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Waste management in leaf-cutting ants

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Hygienic behaviour is an important aspect of social organisation because living in aggregations facilitates the spread of disease. Leaf-cutting ants face the additional problem of an obligatory dependency on a fungus, which itself is also susceptible to parasites. In this study we provide evidence for the importance of effective waste management in colonies of several Panamanian species of *Atta* and *Acromyrmex* leaf-cutting ants, differing in colony size and typical mode of waste accumulation (external or internal dumps). We show that: (1) waste is dangerous for the ants, which die at a higher rate in the presence of waste; (2) waste is dangerous for the mutualistic fungus because waste in field colonies is infected with the specialised fungal parasite *Escovopsis*; (3) the ants allocate considerable effort to active management of waste in order to reduce these dangers. This management follows a "conveyer belt" model according to which increasingly dangerous tasks are performed by older workers, who are less valuable to their colony. Our approach is kaleidoscopic, as different species of leaf-cutting ants are unequally suitable for direct observation and experimental manipulation, and suggests that more in depth studies of waste management in attine ants would be highly rewarding.

KEY WORDS: hygiene, leaf-cutting ants, pathogens, decomposition, *Escovopsis*

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INTRODUCTION

An increased risk of contracting infectious diseases is a significant cost of social organisation because group living facilitates disease-transmission between interacting individuals (SCHMID-HEMPEL 1998). An independent additional factor impairing the control of diseases in societies is the accumulation of refuse, such as decaying food remains, faeces and corpses, which tend to contain abundant pathogenic micro-organisms. In human societies, for instance, faecally transmitted diseases can have a major detrimental effect on public health. Human sanitation coupled with hygienic behaviour to minimise faecal contact is known to reduce the prevalence of diseases (EWALD 1994, CURTIS et al. 1999). Similarly, hygienic behaviour is known to occur in many social insects, but studies have so far mainly concentrated on grooming behaviour and necrophory, the removal of dead nestmates from the nest environment, in honey bees and ants (ROBINSON & PAGE 1988, HÖLLDOBLER & WILSON 1990).

Leaf-cutting ants (Attini Formicidae) are known to suffer from various parasites (see SCHMID HEMPEL 1998 for a recent review). None of the known insect parasites seem to be specifically adapted to attine ants, but there is no doubt that the virulence or toxicity of these parasites is detrimental to ant fitness, so that recognition and control of the sources of infection is important. As the attine ants have an obligatory dependency on an ectosymbiotic fungus (Basidiomycota Agaricales) (CHAPELA et al. 1994), they are also expected to be affected by any parasites that attack their symbionts. One such specialised parasite, a fungus of the genus *Escovopsis* (Ascomycota: anamorphic Hypocreales) (CURRIE et al. 1999a) has recently been studied in detail. *Escovopsis* species are only found in colonies of fungus growing ants. They do not harm the ants directly, but are specifically adapted to parasitising fungus gardens, reducing garden growth and occasionally causing colony death after the entire garden has been overgrown (CURRIE et al. 1999a). Prevalence of *Escovopsis* in field colonies is high (CURRIE et al. 1999a), but leaf-cutting ants can normally control the parasite under laboratory circumstances when continuous re-infection is absent (C. CURRIE pers. comment).

Waste accumulation problems are most severe in large societies, both in humans and in insects. Mature colonies of the genus *Acromyrmex* contain up to ca 35,000 workers and one to several fungus gardens (WEBER 1972, LEWIS 1975, WETTERER 1999). However, this considerable number is still small compared to mature *Atta* colonies, which can contain millions of workers and several hundreds of fungus gardens (WEBER 1972, HÖLLDOBLER & WILSON 1990). That such large colonies produce significant amounts of waste has been documented by AUTUORI (1947), who excavated an *Atta sexdens rubropilosa* colony, which yielded 296 underground refuse chambers containing a total of 475.2 kg of refuse material.

The active management of hazardous waste material by leaf-cutting ants has two important features. First, waste is not scattered around outside the nest, but is concentrated in one or a few locations. Most *Atta* species have underground refuse chambers (STAHEL & GEIJSKES 1939, AUTUORI 1947, MOSER 1963), but some dispose of their refuse material on a single dump-pile above ground (WEBER 1972) at a safe distance from the colony. Workers that are not involved in waste management have been observed to actively avoid coming in contact with refuse material (ZEH et al. 1999). The second special feature is that waste transport from the fungus gardens to the dump is subject to task partitioning. In *Atta cephalotes* workers from the fungus garden place refuse particles on a cache just outside the dump, from which it is further transported by dump workers (HART & RATNIEKS in press). Furthermore, dump workers do not appear to leave the dump. HART & RATNIEKS never found marked dump workers outside the garbage chamber and only 3 of 612 observed workers were seen to leave the chamber. This form of task partitioning appears to correspond to a conveyor belt model (SCHMID-HEMPEL 1998). Young workers are active on the fungus garden, but as they grow older they become involved in functions away from the centre of the colony. Near the end of their lives they engage in risky tasks such as foraging or possibly waste management. As a consequence of this type of task partitioning only the older and less valuable workers are exposed to infectious agents from the outside environment while the reproductive centre of the colony is relatively protected.

The purpose of this paper is to identify the hazard that waste material imposes on leaf-cutting ant societies. We do so by examining the impact of waste accumulation on ant mortality and by investigating the presence of the garden parasite *Escovopsis* in refuse material to document the danger of waste for the fungus garden. We also address the variation in waste-management behaviour in *Atta*. It is known that *Atta colombica* has above ground dumps without elaborate waste management after transport to the dump (WEBER 1972). In this paper we describe how workers of *Atta cephalotes* actively manage waste after transport to a dump chamber in a laboratory nest, mimicking an underground dump chamber of a field colony.

MATERIAL AND METHODS

Model systems for the study of Attine waste management

Not every *Acromyrmex* or *Atta* species is equally suitable for studying the various relevant aspects of waste management in leaf-cutting ants. Variation in colony size and in the nature of waste storage provides different opportunities, but also imposes specific constraints on what is possible (Table 1). With the Panamanian species that were available to us, we were able to study waste management of one *Atta* species in the field and another *Atta* species plus a third *Acromyrmex* species in the laboratory. The arguments for this selection of model systems are set out below.

Atta colombica is the only species in Panama that maintains above ground dumps (WEBER 1972, HAINES 1978) and is thus suitable for non-destructive field studies on waste management. We used this species to study the presence of the specialised garden parasite *Escovopsis* in refuse material. *Atta colombica* workers usually carry refuse material on to a branch or a tree trunk and let it fall down on to the dump, after which they do not engage in elaborate further waste management (WEBER 1972). Other *Atta* species that maintain underground dumps may have different waste management behaviours, but these can not be observed in the field. For this reason, a laboratory colony of *Atta cephalotes* was used for behavioural observations of workers in an artificial dump chamber. Finally, the effect of accumulating refuse material on ant-mortality can only be studied experimentally and *Acromyrmex* colonies are best suited for such work because entire mature laboratory colonies are of manageable size and can be used as replicates. Three colonies of the species *Acromyrmex echinatior* were therefore used to address questions of this kind.

Table 1.

Characterisation of the most common leaf-cutting ants in Gamboa, Panama and their suitability as model systems for the study of waste management in the field and in the laboratory.

Species		Field	Laboratory
Atta colombica *Atta cephalotes*	Advantages:	Mature colonies are large and produce large amounts of refuse Easy sampling of refuse in species that have above ground dumps outside the nest (*A. colombica*)	Experimental equivalents of underground dump chambers can be offered, allowing the detailed study of the waste management behaviour of workers (*A. cephalotes*)
	Problems	Species with underground dump chambers are unsuitable for behavioural studies of waste management Species with above ground dumps do not manage their refuse after deposition	Colonies cannot normally be maintained at their typical mature size
Acromyrmex octospinosus *Acromyrmex echinatior*	Advantages	None	Colonies can be kept and maintained at their typical mature size; mature colony sizes of ca 10,000 workers and 1-2 fungus gardens make experimental manipulation and replication relatively easy
	Problems	Mature colonies are much smaller and produce low amounts of refuse compared to *Atta* Refuse has occasionally been observed, but always in a single relatively small underground dump chamber (A.N.M. Bot unpublished); direct behavioural observations on waste management are therefore difficult	Colonies can be induced to use experimental equivalents of underground dump chambers, but these are small and less suitable for obtaining quantitative data on waste management behaviour than *Atta* dumps

Colony collection and maintenance

Field studies were conducted in the canal zone of the Republic of Panama. Laboratory colonies were maintained in open nest-boxes in a climate room at the University of Aarhus, Denmark (25 °C, RH = 70%) (BOT & BOOMSMA 1997). Food was provided 3 times a week. In summer the ants were given a variety of leaves, but in winter their diet consisted mostly of bramble leaves (*Rubus fruticosus*). All colonies were apparently healthy at the start of experiments.

Escovopsis *in waste material of* Atta colombica

Sterilised forceps were used to collect the load carried by refuse transporting workers as they emerged from the nest opening. Twenty-eight pieces with a diameter of approximately 5 mm were sampled from different ants from each of 23 colonies in Gamboa, Panama in September 1998. In addition, a single fungus garden from each colony was excavated and returned to the lab in a small plastic container following CURRIE et al. (1999a). Twenty-eight pieces of similar size as the collected refuse particles were sampled from various locations of the garden. Refuse and fungus garden samples were placed on potato dextrose agar medium with 50 mg/l antibacterial substances (Penicillin-G and Streptomycin sulphate). The number of samples from which *Escovopsis* was isolated was recorded.

Waste management behaviour in Atta cephalotes

The observation colony was collected as an incipient colony in January 1996 at El Llano, Panama (MUELLER & WCISLO 1998) and set up in a perspex observation nest. The nest consisted of four 4-litre chambers, connected by horizontal tubes. The ants used two of these chambers for cultivating fungus and, shortly after they had settled in the observation nest they started used the other chambers for waste disposal. The colony consisted of approximately 25,000 workers and 4 litres of fungus garden.

Two different types of behavioural observations were made: first, during 7 consecutive days, the number of workers present on the surface of the dump was determined by scan sampling once an hour for seven 8-hr periods. Differences in the number of ants present between the different 8-hr periods were tested by means of a repeated measures ANOVA, using the periods as repeated factor and the observations within periods as replicates. Second, workers on the dump were observed for 3-min periods. In total 312 observations were conducted over a time span of 1 month. The first activity observed during each 3-min period was categorised and these observations were ranked according to frequency of occurrence. To test whether ants were specialised for any specific task, the frequencies of combinations expected under random pairing of activities were calculated for the 212 ants that performed more than one task during the observation period. Subsequently, in order to avoid small sample sizes, the observations were pooled into two classes: behaviours that were followed by an identical behaviour and behaviours that were followed by any different type of behaviour. Differences between observed and expected frequencies were tested with a goodness of fit test (SOKAL & ROHLF 1995).

Refuse material and worker mortality in Acromyrmex echinatior

Two experiments were carried out to establish the effect of refuse material on worker mortality. For the first experiment three *Acromyrmex echinatior* colonies (#6, 10 and 12) collected in Gamboa, Panama in April 1993 were used. Colonies consisted of one fungus garden positioned on a tray underneath a plastic beaker and covered by a plastic flowerpot to exclude light. In April 1994, petals and leaves of cultivated flowers such as roses were used to supplement the regular leaves that were fed to the ants, which were dry and blighted after several frost periods. These possibly contained fungicides that could have had a negative impact on the fungus. Infection with *Escovopsis* can be excluded as an additional stress factor for the colonies, because all colonies were continuously growing and accumulating fungal mass, a characteristic that is incompatible with *Escovopsis* infection (CURRIE in press).

Refuse was allowed to accumulate in the nest boxes for periods of 16, 4 and 7 days, respectively. This specific order in the accumulation periods assured that the results do not describe a general pattern of increased mortality. After the experimental period the refuse material was collected and the number of dead ants in it was determined and divided by the number of days of waste accumulation to estimate daily mortality. The experiment was repeated in August 1994 when leaves were of optimal quality and no food supplements were required. Because each of the *Acromyrmex* colonies used contained over 10,000 workers, we could only determine how many ants died, but not how many survived. This precluded analysis of the proportion of surviving workers (survival analysis) and led us to decide to use a repeated measures ANOVA on the observed absolute daily mortality, with the time of year during which the experiment was conducted as a factor and refuse accumulation periods as a repeated factor. In a later trial experimental manipulation of the amount of refuse material in the colonies was attempted, but when handling the refuse material, infective agents apparently spread through the air and caused an increase in mortality in all nearby colonies, including the controls, which were not supposed to be exposed.

Where the purpose of the above experiment was to assess the hazards of unmanaged waste lying around in the foraging arena of intact laboratory colonies, the objective of the second experiment was to determine the direct effect of waste on mortality of individual workers. The three *A. echinatior* colonies used for this experiment (#30, 47 and 48) had been maintained in the laboratory since 1996 and contained two 1-litre fungus chambers and a special chamber for the disposal of waste material. In March 2000, healthy young fungus garden workers were collected from each of these colonies and divided over four petri dishes, so that the different size classes were equally represented in each petri dish. The two treatment dishes contained 1.5 g of moist refuse material from the colonies' own dump and the two controls contained two moist cotton plugs to secure a humidity equivalent to that produced by the dump material. A piece of filter paper was placed between the dish and the lid for ventilation. The workers were kept without any form of nutrition and worker mortality was scored every day until day 15. The data were analysed with the non-parametric Cox Multivariate Regression (JMP) providing likelihood-ratios and χ^2 statistics. Ants that had not died by day 15 were included as censored data.

RESULTS

Escovopsis *is abundant in waste material of* Atta colombica

We found *Escovopsis* in the refuse of 16 out of 23 colonies (70%). Within *Escovopsis* infected colonies a mean of 13.5% of particles were infected (mean number of particles yielding *Escovopsis* = 3.77, SD = 2.64, n = 28). In only one colony was *Escovopsis* not detected in the refuse material, when it was isolated from the fungus garden. In six colonies the parasite was detected in the dump but not in the fungus garden. Statistical comparisons between garden and refuse samples were not made, as these two types of samples were not directly comparable. The refuse material originates from many different gardens with, presumably, different levels of infection, whereas only one garden per colony was sampled.

Atta cephalotes *workers do manage waste*

Seven different types of behaviour were observed in the intra-nest dump chamber (Table 2). Seventy-one percent of dump-workers were transporting refuse

Table 2.

The different types of behaviour, performed by *Atta cephalotes* workers on the dump. The number of observations indicate the number of times in which the respective behaviours were recorded as the first observed behaviour during three minute observation periods.

Task	Specification	Number of observations
Transporting fresh fungal refuse	Antennating fresh refuse on cache near dump entrance, picking up refuse particle, carrying it inside, putting it down, walking back to cache	125
Transporting old fungal refuse	Antennating old refuse inside dump, picking up refuse particle, carrying it to different place on dump surface, putting it down, walking back to original site	98
Rearranging	Antennating refuse, picking particle up, putting it down near original site while rocking it backward and forward with forelegs and mandibles	29
Undertaking	Antennating ant corpses, picking up a corpse, moving it, putting it down, sometimes rocking it	25
Unidentified	Performing no obvious behaviour	19
Grooming	Scraping antennae and legs with forelegs, licking forelegs with mouth parts. Sometimes licking cuticle of other workers	13
Dying	Ant falls over and legs fold to ventral side of thorax	3
Total		312

particles, 9% were rearranging refuse and 8% were undertaking, i.e. handling dead ants (see Table 2). Four percent of ants spent the 3-min observational period grooming either themselves or other workers. One percent of the workers died during the observational period. A system of tunnels was present in the heap and 11% of the refuse transporting ants and undertakers were observed entering tunnels with particles. Tunnels were also observed in the dumps of over 30 laboratory colonies of *Acromyrmex* and in three further laboratory colonies of *A. cephalotes*.

Scan sampling showed that a mean of 21.5 workers were present on the surface of the dump but there was significant variation in this number between the 7 different days (8-hr periods) of observation (repeated measures ANOVA, $F_{6,42} = 6.17$, $P < 0.001$). These workers perform on average 1.1 behavioural sequences per minute. Workers that concluded more than one discrete behavioural sequence during the observational period showed a high degree of specialisation (Table 3). They were significantly more likely to repeat the same task than to perform a different task ($G = 42.12$, $df = 1$, $P < 0.001$).

Waste kills workers of Acromyrmex echinatior

Accumulation of refuse material in nest-boxes increased worker mortality in April 1994 (Fig. 1). Daily mortality was 6 times higher when refuse material was

Table 3.

The combinations of tasks that were observed for 212 workers of *Atta cephalotes* that performed two or more behavioural sequences during the 3-min observation periods. Only the four dump-related behaviours are presented in this table. No worker was observed to perform more than two different types of behaviour.

	Transporting fresh waste	Transporting old waste	Undertaking	Rearranging	Total
Transporting fresh waste	80	43	2		126
Transporting old waste		71	0		72
Undertaking			9	0	9
Rearranging				5	5
Total					212

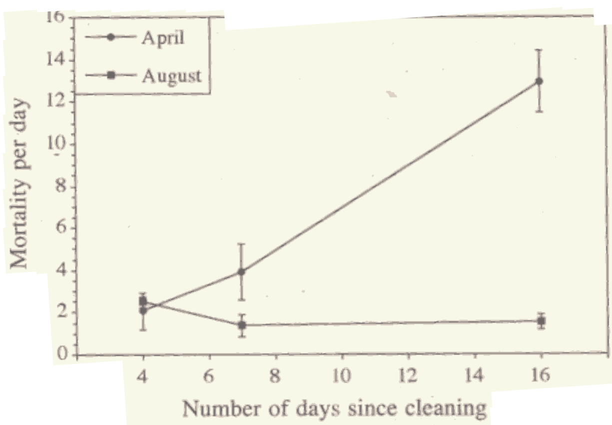

Fig. 1. — The effect of the presence of refuse material in the foraging arena on ant-survival in laboratory colonies of *Acromyrmex echinatior*. Error bars represent standard errors (n = 3).

left to accumulate in the nest-boxes for 16 days versus 4 days. However, there was no increase in mortality when the experiment was repeated in August 1994. The difference between April and August was tested by investigating the statistical interaction between the period during which the experiment was conducted and the number of days since the colonies were cleaned. The interaction term in this analysis is highly significant (repeated measures: $F_{2,8} = 30.2$; $P < 0.001$), showing that waste accumulation had a significant larger effect in April than in August.

Fig. 2 shows the effect of waste on worker mortality in the smaller scale experiment where all other factors were held constant. Workers that were kept in the presence of refuse material died significantly faster than those that were kept in a sterile environment, but this result was also influenced by worker-caste (two-way interaction: $\chi^2 = 74.7$, df = 2, $P < 0.0001$) and colony of origin (two-way interaction: $\chi^2 = 30.5$, df = 4, $P < 0.0001$). The three-way interaction between treatment, worker-caste and colony of origin was not significant ($\chi^2 = 6.7$, df = 4, $P = 0.14$).

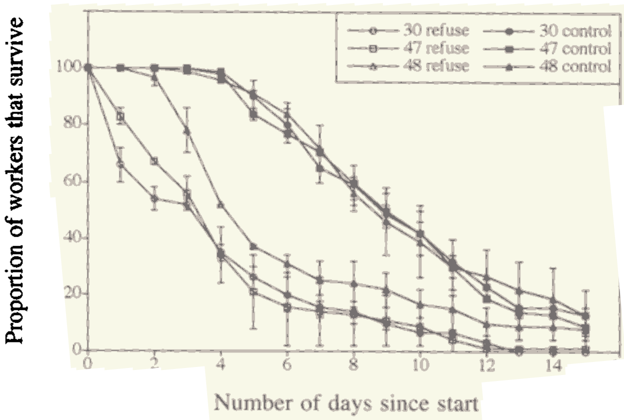

Fig. 2. — The difference in the proportion of ants that survived when kept in the presence of their own refuse material and when kept in a sterile environment for three laboratory colonies of *Acromyrmex echinatior*. Error bars represent standard errors (n = 2).

but there was a significant effect of sub-colonies nested within both colony and treatment (χ^2 = 26.9, df = 6, P = 0.0002). Minor workers died within the first 5 days when kept in the vicinity of waste material, whereas some major workers managed to survive until the experiment ended. The survival of small workers was also lower in the controls, but differences in survival between worker-castes were much less pronounced than in the treatment groups. Although idiosyncratic differences between colony dumps in the laboratory may be of little relevance to the natural situation, such factors did seem to affect worker mortality in our experimental colonies. Large numbers of mites were observed in the dumps of colony 30 and 47, whereas the dump of colony 48, which had lower worker mortality (Fig. 2), did not contain mites but gray fungal hyphae.

DISCUSSION

The danger of waste and its management according to a conveyor belt model

The presence of the micro-fungus *Escovopsis* in the refuse material of *Atta colombica* suggests that the dump is a source of re-infection or spread of parasites of the fungal garden. *Escovopsis* is horizontally transmitted between colonies and has the potential to devastate gardens, leading to colony death (Currie et al. 1999a). In addition, this fungus can have direct and indirect negative effects on colony growth rate (Currie in press).

Our results also show that refuse is a hazard to the ants. *Acromyrmex* workers that were kept in the vicinity of their refuse material died significantly sooner than ants that were kept in a sterile environment. However, the effect of waste material on ant survival may not have been solely due to microbial infections, as toxic microbial products may also have been important. Under some circumstances, we were able to show that refuse accumulation had an effect on colony-level worker mortality. When refuse was allowed to accumulate within nest boxes of *Acromyrmex echinatior*, worker mortality was significantly higher during a period when colonies were nutritionally stressed. However, in another period of the year the ants were apparently able to suppress the presence of infectious agents, or were less sensitive to them. During this period, the built-up refuse material did not result in an increase in ant mortality. Leaf-cutting ants have many ways of dealing with the presence of pathogenic bacteria and fungi. They have large metapleural glands which produce antibiotics (MASCHWITZ et al. 1970; SCHILDKNECHT & KOOB 1970, 1971; BEATTIE et al. 1985, 1986; VEAL et al. 1992; BOT & BOOMSMA 1997). They can facultatively increase the growth of mutualistic bacteria that are attached to their cuticle (CURRIE et al. 1999b). In addition they may, like most other insects, have an active innate immune system (BOUCIAS & PENDLAND 1998). However, all these defense mechanisms are potentially costly, suggesting that the increase in food quality by August may have resulted in an increase in the ants' ability to deal with the infective agents that were present in the refuse material.

Our results indicate that waste material is potentially harmful to the ants themselves and to the fungi on which they have an obligatory dependence. We thus predict that the ants engage in hygienic behaviours that reduce contact between refuse and the productive part of the colony. It appears that leaf-cutting ants accomplish this by partitioning waste transportation tasks, ranging from benign (close to the fungus garden) to hazardous (close to the dump). This is in accordance with the conveyor belt model (SCHMID-HEMPEL 1998). According to this model, the hazardous nature of waste material implies that working on the dump is expected to be the final task. This is supported by the fact that dump workers never seem to leave the dump in *A. cephalotes* (A.G. HART & F.L.W. RATNIEKS submitted manuscript). Transportation of dead ants between the fungus garden and the dump was never observed, suggesting that old and sick workers become dump workers before they die. This may partly explain the observed high mortality rate among dump workers (1% of 312 workers during a given 3-min period).

The function of dump workers

Although all three leaf-cutting ant species examined treat their waste material with great caution, they show significant differences in their natural waste management behaviour. *Atta colombica*, for example, dumps its waste on a single large heap outside the colony and does not process it much further. *Atta cephalotes*, on the other hand, has underground dump chambers and always has active workers on the dump. Our results indicate that only 6% of the dump workers are inactive, whereas the others perform a mean of 1.1 behavioural sequences per minute. These workers show a high level of task specificity for one of the four main behaviours observed in the dump (transporting fresh waste, transporting old waste, rearranging and undertaking), which suggests that even the dump tasks are partitioned according to risk.

Dump workers can have various functions. First, they can cover potentially hazardous material, thereby reducing the likelihood of re-infection of the colony with pathogenic micro-organisms. They can do so by burying material or by placing it in the extensive tunnel system that penetrates the dump. Eleven percent of the *A. cephalotes* workers entered the tunnels with garbage particles during our behavioural observations, and large items such as dead soldiers were always taken into tunnels. Second, the tasks that dump workers engage in may promote the rate of refuse decomposition. The tunnels they build may help maintain an optimal humidity within the dump, just as ventilation channels are used to regulate climatic parameters in the nest as a whole (WEBER 1972). This process may also oxygenate the dump and allow toxic fumes to escape, thus facilitating aerobic decomposition and creating a suitable climate for the many commensal micro-organisms and arthropods that are associated with this habitat under natural conditions (HÖLLDOBLER & WILSON 1990).

Variability in waste management behaviour

Attine ant species with underground dumps will benefit most from waste management on the dump for several reasons. First, they store their waste material relatively close to the fungus gardens, so that efforts to reduce the hazardous nature of waste are most rewarding. Second, their dumps are relatively sheltered, so that the efforts of dump workers is at no risk of being destroyed by biotic or abiotic factors such as large animals or rain. Third, a higher rate of decomposition owing to active waste management will allow for a more effective use of limited space in underground dump chambers, which are energetically costly to excavate and maintain. Species-specific differences in the type of dumps used or in the management of waste performed may ultimately be maintained by differences in habitat. Factors such as soil texture and ground water levels influence the ability to built deep chambers, so that a need for underground dump chambers imposes constraints to the nesting habitat. For example, in Panama, *Atta cephalotes* is restricted to well-drained soils, whereas *Atta colombica* also uses locations where the groundwater table is relatively high. Colony size interacts with habitat. Mature *Acromyrmex* colonies are of a size that does not require deep soils even when dump chambers are internal, so that *Acromyrmex* species are perhaps unlikely to evolve a waste management system with external dumps. In addition, the maintenance of many virulent diseases requires a minimum threshold population size (ANDERSON & MAY 1979, SCHMID HEMPEL 1998), so that the most elaborate forms of waste management are only to be expected in the largest societies.

There are a number of interesting parallels between waste management in agricultural societies of ant and humans. Both isolate waste in specific places: ants use refuse chambers or piles where we use landfill sites. Although there are differences in waste management behaviour across human cultures, natural selection has probably led to a series of general attitudes of disgust towards different types of waste products (CURTIS 1999). Both ants and humans face increasing problems with waste as societies become more populous. Apparently, natural selection and evolution have produced converged methods by which to address these problems in both human and insect societies.

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REFERENCES

ANDERSON R.M. & MAY R.M. 1979. Population biology of infectious diseases: part 1. *Nature* 280: 361-367.

AUTUORI M. 1947. Contribuição para o conhecimento da sauva (*Atta* spp. IV). Osauveiro depois da primeira revoada (*Atta sexdens rubripilosa* Forel, 1908). *Arquivos do Instituto Biologico* 18: 39-70.

BEATTIE A.J., TURNBULL C., HOUGH T., JOBSON S. & KNOX R.B. 1985. The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *American Journal of Botany* 72 (4): 606-614.

BEATTIE A.J., TURNBULL C., HOUGH T. & KNOX R.B. 1986. Antibiotic production: a possible function for the metapleural glands of ants (Hymenoptera: Formicidae). *Annals of the Entomological Society of America* 79 (3): 448-450.

BOT A.N.M. & BOOMSMA J.J. 1996. Variable metapleural gland size-allometries in *Acromyrmex* leafcutter ants (Hymenoptera: Formicidea). *Journal of the Kansas Entomological Society* 69 (4 suppl.): 375-383.

BOUCIAS D.G. & PENDLAND J.C. 1998. Principles of insect pathology. *Boston, Dordrecht, London: Kluwer Academic Publishers.*

CHAPELA I.H., REHNER S.A., SCHULTZ T.R. & MUELLER U.G. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* 266: 1691-1695.

CURRIE C.R. 2001. Prevalence and impact of a virulent parasite on a tripartite mutualism. *Oecologia, Berlin* (in press).

CURRIE C.R., MUELLER U.G. & MALLOCH D. 1999a. The agricultural pathology of ant fungus gardens. *Proceedings of the National Academy of Science USA* 96: 7998-8002.

CURRIE C.R., SCOTT J.A., SUMMERBELL R.C. & MALLOCH D. 1999b. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature* 398: 701-704.

CURTIS V., VONCKEN N. & SINGH S. 1999. Dirt and disgust: a Darwinian perspective on hygiene. *Medische Antropologie* 11 (1): 143-158.

EWALD P.W. 1994. Evolution of infectious disease. *New York: Oxford University Press, Inc.*

HAINES B.L. 1978. Element and energy flow through colonies of the leaf-cutting ant, *Atta colombica*, Panama. *Biotropica* 10: 270-277.

HART A.G. & RATNIEKS F.L.W. 2001. Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leaf-cutting ant *Atta cephalotes*. *Behavioral Ecology and Sociobiology* (in press).

HÖLLDOBLER B. & WILSON E.O. 1990. The ants. *Berlin, Heidelberg, London, Paris, Tokyo, Hong Kong: Springer-Verlag.*

LEWIS T. 1975. Colony size, density and distribution of the leaf-cutting ant, *Acromyrmex octospinosus* (Reich) in cultivated fields. *Philosophical Transactions of the Royal Society, London* 127 (1): 51-64.

MASCHWITZ U., KOOB K. & SCHILDKNECHT H. 1970. Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. *Journal of Insect Physiology* 16: 387-404.

MOSER J.C. 1963. Contents and structure of *Atta texana* nest in summer. *Annals of the Entomological Society of America* 56: 286-291.
MUELLER U.G. & WCISLO W.T. 1998. Nesting biology of the fungus-growing ant *Cyphomyrmex longiscapus* Weber (Attini, Formicidea). *Insectes Sociaux* 45: 181-189.
ROBINSON G.E. & PAGE R.E. JR 1988. Genetic determination of guarding and undertaking in honey-bee colonies. *Nature* 333: 356-358.
SCHILDKNECHT H. & KOOB K. 1970. Plant bioregulators in the metathoracic glands of myrmicine ants. *Angewente Chemie Internationale Edition* 9 (2): 173.
SCHILDKNECHT H. & KOOB K. 1971. Myrmicacin, the first insect herbicide. *Angewente Chemie Internationale Edition* 10 (2): 124-125.
SCHMID-HEMPEL P. 1998. Parasites in social insects. *New Jersey: Princeton University Press.*
SOKAL R.R. & ROHLF J. 1995. Biometry. *New York: W.H. Freeman and Company.*
STAHEL G. & GEIJSKES D.C. 1939. Ueber den Bau der Nester von *Atta cephalotes* L. und *Atta sexdens* L. (Hym. Formicidae). *Revista de Entomologia, Rio de Janeiro* 10 (1): 27-78.
VEAL D.A., TRIMBLE J.E. & BEATTIE A.J. 1992. Antimicrobial properties of secretions from the metapleural glands of *Myrmecia gulosa* (the Australian bull ant). *Journal of Applied Bacteriology* 72: 188-194.
WEBER N.A. 1972. Gardening ants: the attines. *Philadelphia: American Philosophical Society.*
WETTERER J.K. 1999. The ecology and evolution of worker size-distribution in leaf-cutting ants (Hymenoptera: Formicidae). *Sociobiology* 34: 119-144.
ZEH J.A., ZEH A.D. & ZEH D.W. 1999. Dump material as an effective small-scale deterrent to herbivory by *Atta cephalotes*. *Biotropica* 31 (2): 368-371