Hitchhiking and the removal of microbial contaminants by the leaf-cutting ant *Atta colombica*

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Abstract. 1. The ecologically dominant leaf-cutting ants exhibit one of the most complex forms of morphological caste-based division of labour in order to efficiently conduct tasks, ranging from harvesting fresh leaf material to caring for the vulnerable fungal crop they farm as food. While much of their division of labour is well known, the role of the smallest workers on foraging trails is puzzling. Frequently these minim workers hitchhike on leaf fragments and it has been suggested that they may act to reduce the microbial contamination of leaf material before they enter the nest. Here we investigated this potentially important role of minims with field colonies of *Atta colombica*.

2. We experimentally increased the microbial load of leaf fragments and found that this resulted in minims hitchhiking on leaf fragments for longer. Furthermore, we show that leaves naturally have a significant microbial load and that the presence of hitchhikers reduces the microbial load of both experimentally manipulated and natural leaf fragments.

3. Intriguingly, the microbial load of leaves high in the canopy where ants were foraging was much lower than closer to the ground where the ants avoided cutting leaves. This suggests that the often perplexing foraging patterns of leaf-cutting ants may in part be explained by the ants avoiding leaves that are more heavily contaminated with microbes.

4. The removal of microbial contaminants is therefore an important role of hitchhiking minim workers in natural colonies of *Atta* leaf-cutting ants, although other tasks such as trail maintenance and defence also explain their occurrence on trails.

Key words. Castes, division of labour, hygienic behaviour, social insect, symbiosis.

Introduction

Leaf-cutting ants (*Atta* and *Acromyrmex* species) are dominant herbivores of the Neotropics and have large, complex societies based upon division of labour (Hölldobler & Wilson, 1990). Division of labour is the hallmark of social insects and is integral to their ecological success, allowing multiple tasks to be conducted simultaneously, individual specialisation and an overall increase in work output. In many species, division of labour is determined by experience or temporal patterns often correlating with age, but in approximately 20% of ant species, it is also influenced by morphological castes (Oster & Wilson, 1978). The *Atta* leaf-cutting ants exhibit one of the most extreme forms of such polymorphism, with workers displaying an 8-fold range in head width and a 200-fold range in dry body mass (Hölldobler & Wilson, 1990). Although the size distribution is continuous, workers can be divided into four main castes: minims, medias, foragers, and soldiers (Wilson, 1980). Minims are generally considered to work within the nest, medias are within-nest generalists, foragers cut leaves and soldiers defend against vertebrate predators. Leaf-cutting ants may have a particular need for such alloethism, because they use almost exclusively fresh vegetation as a substrate for their mutualistic fungal crop and their large colonies exhibit complex social organisation to harvest it (Weber, 1972). This presents leaf-cutting ant colonies with a variety of challenges, from harvesting leaf material to protecting the vulnerable fungal crop from competitive or pathogenic microbes.

While the broad patterns of division of labour in leaf-cutting ant colonies are well known, there is an intriguing anomaly

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that remains unexplained. The smallest workers work most commonly within the nest, caring for the brood and fungal crop (Weber, 1972; Wilson, 1980; Hughes *et al*., 2003). However, minims are also seen in significant numbers on the foraging trails of *Atta* where their role is unclear, because they are too small to contribute to the cutting or transport of leaves. Many of these minims on trails may 'hitchhike' on the leaf fragments being transported by their larger nestmates, and the leading explanation for the presence of minims on *Atta* trails is that these hitchhikers defend against parasitoid phorid flies (Feener & Moss, 1990). Phorids often land on leaf fragments before ovipositing into the head of the transporting worker and the presence of hitchhiking minims appears to hinder this. Accordingly, the presence of phorid flies can result in an increase in the occurrence of hitchhiking (Feener & Moss, 1990; Braganca *et al*., 1998).

However, several pieces of evidence suggest that defence against phorids cannot provide the entire, or even the main, explanation for hitchhiking or the presence of minims on foraging trails. Most minims on trails do not hitchhike, and the role of these ants appears instead to be trail maintenance or the detection of threats in general (Hughes & Goulson, 2001; Evison *et al*., 2008). Phorids are diurnal (Orr, 1992; Feener & Brown, 1993), yet hitchhiking is also common at night and can even be more common than during the day (Linksvayer *et al*., 2002; Yackulic & Lewis, 2007). Parasitism rates by phorids of foraging ants also appear to generally be very low (<1%; Yackulic & Lewis, 2007). In addition, defence against phorids is characterised by ants adopting a head-up posture with open mandibles (Feener & Moss, 1990), but hitchhikers are frequently seen in a head-down posture (Linksvayer *et al*., 2002; Vieira-Neto *et al*., 2006). It is unlikely that minims hitchhike to conserve energy because they frequently move around on leaves and disembark before reaching the nest entrance (Linksvayer *et al*., 2002; Vieira-Neto *et al*., 2006). They may possibly imbibe leaf sap (Stradling, 1978; Vieira-Neto *et al*., 2006), but the position and behaviour of hitchhikers in the field suggest this is only a subsidiary function (Linksvayer *et al*., 2002).

One likely explanation for hitchhikers is that they clean leaf fragments prior to entry into the colony. The fungal crop of leaf-cutting ants is highly vulnerable to competitive and pathogenic microbes such as the fungal weed *Escovopsis* (Currie *et al*., 1999; Currie, 2001; Currie & Stuart, 2001). Minims are known to play the main role in decontaminating leaf material within nests, as well as in general providing the main line of defence against parasites (Weber, 1972; Wilson, 1980; Jaccoud *et al*., 1999; Hughes *et al*., 2002; Poulsen *et al*., 2002a, 2006). In keeping with this, hitchhikers are frequently seen in a head-down posture on leaves (Linksvayer *et al*., 2002), and cornflakes experimentally contaminated with fungal spores have more hitchhikers than uncontaminated cornflakes (Vieira-Neto *et al*., 2006). However, what effect, if any, hitchhikers actually have on the microbial contamination of leaves is unknown, as well as whether the natural loads of leaves are even significant enough to require decontamination.

Here we investigate in the field the hypothesis that hitchhiking minims reduce the microbial load of leaf fragments before they enter the colony. We experimentally increase the microbial load of leaf fragments to examine how this affects hitchhiking frequencies and examine the natural microbial loads of leaves. We also determine the effect of hitchhiking on the microbial loads of leaves, both those manipulated experimentally and those with natural microbial loads. We used the entomopathogenic fungus *Metarhizium anisopliae* as the model microbe in our experimental manipulations, because it is a virulent, natural parasite of *Atta* leaf-cutting ants in the area studied (Hughes *et al*., 2004a,b). Finally, we investigate the behaviours of minims on trails to determine the extent to which their presence on trails can be explained by hitchhiking.

Materials and methods

Experiments were conducted in Panama during June and July 2009. Five mature (mound size greater than 10 m^2) *Atta colombica* colonies were used: one located on the Donato trail, Barro Colorado Island (Donato; 9°09.783′N, 79°50.228′W) and the remaining four on Pipeline Road, Gamboa (Pipeline A: 9°08.061′N, 79°43.327′W; Pipeline B: 9°08.055′N, 79°43.288′W; Pipeline C: 9°08.012′N, 79°43.204′W, Pipeline D: 9°07.962′N, 79°43.191′W).

Experiment 1: Metarhizium *spore load and hitchhiking*

For each of the five colonies, the trail with the greatest proportion of leaves carrying hitchhikers was identified based on a 3-min traffic count for consistency. Workers transporting leaf fragments on this trail were then timed to establish the distance from the nest entrance that took approximately 10 min for a leaf-laden worker to travel. At this point, 'normal' leaf fragments (freshly cut by the ants from a living leaf, not obviously larger or smaller than the majority of fragments being transported; mean ± SE area 110.2 ± 2.6 mm^2), lacking a hitchhiker, were removed from the trails (along with the transporting individual) using sterile forceps. Leaf fragments had either 1 μl applied to their dorsal surface of: (i) a low or (ii) high dose of *Metarhizium* spores, or (iii) 0.05% Triton-X control solution, or (iv) were left as blank controls. The *Metarhizium* used was strain KVL02-73 of *Metarhizium anisopliae* var. *anisopliae* that had been isolated from soil collected near leaf-cutting ant colonies in Gamboa, Panama (Hughes *et al*., 2004b). The low and high dose spore suspensions were made from a freshly sporulating plate and contained either 1.6×10^6 spores ml^{-1} or 6.8×10^7 spores ml^{-1}, respectively, in 0.05% Triton-X solution. A small triangle was cut from the leaf fragment to enable identification and the fragment was then replaced on the foraging trail at the same point as where they were collected from. Fragments were transported by ants soon after placement and were observed for the duration of their journey to the nest entrance. We recorded both whether or not leaf fragments had a hitchhiker during this time and the cumulative time leaf fragments had hitchhikers. Hitchhiking events were only recorded if an ant spent >2 s on the leaf fragment. Treatments were repeated six times

with each of the five colonies over different days ($n = 30$), with the order of treatments being changed systematically between days. The time of day of each replicate was recorded as: morning (10.00–12.00 hours), early afternoon (12.01–14.00 hours), late afternoon (14.01–16.00 hours) or evening (16.01–18.00 hours).

Simultaneously with the high dose *Metarhizium* treatment above, a second set of leaf fragments were treated with the high dose of *Metarhizium* and placed to one side of the foraging trails, such that they did not come into contact with the ants ($n = 30$). One set of leaf fragments treated with a high dose of *Metarhizium* was thus exposed to hitchhikers while the second set was not. Immediately before the leaf fragments exposed to hitchhikers were taken into the nest entrances, they were removed. The paired leaf fragments that had not been exposed to hitchhikers were also collected at the same time and both the exposed and unexposed leaves were placed into individual Eppendorf tubes using sterile forceps. Within 5 h of this treatment, 1 ml of sterile water was added to each tube and the tube then vortexed thoroughly for 1 min. The concentration of *Metarhizium* spores in the suspensions was then quantified using a haemocytometer.

Experiment 2: natural microbial load on leaves

At each of the five experimental colonies, 20 normal leaf fragments were taken from foraging workers at two locations: (i) in the canopy close to where workers were cutting leaves and (ii) from individuals on the same foraging trail close to the nest entrance. Before collection, the proportion of leaf fragments carrying hitchhikers at each location was determined over three observation periods of 3 min each. In addition, 20 whole leaves were taken from lower down the same tree, within 1 m of the ground and thus below the height at which the ants were foraging. All leaves and leaf fragments were collected using sterile forceps and stored individually in Eppendorf tubes or Ziploc®bags.

A 5×5 mm section was cut from each whole leaf or leaf fragment using a sterile scalpel blade, with sections cut from whole leaves being taken from the middle, 1 cm to the left side of the central vein. The leaf sections were then placed in individual Eppendorf tubes with 1 ml of sterile water and vortexed for 1 min. Twenty microlitres of each solution was then spread on to 90 mm potato dextrose agar (PDA) plates, with two samples from the same colony and location being plated on to each half of each plate. Plates were stored at ca 28 °C, and the numbers of fungal and bacterial colony forming units (CFUs) were recorded after 24 and 48 h. All leaf material was prepared and plated inside a laminar flow cabinet to prevent contamination.

Experiment 3: minim behaviour on trails

Minim workers were observed on trails either close to, or 10 m from, nest entrances. Individual minims were observed continuously for up to 5 min, and their first behaviour recorded. These behaviours were either: hitchhiking, self-grooming, allogrooming, trail maintenance (during which the ant inspected the trail for periods of greater than 5 s, moved soil particles to fill holes or removed obstructions), defence (characterised by alarm behaviours such as gaping mandibles directed at another organism; Hughes & Goulson, 2001; Hughes *et al.*, 2001), inspection (the sustained or repeated antennation of a nestmate), or walking (where the focal individual carried out none of the aforementioned behaviours but continued to walk for the duration of the 5 min observation period). These observations were carried out for 15 individuals at each location (close to, and 10 m from, nest entrances) for three colonies (Donato, Pipeline A and Pipeline B), which were summed to give 45 replicates per location.

Statistical analysis

Generalised linear models using a binomial distribution and a logit link function were used to examine how the proportion of leaf fragments carrying hitchhikers was affected by treatment, colony, time of day and leaf fragment size in Experiment 1, and by location (canopy or close to nest entrance) and colony in Experiment 2. A linear model using arcsine square-root transformed data was used to test the effects of treatment, colony, time of day, and leaf fragment size on the proportion of total journey time that a leaf fragment carried a hitchhiker. Linear models using $\log_{10}(x + 1)$ transformed data were used to investigate the numbers of *Metarhizium* spores found on leaf fragments in both Experiments 1 and 2. All linear and generalised linear models were performed in R (R Development Core Team 2008, Vienna, Austria; available from http://www.r-project.org) and model assumptions were met. Where interaction and single terms were non-significant, they were removed from the model through stepwise deletion until a minimum adequate model was obtained. A Fisher's Exact test was used in Experiment 3 to test for a difference between locations in the frequencies of observed behaviours.

Results

Experiment 1: Metarhizium *spore load and hitchhiking*

There were no significant interactions between treatment, colony, time of day, and leaf fragment size for either the proportion of leaf fragments carrying a hitchhiker or the proportion of journey time leaf fragments had hitchhikers ($P > 0.05$ in all cases). Treatments did not differ significantly in the proportion of leaf fragments with hitchhikers, with these being only slightly higher when *Metarhizium* had been applied to leaf fragments ($Z_3 = 1.178$, $P = 0.239$; Fig. 1a). However, the application of a low or, especially, a high dose of *Metarhizium* spores to leaf fragments did result in them having hitchhikers for a significantly greater proportion of journey time ($F_{3,112} = 3.05$, $P = 0.032$; Fig. 1b). Colonies differed significantly in both the proportions of leaf fragments with hitchhikers ($Z_4 = 3.129$, $P = 0.001$; Fig. S1a) and the proportion of journey time leaf fragments had hitchhikers
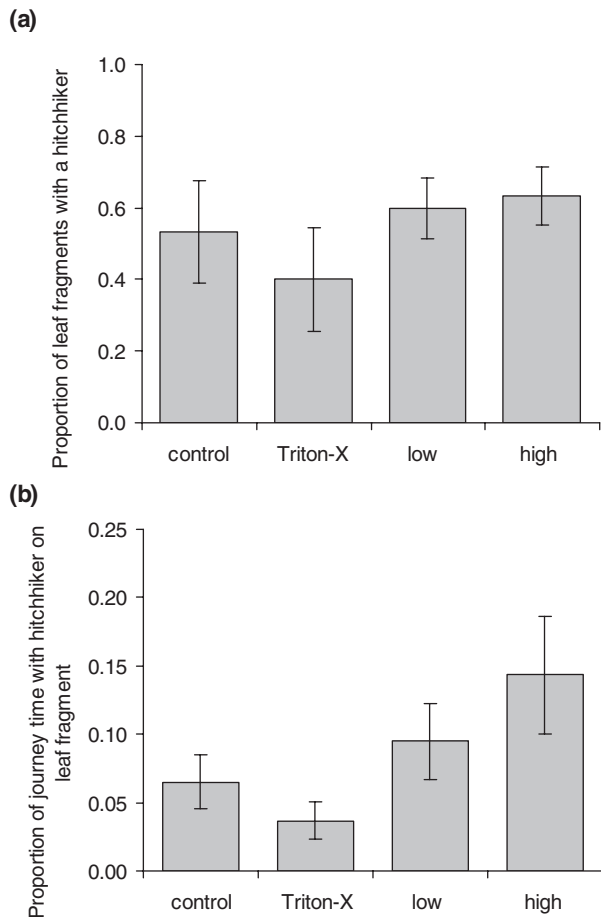
(a)

(b)

Fig. 1. The effect of treatment in Experiment 1 on (a) the mean ± SE proportion of leaf fragments carried by *Atta colombica* that had a hitchhiker and (b) the mean ± SE proportion of journey time during which a leaf fragment had a hitchhiker.

($F_{4,111} = 2.69$, $P = 0.035$; Fig. S1b), with the Donato colony having much lower hitchhiking rates than the Pipeline Road colonies. Time of day did not affect the proportion of leaf fragments with hitchhikers ($Z_3 = 0.01$ $P = 0.625$), but did affect the proportion of the journey time that leaf fragments had hitchhikers ($F_{4,111} = 3.98$, $P = 0.005$), with those transported during early afternoon and evening carrying hitchhikers for less time than those transported during morning or late afternoon (Fig. S2). Leaf fragment size has no significant effect on the proportion of fragments carrying hitchhikers ($Z_1 = 0.435$ $P = 0.664$) or the proportion of the journey leaf fragments had hitchhikers ($F_{1,96} = 0.40$, $P = 0.530$).

The number of *Metarhizium* spores on leaf fragments was significantly lower when they had been exposed to hitchhikers ($F_{1,56} = 21.41$, $P < 0.001$; Fig. 2a). The relationship between the length of time that leaf fragments had hitchhikers and the numbers of *Metarhizium* spores on the fragments was not significant ($F_{4,24} = 0.32$, $P = 0.86$), although there was a negative correlation with a medium effect size ($r = -0.26$; Fig. 2b). Most of the leaf fragments carried minims for only a

very short period of time during this experiment, which limited the statistical power of the analysis. Whenever minims stayed on leaf fragments for >3 min (6 out of 30 replicates), very few spores were found to remain on the leaves (Fig. 2b). The numbers of *Metarhizium* spores on leaf fragments did not differ between colonies, and there was also no significant interaction between colony and hitchhiker exposure ($P > 0.05$ in both cases).

Experiment 2: natural microbial load on leaves

The number of microbial CFUs found on leaf fragments taken from foraging ants in the canopy was generally higher (except in the Pipeline D colony) than for leaf fragments taken from ants near the nest entrance (Fig. 3a), but did not differ significantly ($F_{1,177} = 0.13$, $P = 0.71$). The colonies differed significantly in the microbial load of their fragments ($F_{4,174} = 14.72$, $P < 0.001$), with the Pipeline A colony having a lower load than the other colonies. There was a significant interaction between colony and location for the proportions of leaves with hitchhikers ($Z_4 = 5.59$, $P < 0.001$). A greater proportion of the leaf fragments collected near the nest entrances had hitchhikers than those collected in the canopy, for all colonies apart from Donato (Fig. 3b). For whole leaves collected either from low vegetation or high in the canopy where the ants were foraging, there was a significant interaction between the effects of colony and location on the microbial load of leaves ($F_{4,184} = 10.21$, $P < 0.001$). All colonies apart from Pipeline B had far more microbial CFUs on leaves collected from low vegetation than on leaves collected from high in the canopy (Fig. 3c).

Experiment 3: minim behaviour on trails

Hitchhiking was not a commonly observed behaviour either close to, or 10 m from, the nest entrances (Fig. 4). Trail maintenance was by far the most frequent behaviour performed by minims at both locations. The frequency of behaviours differed significantly between minims close to and 10 m from the nest entrance (Fisher's = 14.9, $P = 0.008$). Both hitchhiking and self grooming were more common 10 m from the nest entrance, while allogrooming and trail maintenance were more common close to the nest entrance (Fig. 4).

Discussion

This study investigated the role of minim workers on the foraging trails of *A. colombica* leaf-cuttings ants in the field and, in particular, their possible importance in the removal of microbial contaminants from leaf material. We found that the experimental addition of fungal spores to leaf fragments resulted in greater hitchhiking. Hitchhiking did reduce microbial contamination, with leaf fragments exposed to hitchhikers having fewer microbial CFUs than those which had not been available to hitchhikers. Importantly, we found that the microbial load of natural leaves was significant and
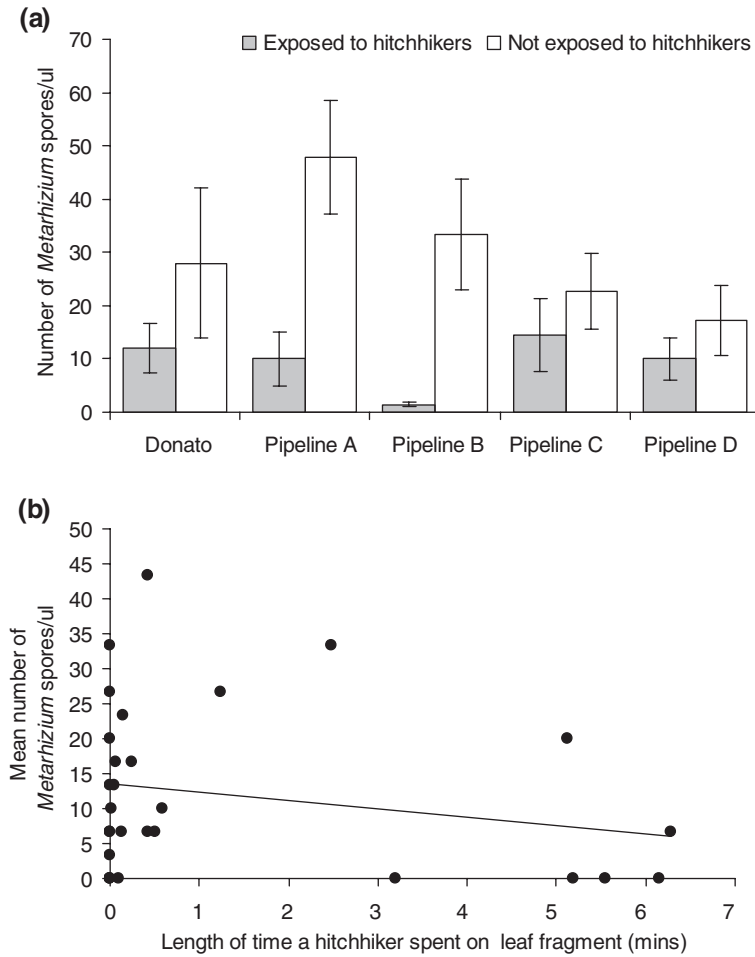
Fig. 2. The effect of hitchhiking on leaf fragment spore load in Experiment 1. (a) The mean $\pm$ SE number of *Metarhizium* spores μl^{-1} on leaf fragments treated with *Metarhizium* and then either exposed to hitchhikers or kept unexposed. (b) The relationship between the length of time that a leaf fragment had a hitchhiker and the number of *Metarhizium* spores μl^{-1} on it. The line of best fit is $y = 13.6 - 1.2x$; $r^2 = -0.07$.

lower on vegetation high in the canopy where the ants were foraging. Finally, we observed that only a relatively small proportion of minims on trails engage in hitchhiking, with most minims on trails engaging in other tasks, notably trail maintenance.

The fungal inoculation experiment showed that hitchhiking by minims is related to microbial contamination. Leaf fragments to which we had applied *Metarhizium* fungal spores carried hitchhikers for approximately double the length of time of the control leaf fragments. Treatment did not significantly affect the proportion of leaf fragments carrying hitchhikers, although the data showed the same pattern. These results indicate that there is a behavioural response by hitchhiking minims to fungal contaminants, but only once were they hitchhiking on a leaf fragment. It will presumably be difficult for them to sense the presence of contamination prior to that point, unless the transporting workers signal it. Such a signal may explain the disparity with the study by Vieira-Neto *et al.* (2006), which found a greater proportion of hitchhiking on cornflakes with added fungal spores. In our study the fungal spores were added to leaf fragments already in the process of being transported to the nest, and the transporting ants may therefore not have investigated the leaf fragments or sensed the presence of the

contamination. In the study by Vieira-Neto *et al.* (2006), in contrast, the cornflakes were inoculated in the foraging area prior to being selected for transport by the ants. Hence, transporting ants may therefore have investigated the cornflakes and signalled the presence of contamination, perhaps by stridulation (Roces & Hölldobler, 1995).

The effect of hitchhikers on microbial contamination is significant. After experimental inoculation with *Metarhizium*, the number of spores remaining on leaf fragments was substantially reduced when the fragments were exposed to hitchhikers. There was also a negative relationship between the duration of hitchhiking and the numbers of spores, although lack of power meant this was not statistically significant in spite of its moderate effect size. The level of reduction in fungal spores did vary considerably between colonies. This may have been due to variation between colonies in hitchhiking frequency (Feener & Moss, 1990; Linksvayer *et al.*, 2002; Vieira-Neto *et al.*, 2006), or to the behaviour of the minims. For example, colonies under greater attack from phorids, or which were foraging on leaves with greater natural contamination, may have hitchhikers that are less effective at removing the fungal spores that were experimentally applied. The impact of hitchhikers on the microbial contamination on
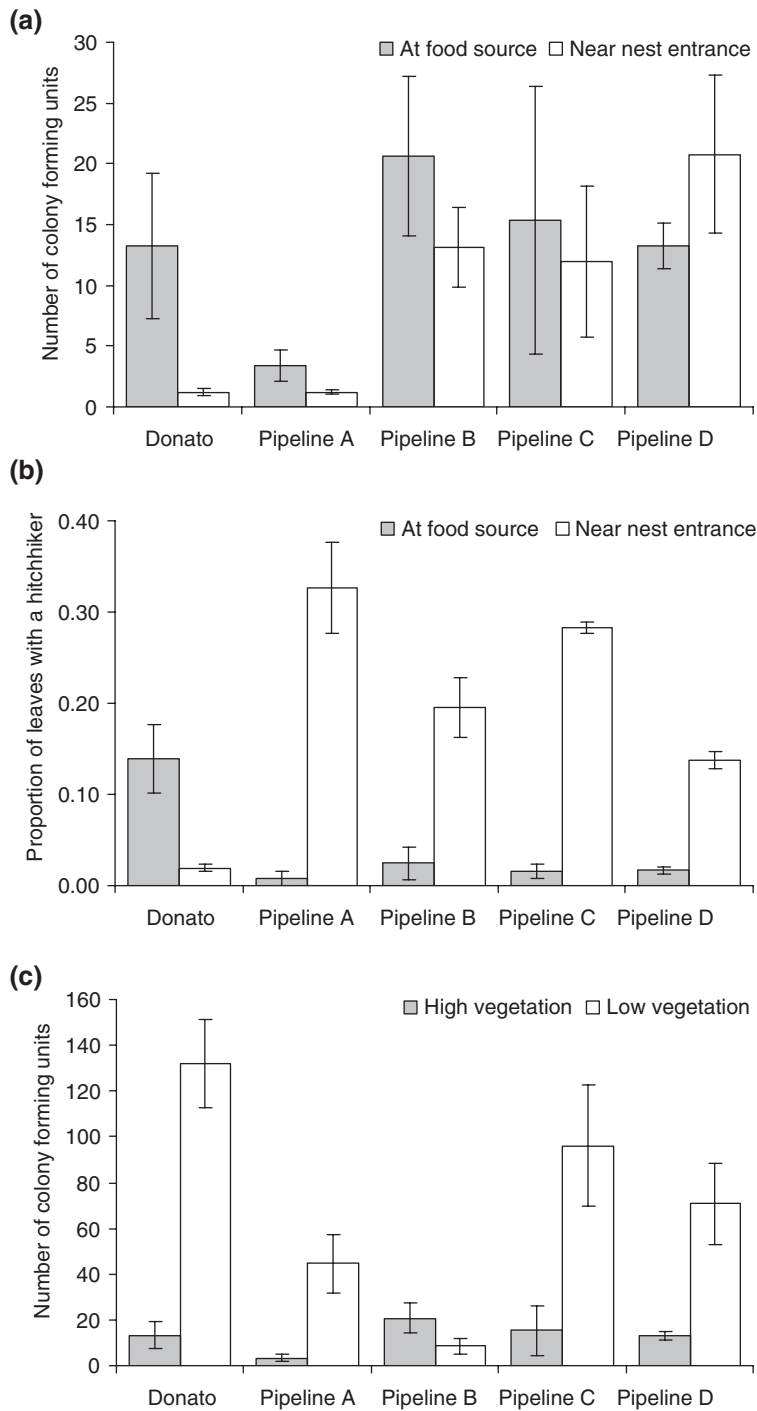
Fig. 3. Natural microbial loads and hitchhiking. (a) The mean ± SE number of colony forming units of fungi and bacteria and (b) the proportion of leaves with hitchhikers for leaf fragments taken from workers at the food source or close to the nest entrance. (c) The mean ± SE number of colony forming units of fungi and bacteria for whole leaves taken from high vegetation where workers were foraging (grey columns) or low vegetation where workers were not foraging (white columns).

leaf fragments may in fact be even greater than these data suggest. Cleaning has been generally assumed to take place through the removal of microbial contaminants (Linksvayer *et al.*, 2002; Vieira-Neto *et al.*, 2006), but it is also possible that minims spread antimicrobial secretions across leaf fragments while hitchhiking and that these may inhibit the viability of microbial contaminants. The secretion of the metapleural gland, in particular, inhibits the viability of bacteria and

fungal spores (Bot *et al.*, 2002; Poulsen *et al.*, 2002b), and leaf-cutting ants actively groom this gland when they detect microbial threats (Fernandez-Marin *et al.*, 2006, 2009). Leaf-cutting ants, and their minim workers in particular, have disproportionately large glands for their body size (Wilson, 1980; Bot & Boomsma, 1996; Hughes *et al.*, 2008, 2010), and it is possible this may be an adaption for decontaminating leaf material prior to its incorporation in the fungal crop.
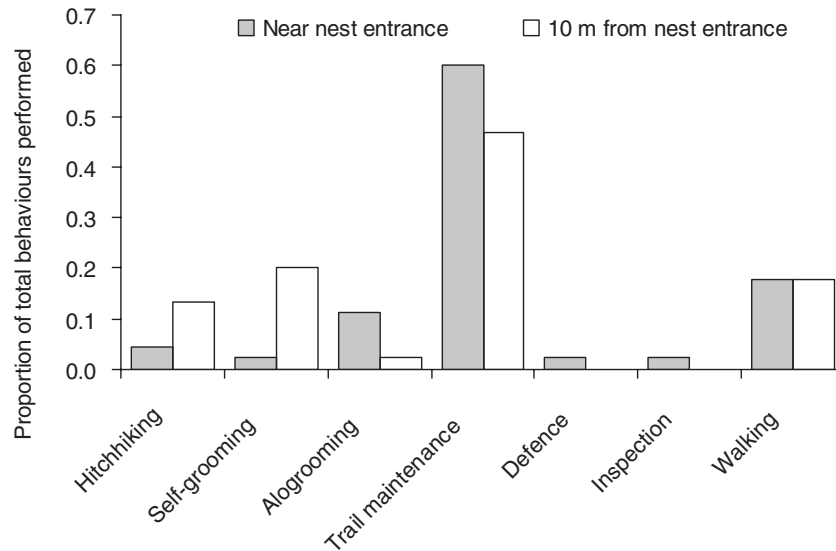
Fig. 4. The behaviours performed by minim workers close to the nest entrance or 10 m from the nest entrance.

The importance of hitchhiking to remove microbial contamination is demonstrated by the data on natural microbial loads. The numbers of CFUs of bacteria and fungi on leaves were considerable, both for leaf fragments collected from transporting workers in the canopy and near to the nest entrance. Intriguingly, there were significantly fewer CFUs on whole leaves collected from the canopy than from vegetation within 1 m of ground level. Rain splash has a major role in the dispersal of microorganisms, particularly fungal spores, through the air column and between vegetation (Fitt *et al.*, 1989; Madden, 1997). Droplets will accumulate microbes as they descend from leaf to leaf through the canopy and, as a result, leaves closer to ground level will have greater microbial loads, as we found. All of the colonies in our study were cutting leaves at least 5 m in height and ignoring vegetation closer to the ground or nest entrance, which appeared suitable for harvesting. Microbial contaminants may pose significant threats to leaf-cutting ant colonies and their removal or inhibition with antimicrobial compounds will be energetically costly (Currie *et al.*, 1999; Currie, 2001; Currie & Stuart, 2001; Hughes *et al.*, 2002, 2004b; Poulsen *et al.*, 2002b; Fernandez-Marin *et al.*, 2006, 2009; Van Bael *et al.*, 2009). While foraging higher in the canopy will also be energetically costly, it may be that this is outweighed by the benefit of obtaining leaf material less laden with microorganisms. Many hypotheses have previously been suggested to explain the often perplexing selection of plants by foraging leaf-cutting ants (Cherrett, 1972; Rockwood, 1976; Shephard, 1982; Hubbell *et al.*, 1984). Our results suggest that the level of microbial contamination on leaves may also play a role in the selection of foraging material by leaf-cutting ants.

It is important to place hitchhiking to remove microbial contaminants in the context of the overall behaviours exhibited by minims on foraging trails. Although minims are most frequently described as being on trails to defend against phorid parasitoids by hitchhiking (Feener & Moss, 1990; Hölldobler & Wilson, 1990; Orr, 1992), they have also been suggested to play the major roles in maintaining pheromone trails (Evison *et al.*, 2008), and in detecting and responding to threats or disturbance in general (Hughes & Goulson, 2001). In our study, only 4–13% of minims on trails were engaged in hitchhiking. This supports them having other functions. Trail maintenance was the most frequently observed behaviour, with approximately half of all minims on trails engaging in this. This behaviour included the inspection of the trail and movement of soil particles, as well as the removal of obstructions. Possibly minims may be particularly useful for such tasks, because their small size allows them to be allocated in large numbers, makes them unattractive targets for parasitoids and will allow forager ants to walk over them without impediment. In addition to trail maintenance, minims engaged in more allogrooming close to the nest entrance, which is in keeping with their hypothesised role in defending the colony against parasites and other microbes (Jaccoud *et al.*, 1999; Hughes *et al.*, 2002; Poulsen *et al.*, 2002a, 2006).

Minims in *Atta* leaf-cutting ants, as well as other ants, are principally thought of as being engaged in within-colony tasks and their role on foraging trails has most commonly been explained as providing defence against phorid parasitoids via hitchhiking (Feener & Moss, 1990; Hölldobler & Wilson, 1990; Orr, 1992). The results presented here demonstrate that hitchhiking minims also have a significant role in sanitising leaf material by removing microbial contaminants, and this may potentially be a more important role than defence against parasitoids. However, the majority of minims on foraging trails engage in other tasks, notably trail maintenance. Together with previous studies showing the role minims play in maintaining pheromone trails (Evison *et al.*, 2008), and general response to threats (Hughes & Goulson, 2001), these results therefore show that *Atta* minims on foraging trails engage in a diversity of

tasks, providing yet another example of the strikingly complex social organisation displayed by leaf-cutting ant societies.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-2311.2010.01212.x

Figure S1. Variation between colonies in Experiment 1 in (a) the proportion of leaf fragments carried by *Atta colombica* that had a hitchhiker and (b) the mean ± SE proportion of journey time during which a leaf fragment has a hitchhiker.

Figure S2. The effect of time period during which observations were made on the mean ± SE proportion of journey time during which a leaf fragment has a hitchhiker.

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