (Fig. 2e,f).

Immunity and resistance

Surprisingly, the addition of various initial levels of immunity before the introduction of disease did not impact overall survival (Fig. 3a,b), independent of whether exposure was constant or periodic. In both scenarios, the level of immunity quickly stabilized (Fig. 3c,d) and early differences in protection were not sufficient to alter ultimate survival outcomes.

Colony demography, worker spatial distribution, and disease resistance

The direction of demographic skew in colonies with uneven demographies made no difference to overall survival in either periodic- or constant-exposure scenarios. However, colonies with initially skewed demographies were significantly less successful than colonies with evenly heterogeneous demographic distributions, though the difference in survival was small (Fig. 4a,b). The spatial grouping of young immatures near the nest center and the distribution of older individuals more distally in proportion to their age had a marginally protective value (~3% of overall colony survival) early after exposure under constant disease presence, but the protective value of location was absent at later iterations (Fig. 4c,d). In contrast, location had a small benefit (~7% of overall colony survival) in the periodic exposure scenario (Fig. 4c,d), though neither of these differences was significant.

Discussion

We modeled the relative importance of individual and social components of infection control to study how preadaptive disease resistance traits might have been expressed within a group-living framework. Nest hygiene and immunity, which we can reasonably assume were basal traits in eusocial insect lineages, affected the survival outcome of colony populations on different temporal scales. While the long-term protective effects of immunity provided a greater overall benefit, the impact of nest hygienic behavior on infection risk was immediate. Immunity and nest hygiene acted in concert with colony demography and the spatial distribution of workers to affect resistance. The nature of pathogen presence, either continuously endemic or periodically

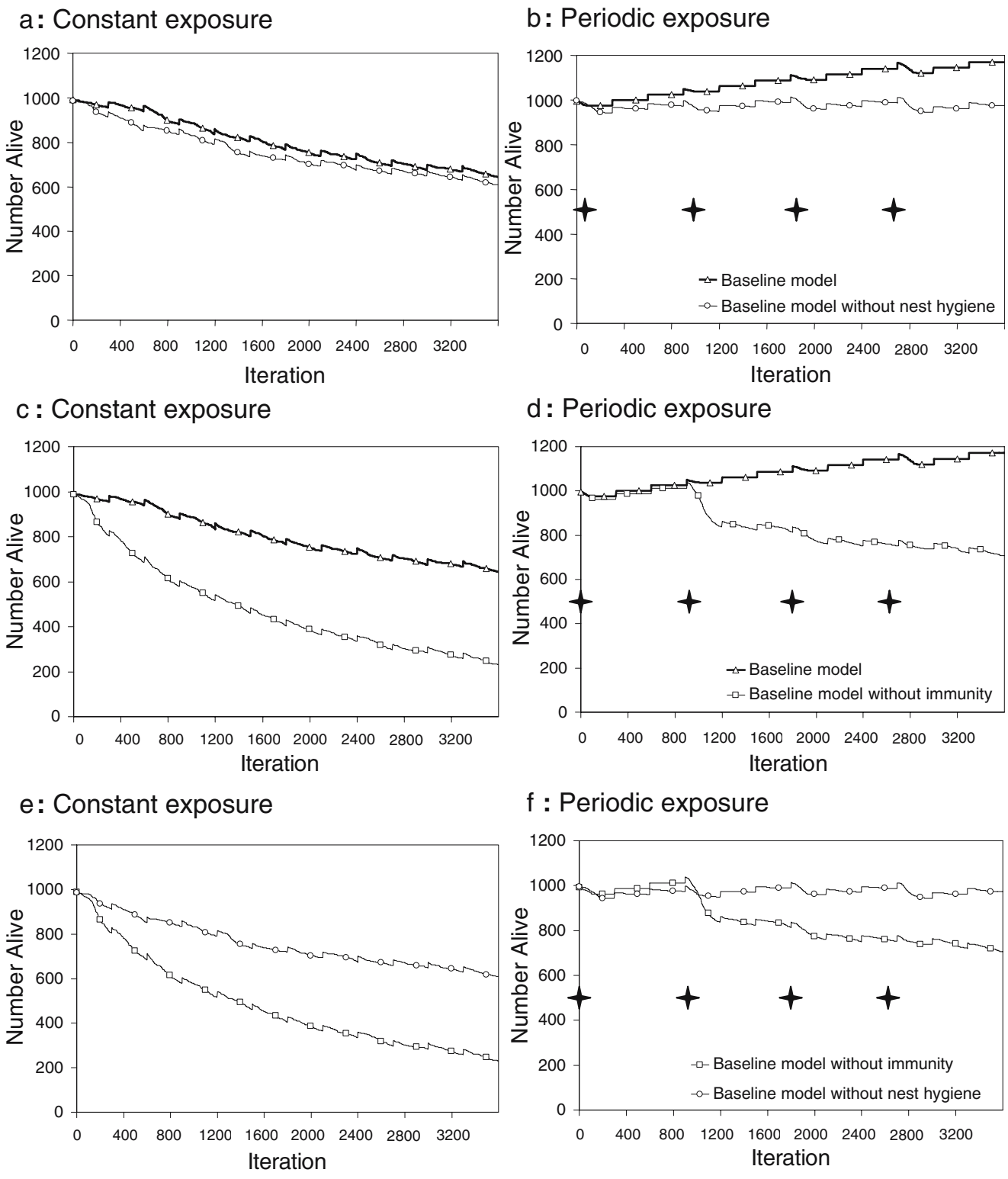

Fig. 1 The impact of immunity and nest hygiene on colony survival. *Four-point stars* mark the iterations at which a primary pathogen was introduced during the periodic exposure scenario

epidemic, influenced the efficacy of allogrooming, demography, and worker spatial distribution, and thus affected the evolution of social modes of disease resistance. Our results offer insight into how methods of infection control that likely characterized presocial species could have served as highly effective preadaptations for disease resistance in eusocial insects and how sociality could have augmented the physiological and

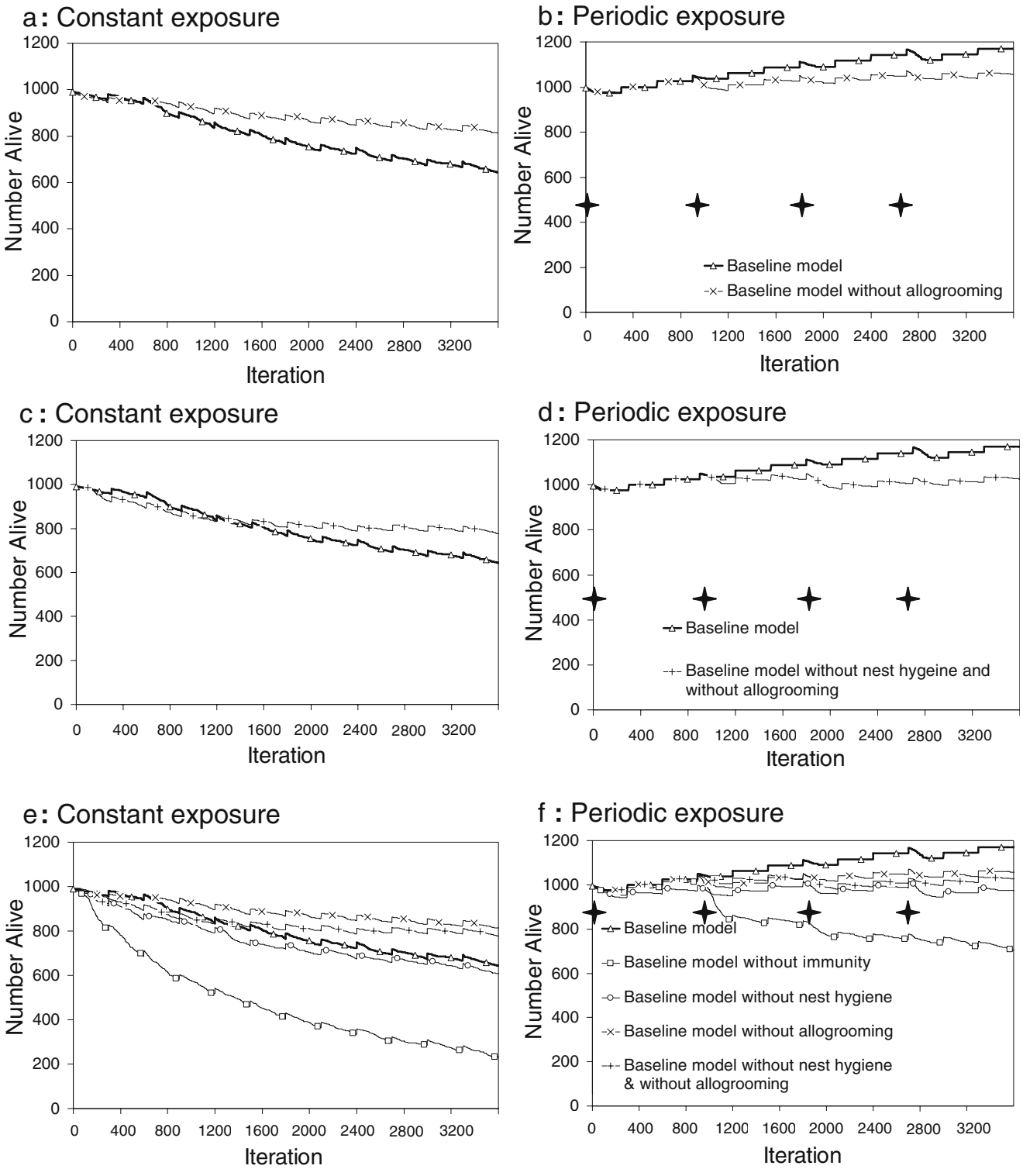

Fig. 2 The comparative impact of allogrooming on colony survival. *Four-point stars* mark the iterations at which a primary pathogen was introduced during the periodic exposure scenario

behavioral adaptations of ancestral forms to enhance resistance. Below we consider the implications of our models for the study of infection prevention and resistance in social insects.

Nest hygiene and pathogen transmission

Based on the behavior of extant presocial insects (Cane et al. 1983; Strohm and Linsenmair 2001; Kaltenpoth et al.

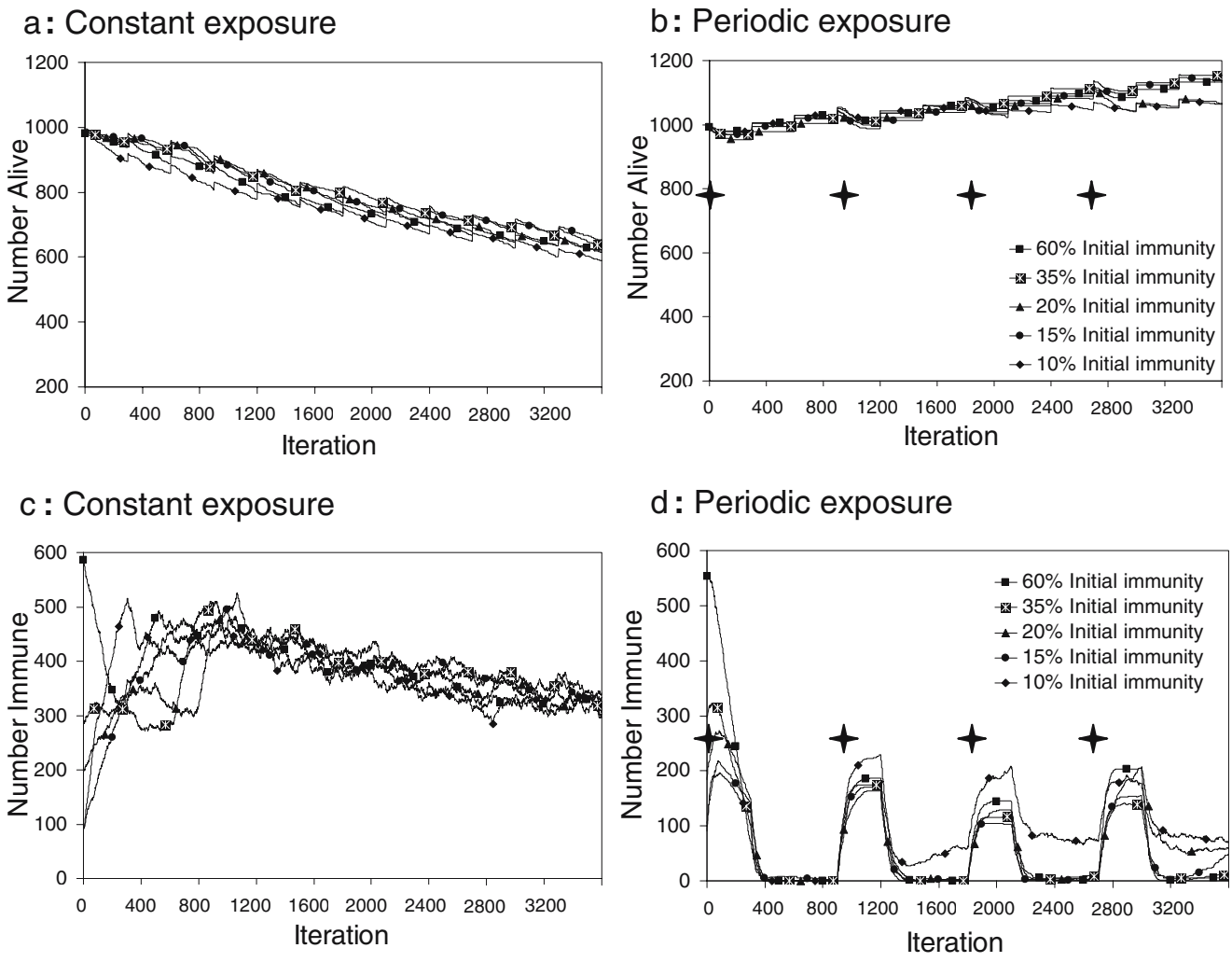

Fig. 3 Levels of colony survival and immunity over time. *Four-point stars* mark the iterations at which a primary pathogen was introduced during the periodic exposure scenario

2005), we can infer that nest hygiene was a basal infection control mechanism. Our findings suggest that nest hygiene alone could have provided an immediate and effective method of colony-wide disease control in evolving social species and colony demographics could have influenced the frequency and nature of hygienic behaviors. Our models predict that adults might cull younger dependent stages to prevent an epizootic once secondary disease transmission is prevalent in a colony even if immatures are not yet infected. The use of nest hygienic behaviors, including cannibalism, as a prophylactic measure in evolving social forms may have depended on the potential trade-offs between disease-related mortality, reproduction, and colony growth. Decisions regarding larvicide were likely influenced by the identity, pathogenicity, and virulence of a given pathogen or parasite. Empirical studies suggest a significant association among age (developmental stage), exposure to varying pathogen loads, and cannibalism (Rosengaus and Traniello 2001).

We interpret the different levels of protection provided by nest hygiene for our two disease-exposure scenarios in the following way. If there is a constant presence of disease in the nest, the behavioral and physiological attributes of immatures, as well as their spatial distribution, will not increase the duration of pathogen presence, but could increase exposure. Therefore, this would have constituted a greater disease threat for eusocial species than for species that had not yet evolved cooperative brood care due to the increased exposure consequential to nursing. Under both the constant and periodic disease scenarios modeled, the elimination of diseased individuals provided colony-wide survival benefits. These results suggest that eusocial species greatly benefited by hygienically removing young, or otherwise eliminating or quarantining diseased nestmates. Nest hygiene would have provided an immediate prophylactic benefit for species at all stages of the evolutionary progression to eusociality, operating more rapidly and

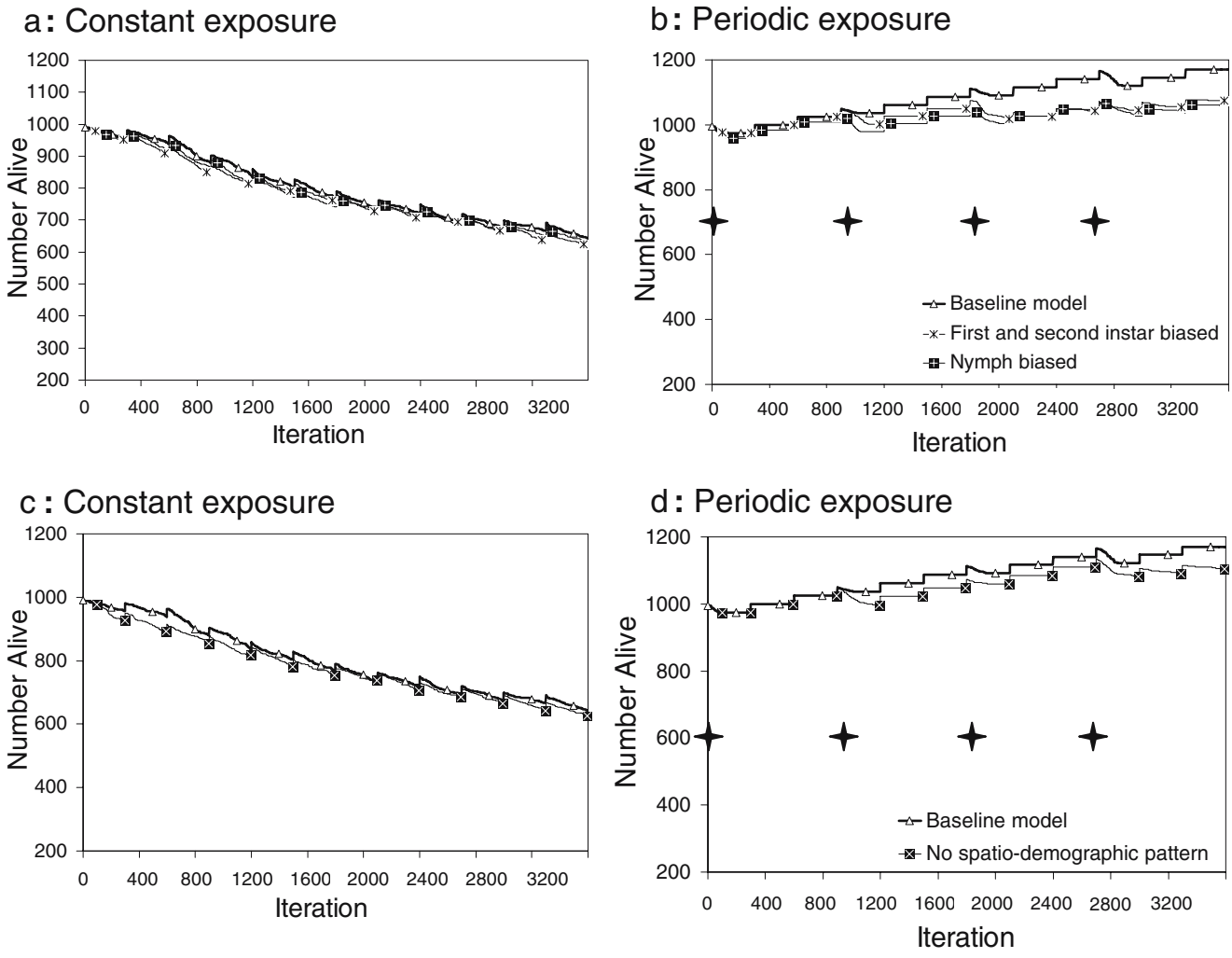

Fig. 4 The impact of altered demographic colony characteristics on colony survival. *Four-point stars* mark the iterations at which a primary pathogen was introduced during the periodic exposure scenario

increasing survival more effectively on a shorter time scale than physiological immunity.

Allogrooming and colony survival

During the early stages of social evolution, it is likely that interactions among nestmates were critical to a colony's ability to withstand pathogen exposure. Our modeling thus included allogrooming as a mechanism of both transfer of disease from sick to healthy individuals and protection from immune to naïve individuals. Additionally, exposed but not yet infected individuals could reduce their likelihood of becoming diseased through hygienic social interactions with healthy nestmates. Each of these effects mirrors the apparent risks and benefits of social hygienic behavior and suggests a trade-off between protection and exposure. What is less apparent is the effect of this trade-off on colony-level survival. The surprising exposure-related differences in the costs and benefits of allogrooming suggest that the majority of disease encounters faced by colonies early after the inception of sociality were sporadic or periodic rather than constant, given that allogrooming is virtually universal among eusocial insects.

Immunity and resistance

Innate and acquired immunity have been described in some solitary insects found in taxa that include eusocial relatives (Duwel-Eby et al. 1991). Therefore, physiological immunity was very likely a basal disease-resistance trait in social insects. Our findings indicate that the extent of immune protection conferred on individuals living in social groups can exceed the capacity to resist infection provided solely by physiological immunity. In our models, immune individuals resisted disease for 30 days, after which they reverted to naïve status. Therefore, under conditions of

constant exposure and induction of physiological resistance we expect immunity would have played a significant role in colony survival in ancestral species. In contrast, under conditions of periodic pathogen exposure, immunity would have conferred a smaller colony-wide benefit because of the shorter time period available to immunize a large portion of the colony population before exposure levels declined. For each incidence of primary exposure to disease, subsequent social immunity could result in the infection of extremely few (one to two) individuals, even in the absence of primary exposure. A small number of infected individuals could, in turn, act as a source of inoculation rather than infection, increasing the level of immunity. Repeated pathogen exposure can therefore lead to the maintenance of immunity at the colony level, regardless of its initial level. Over time, socially maintained immunity can provide one of the most effective protective benefits of group life.

The results of our model (illustrated in Fig. 3) lead us to predict that the duration of individual immunity should be only as long as required for a naïve worker to be exposed (either directly or socially), inoculated and become immune. Our models illustrate this balance between the loss of immunity and the gradual induction of newly immune individuals, leading to eventual system stability over time. Therefore, the maintenance of colony-level immunity during periods of infrequent or low-level exposure could be favored by infected individuals increasing their encounter rates with nestmates. This contact could have the consequence of socially inoculating nestmates (Traniello et al. 2002), thereby increasing colony-wide immunity while also increasing the survival of infected individuals due to increased rates of allogrooming (Rosengaus et al. 1998), or other susceptibility-reducing social interactions.

Colony demography, worker spatial distribution, and disease resistance

Our demographic distributions were modeled after the reproductive patterns of some basal termites and ants, but demographies resulting from different oviposition patterns could have occurred in transitional forms (e.g., Seelinger and Seelinger 1983) and await future analysis. In the case of periodic exposure, all colonies with skewed demographic distributions were more vulnerable to disease than were colonies having an initially balanced demography. In the constant exposure scenario, colonies with skewed demographies were not at a disadvantage. In any case, instar-specific susceptibility and virulence can quickly skew colony demography, which may only affect colony-wide survival in instances of extremely brief exposure.

We hypothesized that the demographically defined, centripetal spatial distribution of nestmates that may have characterized colonies of incipiently eusocial species would affect disease transmission and immunity at the colony level due to differential interaction rates among age cohorts. However, the observed impact of the spatial distribution of individuals was negligible. This could be due to the random movement of individuals that homogenize interaction rates among cohorts despite initial spatial demography or result from the random distribution of primary pathogen exposure throughout the nest over time. In view of this lack of effect of spatial distribution, we hypothesize that nest architecture would have little impact within the scope of the disease scenarios examined in this investigation. However, it is also plausible that a more realistic, non-random pattern of individual movement would result in a greater effect based on spatial distribution. Further modeling is required to elucidate the role of spatio-demographic distribution on colony survival in the presence of disease.

In summary, our modeling generates testable predictions concerning the relative efficacy of physiological and behavioral mechanisms of infection control and how they may have been expressed in the colony environments of incipiently eusocial insects. Additional infection control traits such as the production and distribution of antibiotic secretions could be considered in conjunction with the mechanisms we examined and our models could be modified to accommodate the biology of different social species. Empirically, testing the predictions of our models in clades of insects that include a range of social intermediates could advance our understanding of the evolution of disease prevention and resistance in group-living species.

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Appendix 1: Model details

Primary exposure

Constant exposure was defined as the continual presence of infection (such as fungal conidia or bacteria) in 20 cells, chosen at random in each iteration and lasting 1–10 days in each cell. *Periodic exposure* was defined as the presence of an infective agent in 70 cells chosen at random every 900 iterations (3 months). These cells continued to be infectious for 100 iterations (10 days) after which they no longer held primary contagion. The duration of contagion in these cells in both types of primary exposure was arbitrary, thus allowing us to explore the impact of disease over time.

While primary exposure can be thought of as the novel introduction of pathogen into the nest from an external source, it can also be introduced via the internal source of dead individuals. Although we discuss the results of our model as though dead workers were quarantined, removed from the nest or buried and therefore incapable of infecting nestmates via social interaction, our results can also be interpreted as though primary exposure resulted from a failure to remove infected corpses. To examine whether or not our results would differ if all introduction were from external sources, we examined the same scenarios of disease spread while restricting the incidence of primary exposure to the periphery of the nest (within two cells of the maximum *r*). This restriction had no effect on survival, and these models are not presented.

Workers development and mobility

To stimulate normal colony growth, 25 eggs were added every 300 iterations (≅ to 30 days) in accordance with some observed patterns of oviposition in small social insect colonies (Castle 1934), though any pattern of oviposition (e.g., single large groups of eggs with synchronous maturation before subsequent oviposition, or overlapping generations) can be used if it is held constant across experimental models (Fefferman et al., *in preparation*). Workers aged during each iteration and after an appropriate number of iterations in a given developmental stage (Rosengaus and Traniello 2001; see Table 2) workers progressed to the next stage until reaching 'adulthood'. Initially, first and second instar larvae did not move away from the center of the nest, but as they matured, they were allowed to move "outward" by one cell to avoid an artificially dense nest center and emulate the often centripetal age-related movement of workers. These restrictions on movement reflect the limited mobility of young larvae in natural colonies of termites and most species of social *Hymenoptera* (Wilson 1971).

Topology, colony size, and denisty

All models defined a simplified, two-dimensional circular nest with ~10,000 "cells" or discrete areas through which an initial population of 1,000 "workers" could move and interact. No restriction was made on the number of workers occupying a single cell at a time and workers were only able to interact if they occupied the same cell. Colony size should not impact the results of our model because colony size and nest size are seen to vary proportionally in natural settings. Therefore, density and its concomitant effect on nestmate interaction rates should remain constant. To reflect ontogenic changes, our model focused on demographic distribution rather than colony size per se.

References

Abe T, Bignell DE, Higashi M (eds) (2000) Termites: evolution, sociality, symbioses, ecology. Kluwer, Dordrecht

Bienvenu RJ, Atchison FW, Cross EA (1968) Microbial inhibition by prepupae of the alkali bee, *Nomia melanderi*. J Invertebr Pathol 12:278–282

Brown CR, Brown MB (1986) Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). Ecology 67:1206–1218

Bulmer MS, Crozier RH (2004) Duplication and diversifying selection among termite antifungal peptides. Mol Biol Evol 21:2256–2264

Bulmer MS, Crozier RH (2006) Variation in positive selection in termite GNBPs and relish. Mol Biol Evol 23:317–326

Cane JH, Gerdin S, Wife G (1983) Mandibular gland secretions of solitary bees (*Hymenoptera*; *Apoidea*): potential for nest cell disinfection. J Kans Entomol Soc 56:199–204

Castle GB (1934) The dampwood termites of western United States, genus *Zootermopsis* (formerly, *Termopsis*). In: Kofoid CA (ed) Termites and termite control. University of California, Berkeley, pp 273–310

Duwel-Eby LE, Faulhaber LM, Karp RD (1991) Adaptive humoral immunity in the American cockroach. In: Gupta AP (ed) Immunology of insects and other arthropods. CRC, Boca Raton, FL, pp 385–402

Du Pasquier L, Flajnik M (1999) Origin and evolution of the vertebrate immune system. In: Paul W (ed) Fundamental immunology, 4th edn. pp 605–650

Fernandez-Marin H, Zimmerman JK, Wcislo WT (2003) Nest-founding in *Acromyrmex octospinosus* (*Hymenoptera*, *Formicidae*, *Attini*): demography and putative prophylactic behaviors. Insectes Soc 50:304–308

Fernandez-Marin H, Zimmerman JK, Wcislo WT (2004) Ecological traits and evolutionary sequence of nest establishment in fungus-growing ants (*Hymenoptera*, *Formicidae*, *Attini*). Biol J Linn Soc 81:39–48

Fernandez-Marin H, Zimmerman JK, Wcislo WT, Rehner SA (2005) Colony foundation, nest architecture and demography of a basal fungus-growing ant, *Mycocepurus smithii* (*Hymenoptera*, *Formicidae*). J Nat Hist 39:1735–1743

Field J, Brace S (2004) Pre-social benefits of extended parental care. Nature 428:650–652

Hultmark D (1993) Immune reactions in Drosophila and other insects: a model for innate immunity. Trends Genet 9:178–184

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge

Kaltenpoth M, Gottler W, Herzner G, Strohm E (2005) Symbiotic bacteria protect wasp larvae from fungal infestation. Curr Biol 15:475–479

Loehle C (1995) Social barriers to pathogen transmission in wild animal populations. Ecology 76:326–335

McCarthy MA, Thompson C (2001) Expected minimum population size as a measure of threat. Anim Conserv 4:351–355

Moret Y, Schmid-Hempel P (2004) Social life-history response to individual immune challenge of workers of *Bombus terrestris* L.: a possible new cooperative phenomenon. Ecol Lett 7:146–152

Naug D, Camazine S (2002) The role of colony organization on pathogen transmission in social insects. J Theor Biol 215:427–439

Nelson KE, Williams CM, Graham NMH (2001) Infectious diseases epidemiology, theory and practice. Aspen, Maryland

Nunn CL, Gittleman JL, Antonovics J (2000) Promiscuity and the primate immune system. Science 290:1168–1170

Pancer Z, Amemiya CT, Ehrhardt GRA, Celtllin J, Gartland GL, Cooper MD (2004) Somatic diversification of variable lymphocyte receptors in the agnathan sea lamprey. Nature 430:174–180

Pie MR, Rosengaus RB, Traniello JFA (2004) Nest architecture, activity pattern, worker density and the dynamics of disease transmission in social insects. J Theor Biol 226:45–51

Rhodes CJ, Anderson RM (1996) Persistence and dynamics in lattice models of epidemic spread. J Theor Biol 180:125–133

Rosengaus RB, Traniello JFA (1991) Biparental care in incipient colonies of the dampwood termite *Zootermopsis angusticollis* Hagen (Isoptera: Termopsidae). J Insect Behav 4:633–647

Rosengaus RB, Traniello JFA (1997) Pathobiology and disease transmission in dampwood termites *Zootermopsis angusticollis* (Isoptera: Termopsidae) infected with the fungus *Metarhizium anisopliae* (Deuteromycotina: Hypomycetes). Sociobiology 30:185–195

Rosengaus RB, Traniello JFA (2001) Disease susceptibility and the adaptive nature of colony demography in the dampwood termite *Zootermopsis angusticollis*. Behav Ecol Sociobiol 50:546–556

Rosengaus RB, Maxmen AB, Coates LE, Traniello JFA (1998) Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). Behav Ecol Sociobiol 44:125–134

Rosengaus RB, Traniello JFA, Chen T, Brown JJ, Karp RD (1999) Immunity in a social insect. Naturwissenschaften 86:588–591

Sanchez-Villagra MR, Pope TR, Salas V (1998) Relation of intergroup variation in allogrooming to group social structure and ectoparasite loads in red howlers (*Alouatta seniculus*). Int J Primatol 19:473–491

Schmid-Hempel P (1998) Parasites in social insects. Princeton University Press, Princeton

Seelinger G, Seelinger U (1983) On the social organization, alarm and fighting in the primitive cockroach *Cryptocercus punctulatus* Scudder. Z Tierpsychol 61:315–333

Strohm E, Linsenmair KE (2001) Females of the European beewolf preserve their honeybee prey against competing fungi. Ecol Entomol 26:198–203

Thorne BL, Traniello JFA (2003) Comparative social biology of basal taxa of ants and termites. Annu Rev Entomol 48:283–306

Traniello JFA, Rosengaus RB, Savoie K (2002) The development of immunity in a social insect: evidence for the group facilitation of disease resistance. Proc Natl Acad Sci USA 99: 6838–6842

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge

Wilson K, Cotter SC (2004) Density dependent prophylaxis in insects. In: Ananthakrishana TN, Whitman TW (eds) Insects and phenotypic plasticity. Science, Enfield

Wilson K, Reeson AF (1998) Density-dependent prophylaxis: evidence from Lepidoptera–baculovirus interactions. Ecol Entomol 23:100–101

Wilson K, Thomas MB, Blanford S, Doggett M, Simpson SJ, Moore SL (2002) Coping with crowds: density-dependent disease resistance in desert locusts. Proc Natl Acad Sci USA 99:5471–5475