

Research article

Mutualistic bacteria and a possible trade-off between alternative defence mechanisms in *Acromyrmex* leaf-cutting ants

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Summary. Leaf-cutting ants in the genus *Acromyrmex* are obligately dependent upon a mutualistic fungus as their primary food source. One mechanism employed by fungus-growing ants to control general infections of the fungus garden is through secretions of the metapleural glands, which produce a broad spectrum of antibiotics. In addition, the ants carry a mutualistic filamentous bacterium (an actinomycete) on their cuticle, which produces antibiotics that suppress the growth of *Escovopsis*, a specialised parasite of the gardens of fungus-growing ants (Currie et al., 1999a). We show that a clear trade-off between these defence mechanisms exists at the level of individual workers. Major workers have relatively small metapleural glands, proportional to body size, have a high abundance of the mutualistic bacterium, and are, when carrying the bacterium, most abundant in the lower part of the fungus garden, where *Escovopsis*, if present, is also most abundant. Minor workers have relatively large metapleural glands, proportional to body size, but have a relatively low abundance of the bacterium and are most abundant at the top of the fungus garden, where a variety of potentially infectious microbes enter the colony on the substrate used to grow the fungal mutualist. The two sympatric species investigated, *Acromyrmex octospinosus* and *A. echinator*, have quantitatively different combinations of these defence mechanisms, suggesting that the optimal investment in alternative defence mechanisms in different ant species depends on differences in caste allocation or parasite pressure.

Key words: Leaf-cutting ants, pathogens, defence, *Escovopsis*, actinomycetes.

Introduction

Ants belonging to the leaf-cutter genera *Acromyrmex* and *Atta* are the most evolutionary derived members of the tribe Attini. Attine ants are characterised by their obligate symbiosis with mutualistic fungi, that are typically cultivated in underground nest chambers (Weber, 1966). In contrast to other attine ants, leaf-cutters exclusively use fresh plant material as substrate for their fungal cultivars (Weber, 1966, 1972; Hölldobler and Wilson, 1990). The basidiomycetous fungi associated with leaf-cutting ants belong to the family Lepiotaceae (Agaricales: Basidiomycotina) (Chapela et al., 1994) and the history of the association with the ants is perhaps as old as 65 million years (Mueller et al., 2001). The fungus is an essential food source for the ants (Quinlan and Cherrett, 1977; Hölldobler and Wilson, 1990). Ant brood is fed exclusively with fungal hyphae and gongylidia, special fungal cells containing nutrients, whereas adult ants get additional sugars from plant sap (Littleddyke and Cherrett, 1976; Quinlan and Cherrett, 1979). The ants provide the fungus with nutrition and play an essential role in protecting the cultivar from parasites and competitors, which rapidly overgrow fungus gardens when ant-care is absent (Weber, 1972; Currie et al., 1999b; Currie, 2001a).

The complicated mutualistic relationship between leaf-cutting ants and their fungi requires the performance of a large number of specialised tasks in the colony (Weber, 1972; Wetterer, 1999). Leaf-cutting ants have a caste system, which is primarily characterised by size polymorphism of the workers (Hölldobler and Wilson, 1990; Wetterer, 1999). *Acromyrmex* species have essentially two worker castes (Wetterer, 1999). Foraging and defending are primarily tasks of mature major workers, whereas garden tending, brood care, and food

preparation are primarily tasks of minors (Wilson, 1980; Hölldobler and Wilson, 1990) and of younger major workers (A.N.M. Bot, pers. obs.). A third category of media workers has also been distinguished by some authors, but has recently been shown to be merely an extension of the size distribution of minor workers (Wetterer, 1999). In the present study we investigate whether caste differentiation in *Acromyrmex* leaf-cutting ants is also important with respect to the expression of different defence mechanisms against potentially infectious pathogens.

Leaf-cutting ants employ a series of behaviours and chemicals to avoid the establishment and spread of pathogens in the colony, including workers actively cleaning the mutualistic fungus (Wilson, 1980; Currie and Stuart, 2001), and exhibiting effective waste management (Hölldobler and Wilson, 1990; Bot et al., 2001a; Hart and Ratnieks, 2001). In addition, they produce chemical compounds with antibiotic properties in exocrine glands (Schildknecht and Koob, 1970; 1971; Maschwitz et al., 1970; do Nascimento et al., 1996; Ortius-Lechner et al., 2000; Poulsen et al., in review). The paired exocrine metapleural glands are located laterally at the rear of the mesosoma (Hölldobler and Engel-Siegel, 1984). The secretions of these glands are known to inhibit the growth of bacteria, fungal mycelia, and the germination of fungal spores (Maschwitz et al., 1970; Beattie et al., 1985; Beattie et al., 1986; Ortius-Lechner et al., 2000; Bot et al., 2001b). Minor leaf-cutting ant workers have large metapleural glands relative to their body size compared to major workers, indicating that this defence function is most important in ants specialised on fungus garden maintenance (Wilson, 1980; Bot and Boomsma, 1996; Bot et al., 2001b). An additional defence mechanism employed by the ants is through a mutualistic relationship with a filamentous bacterium that is carried on the cuticle of ants, which produces antibiotics against the pathogenic fungus *Escovopsis* (Currie et al., 1999b; Currie et al., submitted). Currie et al. (submitted) found that minor workers generally carried a smaller abundance of the bacterium than major workers and that major workers present in the fungus garden carried a greater abundance of the bacterium than foraging major workers. It thus appears that different age classes and morphological castes may specialise in different defence mechanisms.

In the present study we investigate whether the different caste-specific defences are primarily operating in different parts of the fungus garden. In particular, we test the hypothesis that minor workers, which are apparently specialised in the defence functions via metapleural gland secretions, are more important at the top of the fungus garden where general contaminants enter the colony on and in the plant material used to manure the fungal cultivar. The other prediction tested is that the filamentous bacterial defence function of young major workers is most important at the bottom of the garden, where *Escovopsis* infections, when present, are most abundant (Currie, 2001a).

Materials and methods

Four laboratory colonies from each of two sympatric species, *Acromyrmex echinator* and *A. octospinosus*, were used in the experiment. They were collected in the spring of 1993, 1994, 1996 and 1998 in Gamboa, Panama. The colonies were maintained as described in Bot and Boomsma (1996). They were all healthy and had accumulated fungus over the months immediately prior to the study, suggesting that they were free of infection by the parasite *Escovopsis* (Currie, 2001b). Fungus gardens (1–2 per colony) were built under inverted 1 litre beakers and were thus cylindrical. A vertical segment of approximately one sixth of the total volume was taken out of one fungus garden of each colony. This segment was quickly divided into top, middle and bottom sections of approximately equal volume. For each of these sections workers were sorted into minors (head widths 0.7–1.2 mm) and majors (head widths 2.0–2.4 mm) (Wetterer, 1999). 18.7% of the workers were of intermediate size (head widths 1.3–1.9 mm). This category has previously been referred to as medium, but has since been shown not to represent a distinct caste (Wetterer, 1999). Workers were counted and divided into two classes based on the amount of mutualistic bacterium covering the cuticle. The first class contained workers carrying no bacterium or having it only on the laterocervical plates (the usual minimum presence; after Currie et al., 1999b). The second class contained workers that had their cuticle partially or completely covered with the mutualistic bacterium.

To investigate whether the size castes of workers were homogeneously distributed, the proportion of minor workers (compared to the total number of workers including the ones of intermediate size) was analysed with a logistic analysis (JMP) with species, colony and section of the fungus garden as factors. As minor workers have little or no mutualistic bacterium on the cuticle (Currie et al., submitted), the analyses of the distribution of the bacterium were restricted to the media and major workers. Differences in the proportion of workers with a high abundance of the bacterium (out of the total number of workers of the same size class) were tested using logistic analysis tests with species, colony and section as factors. All logistic analyses performed had a nested design, with colony nested within species and section nested within colony. Finally the correlations between the number of minor workers and the proportion of major workers with a high abundance of the bacterium present on the cuticle for the two species were analysed, after a logarithmic transformation of the data.

Results

The proportion of workers that are minors decreases significantly towards the bottom of the fungus garden (Chi-square = 118.8; DF = 16; $P < 0.0001$) (Table 1). This difference is significant for both species, but the overall proportion of minors in *A. echinator* is significantly higher than in *A. octospinosus* (Chi-square = 59.4; DF = 1; $P < 0.0001$) (Table 1).

For both species the proportion of major workers with a high abundance of the bacterium is significantly higher in the lower part of the fungus garden (Chi-square = 76.9; DF = 16; $P < 0.0001$) (Table 1). The proportion of media workers carrying a high abundance of the bacterium is likewise higher in the lower part of the fungus garden (Table 1), but this difference is not statistically significant. However, the proportion of both media and major workers with a high abundance of the bacterium is significantly lower in *A. echinator* than in *A. octospinosus* (Chi-square = 4.54; DF = 1; $P < 0.033$ and Chi-square = 64.7; DF = 1; $P < 0.0001$ for media and major workers, respectively) (Table 1; Fig. 1).

The correlations between the proportion of major workers with a high abundance of the bacterium and the absolute

Table 1. Numbers of ants and the proportion of ants with a large abundance of the mutualistic bacterium on their cuticle (means ± SE) in each caste for the three sections (top, middle and bottom) in colonies of *Acromyrmex octospinosus* and *A. echinator*

Section of the fungus garden	<i>Acromyrmex octospinosus</i>			<i>Acromyrmex echinator</i>		
	Top	Middle	Bottom	Top	Middle	Bottom
Number of minor workers	76 ± 9.29	78 ± 6.36	61 ± 14.7	157 ± 28.9	119 ± 19.5	76 ± 7.20
Number of media workers	34 ± 3.24	41 ± 10.7	28 ± 10.4	28 ± 4.42	28 ± 7.60	22 ± 4.44
Number of major workers	31 ± 6.26	32 ± 3.25	43 ± 4.42	35 ± 7.01	44 ± 7.16	36 ± 5.26
% of minors with lots of the bacterium	0	0	0	0	0	0
% of media with lots of the bacterium	3.16 ± 2.28	7.79 ± 3.79	17.6 ± 1.62	1.51 ± 0.87	2.36 ± 1.58	4.55 ± 4.55
% of majors with lots of the bacterium	15.2 ± 4.87	23.7 ± 6.91	37.6 ± 6.74	4.23 ± 0.87	4.88 ± 1.88	16.5 ± 3.69

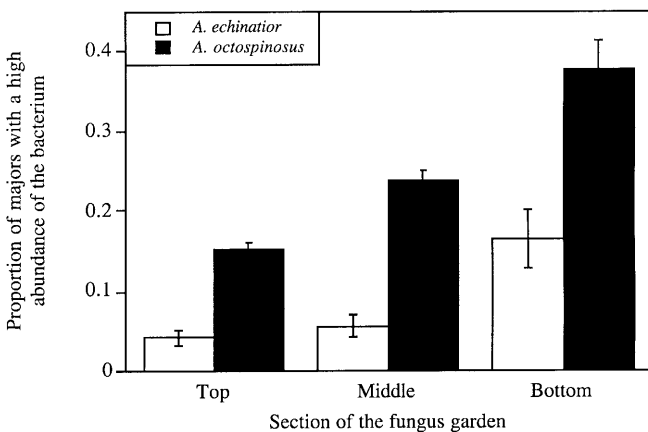


Figure 1. The proportion of major workers with a large abundance of the filamentous mutualistic bacterium on their cuticle for the two species (*Acromyrmex echinator* and *A. octospinosus*) in the three sections of the fungus garden. Error bars represent standard errors (n = 4)

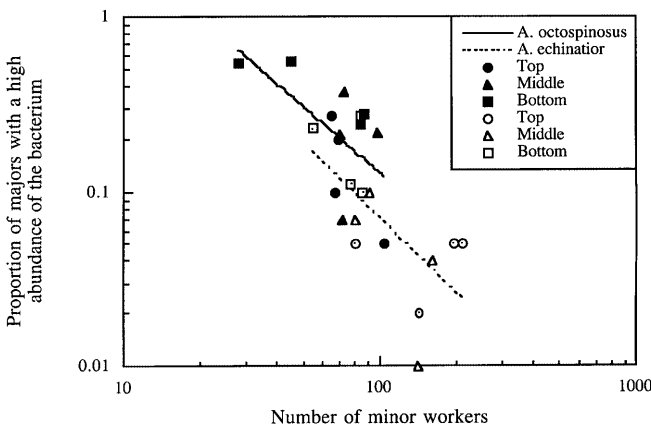


Figure 2. The proportion of major workers with a large abundance of the filamentous mutualistic bacterium on their cuticle as a function of the number of minor workers present for the 2 times 4 colonies of *Acromyrmex echinator* (open symbols) and *A. octospinosus* (black symbols). Each dot represents a section in a single colony. The power equations are $44.22x^{-1.2686}$ and $56.273x^{-1.4482}$ for *A. octospinosus* and *A. echinator* respectively

number of minor workers are negative and significant ($r = -0.602$; $P = 0.038$ and $r = -0.656$; $P = 0.021$ for *A. octospinosus* and *A. echinator*, respectively). This is illustrated in Fig. 2 which also shows that: 1. There are few major workers with a high abundance of the bacterium in the top of the fungus garden where many minor workers are present (bottom right of Fig. 2) and 2. A relatively large proportion of major workers are partially or completely covered with bacterium at the bottom section of the fungus garden where few minor workers are present (top left of Fig. 2). The correlation is similar in both species, although a different range is covered.

Discussion

The results of our study indicate that specialisation in defence mechanisms occurs between worker castes of *Acromyrmex* leaf-cutting ants and that fungus gardens are not homogeneous with respect to the presence of ants of different castes. This heterogeneous distribution of ants of different castes within the fungus garden is likely to be related to the fact that the fungus garden itself has a clear spatial structure. New leaf material is added at the top of the fungus garden. This substrate moves downwards in the colony reaching the bottom of the fungus garden within 6 weeks, from where it is removed as old spent fungus and old substrate. The top of the garden contains a relatively large amount of substrate compared to the biomass of the mutualistic fungus, and is therefore likely to harbour a significantly higher abundance of a variety of non-specialised competing microbes that enter the fungus garden on leaf material incorporated by the ants (Fisher et al., 1996). The metapleural glands are particularly effective as a general defence mechanism against these kinds of microbes (Beattie et al., 1985; Beattie et al., 1986; Bot et al., 2001; Poulsen et al., in review), which is consistent with our result presented here that the minor workers, with their relatively large metapleural glands (Wilson, 1980; Bot and Boomsma, 1996), are most abundant in the top section of the fungus garden. The high abundance of minors in the top section of the fungus garden is thus likely to be a consequence of the role they play both in food preparation and in the general defence via the metapleural glands. The

bottom section of the garden contains more biomass of the mutualistic fungus per unit of substrate (if any still remains), and it is these older regions of the garden that harbour the highest abundance of the specialised fungal parasite *Escovopsis* (Currie, 2001a). We found the mutualistic filamentous bacterium most abundant on workers that are located at the bottom of the fungus garden, as would be predicted by the bacterium being specifically effective against this specialised parasite of the mutualistic fungus (Currie et al., 1999a).

On average major workers of *A. octospinosus* have a higher abundance of the bacterium on their cuticle than major workers of *A. echinator*. Since all colonies of both species were kept under completely standardised conditions, rearing procedures cannot explain this pattern. The difference may be explained by the size distribution of castes in these two sympatric species. *A. echinator* colonies generally contain a higher proportion of minor workers and major workers are usually smaller in this species compared to *A. octospinosus*. Also, major workers of *A. echinator* appear to have a thicker layer of the mutualistic bacterium on the laterocervical plates (M. Poulsen, pers. obs.), which, if true, could mean that the total biomass of the mutualistic bacterium may be the same per volume of fungus garden for the two species. This inter-specific difference may be a secondary effect of ecological differences not related to garden maintenance (e.g., food preferences which may favour smaller major workers in *A. echinator*). Alternatively, if the total allocation towards rearing the mutualistic bacterium per unit of fungus garden volume is indeed different across species, this may reflect a direct effect of different parasite pressures. It has been shown that different isolates of *Escovopsis* apparently have different levels of virulence within gardens of *Atta colombica* (Currie, 2001b), so perhaps these two species of *Acromyrmex* harbour different strains of *Escovopsis* with different degrees of virulence.

Our results show a strong negative correlation between the number of minors and the abundance of mutualistic bacterium across the sections of fungus garden investigated. It was recently shown by Bot et al. (2001b) that minor workers of *A. octospinosus* have fewer metapleural gland cells (ca. 250) than major workers (ca. 550). However, given that major workers weight ca. 15 times as much as minors (respective wet weights are ca. 16.9 mg and ca. 1.1 mg) the allocation of one mg of minors to fungus garden maintenance provides ca. 227 metapleural gland cells, whereas one mg of major workers gives only 14.5% of that (ca. 33 cells). This, combined with the demonstrated fact that only young major workers have an abundance of the mutualistic bacterium (Currie et al., submitted; this study), suggests that there is likely to be a trade-off between general defence mechanisms via the minor workers being active at the top section of the fungus garden and the specific defence mechanisms via the mutualistic bacterium growing on major workers active at the bottom section of the garden. Although it is currently not clear how the pay-offs of these alternative mechanisms interact with the other specific tasks of the two size castes of workers, it seems clear that the two species use a quantitatively different combination of the two defence mechanisms. This implies that

natural selection does not only act on the direct expression of the traits that shape each of these defence mechanisms separately, but also on the relative importance of these defence mechanisms in relation to each other.

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