A colony-level response to disease control in a leaf-cutting ant

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Abstract

Parasites and pathogens often impose significant costs on their hosts. This is particularly true for social organisms, where the genetic structure of groups and the accumulation of contaminated waste facilitate disease transmission. In response, hosts have evolved many mechanisms of defence against parasites. Here we present evidence that *Atta colombica*, a leaf-cutting ant, may combat *Escovopsis*, a dangerous parasite of *Atta*'s garden fungus, through a colony-level behavioural response. In *Atta colombica*, garden waste is removed from within the colony and transported to the midden – an external waste dump - where it is processed by a group of midden workers. We found that colonies infected with *Escovopsis* have elevated numbers of workers on the midden, where *Escovopsis* is deposited. Further, midden workers are highly effective in dispersing newly deposited waste away from the dumping site. Thus, the colony-level task allocation strategies of the *Atta* superorganism may change in response to the threat of disease to a third, essential party.

Introduction

Waste disposal strategies have to be implemented at all levels of biological organisation. This is particularly true for social organisms, where waste can act as a disease reservoir and thus impose a significant cost on social life (e.g., Snow 1855). When social groups are small, waste may not accumulate sufficiently to pose a serious disease threat. However, as groups become larger, waste and associated diseases pose an increasing threat to sociality. Unless strategies are in place to deal with the hazards posed, sociality may breakdown completely as the costs imposed by waste accumulation overcome the benefits of social life.

Social insects face an array of waste-related disease risks, including problems caused by individual faecal products, the disposal of dead colony members, and the management of food-processing waste (e.g., seed-hulls in seed-harvesting ants)(for a review, see Schmid-Hempel 1998). Strategies to deal with such problems include defecation by *Nosema*-infected honey bees outside of the hive to reduce disease-transmission (Schmid-Hempel 1998), specialized necrophoric behaviour to remove diseased individuals from the colony (e.g., Rothenbuhler 1964), and, potentially, midden work to manage large-scale waste. Social insects also exhibit direct responses to the threat of disease, including the communication of disease risk (Rosengaus et al. 1999a) and changes in social behaviour to minimise susceptibility (Rosengaus et al. 1998). Because of the high level of social organization in social insects, such strategies and responses can exist at the level of the individual, group or, potentially, colony. Here we investigate the potential for a colony-level response to waste-related disease risk in the leaf-cutting ant, *Atta colombica*.

Leaf-cutting ants (*Atta* and *Acromyrmex*) culture a basidiomycete fungus on harvested leaves, on which fungus they are completely nutritionally reliant (Weber 1972). Consequently, they must combat both direct disease-risks to themselves, and diseases that attack their fungal food-source. The main disease threat faced by the symbiotic fungus of leaf-cutting ants is *Escovopsis*, a virulent and potentially fatal fungal parasite (Currie et al. 1999a,b). *Escovopsis* invades the fungus gardens of leaf-cutting ant colonies and in extreme cases can overgrow these gardens, leading to colony starvation and death (Currie 2001). To counter the effects of this pathogen, leafcutting ants have entered into another symbiosis with an actinomycete bacterium that lives on the ant cuticle and produces antibiotics against *Escovopsis* (Currie et al. 1999a). However, despite this defence, the pathogen may still succeed in destroying a colony (Currie et al. 1999b, AG Hart pers. obs.).

A large *Atta* colony may produce litres of agricultural fungus waste every day (AG Hart pers. obs.) which is placed in either internal or, in the case of *Atta colombica*, external middens for management (Weber 1972). Such waste poses a mortality cost to workers (Bot et al. 2001). This situation is exacerbated by *Escovopsis* infection, as infected colonies produce waste which is contaminated by the parasite (Bot et al. 2001, Currie and Stuart 2001), and may thus serve as a source of re-infection (AG hart pers. obs.). Consequently, we would expect the functional activity of midden workers to reflect the current waste-related disease threat. Midden workers should perform tasks that reduce the threat posed by *Escovopsis* to the colony, and only be employed when that threat exceeds a certain level.

Here we ask whether allocation of workers to midden work by *A. colombica* colonies is related to the current threat posed by *Escovopsis*. We also investigate the functional role midden workers play in waste management. Together, the answers to these questions allow us to determine whether behaviour and patterns of task allocation may be effective strategies in controlling diseases.

Methods

Midden workers and Escovopsis *presence*

We studied 13 nests of *A. colombica* in the Gamboa district of the former Canal Zone, Panama during April 2000. We counted the number of workers working on the midden of each nest with 3 scan counts at each counting event once a week for 6 weeks. To determine the presence of *Escovopsis* in nests, we followed the protocol of Currie (2001). In week 4, after the first 3 weeks of counts, we used sterile forceps to collect 18 waste pieces from workers carrying waste to the midden. At the same time, three scan counts of the number of midden workers were made. Waste pieces were put onto potato-dextrose agar plates (9 pieces to a plate). The plates were left for 12 hours at 25^{o}C and the presence or absence or *Escovopsis* scored for each waste piece for all colonies. Because the number of midden workers might simply reflect colony size, we controlled for this by using the number of foraging entrances as a relative measure of colony size (Currie

2001). Midden worker numbers were then divided by the number of foraging entrances prior to analyses. The means of these corrected scan counts were used for analyses.

Functional role of midden workers

To determine the role played by midden workers in distributing waste around the midden we soaked sheets of paper overnight in the waste of each of 10 colonies with different numbers of midden workers. These colonies were a subset of the experimental colonies above, chosen for ease of access and to represent a wide range of midden worker numbers, but without respect to their current infection status. One hundred 3mm squares of paper were cut from each sheet and put on the midden of the colony in whose waste the paper had been soaked. The pieces were put at the site where workers were dumping waste, in an area less than 5cm square. The distance each piece had moved after 2 hours was measured. The number of workers on the midden at the time of the experiment was counted with 3 scan counts.

Results

Midden workers and Escovopsis *presence*

There were significantly more workers on middens of colonies infected with *Escovopsis* than on middens of uninfected colonies (t-test for samples with unequal variances, t = 2.327, d.f. = 7.813, $p < 0.05$; Figure 1; uncorrected means: uninfected = 12 ± 5.5 (N=5), infected = 41 ± 12.4 (N = 8)). There was no correlation between the number of midden workers in infected colonies and the degree of infection (Spearman's rank correlation, r = -0.2, $P > 0.6$; number of pieces infected ranged from 1 to 7).

Functional role of midden workers

Midden workers strongly influenced the distribution of the experimental waste around the midden (Figure 2). On middens with more than 20 workers, most of the paper pieces were moved further than 10cm from the dumping site within two hours (mean = 87 pieces, s.d. = 18.2 pieces), whereas on middens with less than 20 workers few pieces were moved (mean = 2.4 pieces moved further than 10cm, s.d. = 2.9 $t = 10.27$, d.f. = 8, $p < 0.001$).

Discussion

Colonies which are infected with *Escovopsis* also have higher numbers of workers on the midden, where *Escovopsis* is deposited. These midden workers, when present in such numbers, are effective in dispersing newly deposited waste away from the dumping site. Together, these results suggest that *Atta colombica* colonies use a behavioural strategy of disease control, i.e., increasing allocation of workers to midden tasks is an attempt to prevent re-infection by the potentially deadly parasitic fungus *Escovopsis*.

Our results suggest that colonies may reduce both the level of contact between waste-transporting ants and infected waste, as well as the gradual backing-up of waste dumps onto colony entrances, by allocating more workers to midden work. Our experimental results showed that when large numbers of midden workers are present, as seen in *Escovopsis* infected colonies, new waste gets rapidly moved away from dumping sites. The absence of a correlation between the degree of infection and the number of midden workers may reflect either an "all-or-nothing" response to infection, or a lack of power in the test. The low number of midden workers on middens free of *Escovopsis* suggests that there may be significant costs associated with midden work. Workers suffer high mortality when they are kept in close contact with waste irrespective of whether *Escovopsis* is present (Bot et al. 2001). Thus, allocating workers to middens is likely to reduce both life span (a demographic cost) and the number of workers available for other tasks (an ergonomic cost). Consequently, resource allocation strategies within the *Atta* colony may change in response to the threat of disease, only investing in costly midden work when it is essential to colony survival. Task allocation in social insects responds to environmental perturbation (Gordon 1996), and recent work by Starks et al. (2000) showed that honey bee colonies respond to infection by inducing 'fever' conditions. Functionally analogous changes in behavioural responses to the direct threat of disease have been demonstrated in groups of termites (Rosengaus et al. 1998, 1999a). Here, we show that colonies may respond, via task allocation strategies, to a disease threat to a third party, in this case the garden fungus of the ants. Such strategies, together with physiological immunity (e.g., Rosengaus et al. 1999b), may result in a disease-response that is integrated across the different organisational levels of social insect colonies.

Our results show the connection between pathogen presence and patterns of worker allocation. In addition, we empirically demonstrate the functional role played by the workers allocated to the threat-management task. However, our study only provides correlational evidence for the association between *Escovopsis* presence and colony allocation to midden work. Further studies, where the presence of *Escovopsis* is manipulated, are required to substantiate the causal link between pathogen and response suggested by our results. *Atta colombica*, with external waste middens and a well-characterized pathogen, is an ideal model system to investigate aspects of disease- and waste-management in a complex social system and this study is a start to characterizing some of the more subtle responses of social insects to the ever-present threat of disease (Schmid-Hempel 1998).

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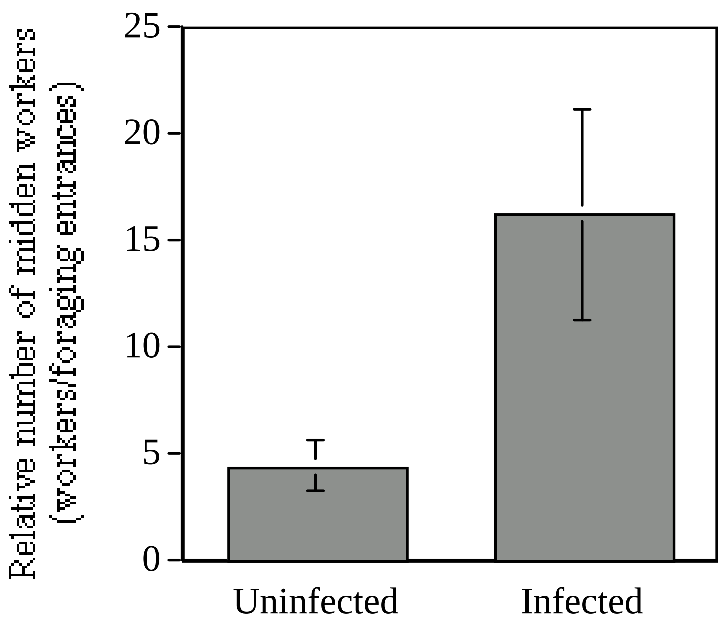
Figure legends

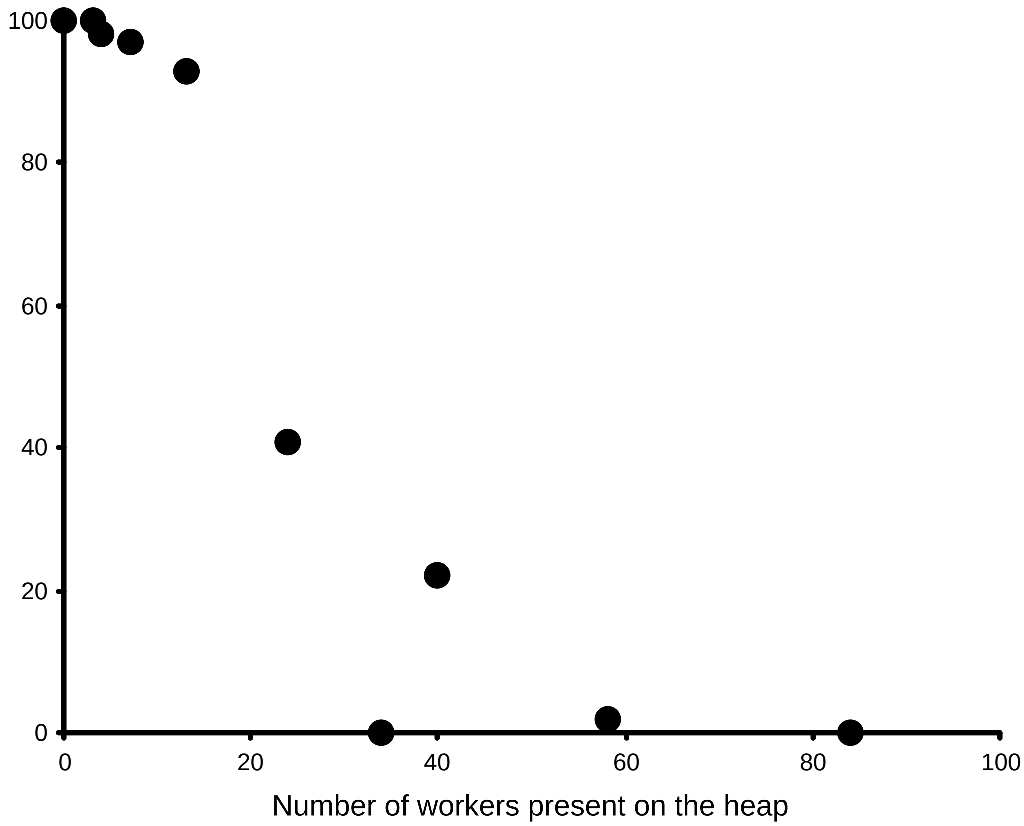
Figure 1. The mean and standard error for the number of midden workers (corrected for colony size, see Methods) on colonies uninfected and infected by the fungal parasite *Escovopsis*. There were significantly more workers on the middens of infected colonies (see Results for statistical analyses).

Figure 2. The number of midden workers at 10 colonies (*x*-axis) plotted against the number of pieces of experimental waste moved >10cm away from its dumping site after 2 hours (*y*-axis). The presence of about 20 or more workers results in redistribution of the waste. Below 20 workers, waste redistribution is almost non-existent.

Figure 1


Relative number of midden workers
(workers/foraging entrances)
25
20
15
10
5
0
Uninfected
Infected

224 Figure 2



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