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Waste management in the stingless bee *Melipona beecheii* Bennett (Hymenoptera: Apidae)

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Abstract

Waste management is important in insect societies because waste can be hazardous to adults, brood and food stores. The general organization of waste management and the influence of task partitioning, division of labor and age polyethism on waste processing were studied in three colonies of the tropical American stingless bee *Melipona beecheii* Bennett in Yucatán, Mexico. Waste generated in the colony (feces, old brood cells, cocoons, dead adults and brood) was collected by workers throughout the nest and taken to specific waste dumps within the nest. During the day, workers based at the waste dumps formed waste pellets, which they directly transferred in 93% of cases, to other workers who subsequently removed them from the nest. This is an example of task partitioning and is hypothesized to improve nest hygiene as it has been found in leafcutting ants, *Atta*. To investigate division of labor and age polyethism we marked a cohort of 144 emerging workers. Workers forming waste pellets were on average 31.2±6.5 days old (X±SD, N= 40, range of 18-45 days). The life span of *M. beecheii* workers was 49.0±14.0 days (N= 144). There was no difference in the life span of workers which formed (52.2±11.6 days, N= 40) or did not form (49.9±11.5 days, N= 97) waste pellets, suggesting that waste work did not increase mortality. Although waste was probably not hazardous to adults and brood, because the dumps are located outside the brood chamber, its presence inside the nests can attract phorid flies and predators, which can harm the colony.

Introduction

Social living can cause problems which are not faced by non-social organisms, or exacerbates problems that are easily dealt with by solitary dwellers. In particular, waste poses a serious problem for social insects living within enclosed nests (Bot et al., 2001; Hart & Ratnieks, 2001, 2002a; Jackson & Hart, 2009). Localizing waste such as in a midden heap (e.g. *Atta colombica* Guérin-Méneville, Hart & Ratnieks, 2002a; *Messor barbarus* (Linnaeus), Anderson & Ratnieks, 2000), or removing waste regularly (e.g. defecating outside the nest and undertaking behavior in *Apis mellifera* Linnaeus, Visscher, 1983; Seeley, 1985) are common strategies to reduce waste building and its negative impact.

Waste management is often a combination of material transport and work organization and adaptations to either can improve effectiveness and efficiency (e.g. Hart & Ratnieks, 2001; Hart & Ratnieks, 2002a; Hart et al., 2002a). Work organization includes the organization of both tasks and the workforce performing them. Division of labor has proved to be an important component of work organization in social insect colonies (Robinson, 1992; Gordon, 1996).

Task partitioning (how tasks are divided into sub-tasks) is also proving to be a powerful organizational principle (Jeanne, 1986; Ratnieks & Anderson, 1999; Anderson & Ratnieks, 2000; Anderson et al., 2001; Hart & Ratnieks, 2002b; Hart et al., 2002a, b; Hart, 2013). Task partitioning describes

situations where a single task, such as foraging, is divided into sequentially linked sub-tasks with a flow of material between them. It has been shown to be important in waste management in leafcutting ants *Atta*. Workers from "clean" parts of the nest, the fungus garden chambers, drop waste outside the "unclean" waste chambers where dedicated waste chamber workers collect it. The two-stage task partitioning (waste transporters coupled with waste chamber workers) with indirect transfer of waste, via caches outside the waste chambers, is hypothesized to help maintain nest hygiene by isolating the waste chambers (Hart & Ratnieks, 2001).

Waste management has not been extensively studied in social insects (Hart & Ratnieks, 2002a). One group which has received some attention are the stingless bees (Meliponini). Stingless bee waste comprises feces, old brood cells, cocoons and both dead adults and brood (Eltz et al., 2001) and waste handling strategies are varied. Workers of *Melipona favosa* (Fabricius) deposit waste in "waste dump areas" within the nest (where workers also defecate) before removing it (Sommeijer, 1984; Bruijn et al., 1989) whereas in some species, e.g. *Cephalotrigona capitata* (Smith) and *Trigona spinipes* (Fabricius), waste accumulates, eventually forming a large mass (scutellum), which can help to regulate nest temperature (Michener, 1974, 2000; Sakagami, 1982). In *Melipona compressipes* (Fabricius) and *Melipona scutellaris* Latreille, Kerr and Kerr (1999) reported that waste (cocoon + feces + wax) are removed from the nests and dropped at a distance of 1 to 45 m according to a Gaussian distribution which reduces the cost of energy and the attraction of predators and pests.

A detailed study of work organization in waste management (division of labor, task partitioning, spatial management and age polyethism) was carried out on the stingless bee *Melipona beecheii* Bennett. This species ranges from Mexico to Costa Rica (Ayala, 1999; Van Veen & Arce, 1999) and colonies typically contain 500 to 2500 workers with a single singly-mated queen (Van Veen & Arce, 1999; Paxton et al., 2001). Natural nests are built in tree hollows and have an entrance hole that connects to the nest cavity. Within the cavity, the brood area consists of multiple horizontal combs of wax cells covered by layers of involucrum (Van Veen & Arce, 1999). Outside that there are many egg-shaped pots made from cerumen (Wille & Michener, 1973; Van Veen & Arce, 1999) which are used for honey and pollen storage.

Material and Methods

Study site and study colonies

The study was carried out at the Department of Apiculture of the Campus of Biological and Animal Husbandry Sciences, Autonomous University of Yucatan, Merida, Mexico, from March to September 2001 during the main nectar flow of "Tsitsilche" (*Gymnopodium floribundum*

Rolfe, Polygonaceae) and many different Fabaceae species (Echazarreta et al., 1997).

Three healthy queenright colonies of *M. beecheii* were studied which originally were housed in log hives. Two months before the study, colonies were transferred into observation nests consisting of a box (30x25x8 cm) with wooden sides and glass top and bottom. This allowed observation of intranidal behavior. Colonies comprised c.900 workers of different ages, 2-4 brood combs and more than 20 honey and pollen pots surrounding the brood area.

General waste dump features

All colonies had two clear areas in which waste was dumped. The waste dump areas were 8-15 cm away from the brood area, outside the involucrum. No other waste dump areas were formed and the original dumps remained in the same place during the study period.

Task partitioning

One waste dump area in each colony was continually observed for 15 min before switching to the second waste dump area. Each dump was observed for a total of c. 170 h. Individuals that were molding waste into pellets were considered "waste dump workers". This behavior was characterized by the worker's body movements when trying to separate small portions of the waste material from the dump (Bruijn et al., 1989). Workers that went to a waste dump to perform a specific activity (e.g. defecation, grooming or dropping material), but did not manipulate the waste to form pellets for removal, were not considered waste dump workers (in contrast to Bruijn et al. (1989) who classified all bees performing activities at the dumps as waste workers).

Workers forming waste pellets were followed to determine the pellet's destination. There were only two destinations. A pellet was either transferred to another worker who removed it from the nest, or it was taken outside the nest directly by the worker that formed it. Task partitioning occurred when a pellet was transferred to another worker. Pellets weighing an average of 18 mg were carried in the mandibles of the workers which represents 25.7% of the weight of the workers (X=70 mg).

Division of labor, age polyethism and worker longevity

Three mature brood combs from one colony were taken from the original log nest and kept in an incubator. All workers that emerged from 28 May to 5 June were individually marked with a numbered tag (Opalithplättchen; 2.1 mg each tag which represents 3% of the weight of the workers) on the notum and introduced into an observation nest formed from the original colony. 144 workers in total were marked and introduced successfully. These marked bees allowed

detailed data on the ages of workers performing waste–related activities to be collected and to determine whether waste-related activities affect worker longevity. They also enabled us to determine whether division of labor into waste workers and non-waste workers was occurring.

Individuals processing waste (e.g. collecting waste and dropping waste at the dumps to form waste caches, molding waste to form pellets) and workers releasing the waste pellets from the nests were recorded and their ages of workers performing these tasks and their longevity by a daily census were determined.

Data analysis

The proportions of pellets transferred to other workers, which then removed the pellets from the nest, were compared in the three colonies using a Chi-square contingency test (Zar, 1999). The life span and survival curves of workers which had or had not formed pellets were compared using Logrank Test (Machin et al., 2006).

All mean values are expressed ± one standard deviations (SD). Statistical analysis were performed using SPSS 11.0 for Windows (SPSS Inc., Norusis, 2002).

Results

General waste dump features

Waste management in *M. beecheii* was a three-stage process. First, workers collected waste from all over the nest, especially from the brood chamber, and deposited it in the waste dumps. This activity was observed during both day and night. Second, workers at the dumps molded waste into pellets during the day. Finally, and again only during the day, waste pellets were removed from the nest.

The average number of waste pellets formed per day in the study nests was 25.3±8.1 (X±SD, N = 40 days study), which represents 454.9±145.9 mg of waste (pellets weighed 18.0±11.1 mg, X±SD, N= 45 pellets).

Task partitioning

The destinations of 416 waste pellets were observed in the three study colonies (Nest 1, N = 112; Nest 2, N = 165; Nest 3, N = 109). In total, 386 workers (92.8%) observed forming pellets subsequently transferred them to other workers which removed the pellets from the nest. There were no significant differences in the occurrence of task partitioning between the study colonies (cross-colony comparison: Chi Squared = 1.17, df= 2, P= 0.56, Table 1). Overall, 92.8% of all waste pellets formed by waste dump workers were transferred directly (from mandibles to mandibles) to another worker, and 7.2% were taken outside by the waste dump worker without transfer. When waste dump workers took waste outside without transfer (i.e. when task partitioning was absent), it was

not because transfer partners were unavailable. In the marked cohort of workers, the only workers that formed pellets and subsequently removed them from the nest were workers that were close to switching tasks from pellet forming to flying outside the nest with waste (Figs 1b and c).

Table 1. Number (%) of waste pellets formed and transferred between waste dump workers and individuals removing pellets from the nest.

	Nest 1	Nest 2	Nest 3	Total
With transfer	112	165	109	**386**
	(91.0%)	(94.3%)	(92.4%)	**(92.8%)**
Without transfer	11	10	9	**30**
	(9.0%)	(5.7%)	(7.6%)	**(7.2 %)**

Cross-colony comparison: χ^2= 1.17, df= 2, P= 0.56

Division of labor, age polyethism and worker longevity

Workers that collected waste within the nest (mainly in the brood area) and then placed it at the waste dump areas were 20.7±5.7 days old (X±SD, N= 46) with a range of 10-31 days (Fig 1a). Workers engaged in this task were removing wax from old brood combs, destroying old brood cells and involucrum, removing dead brood and collecting dead adults. Workers then carried the waste to the dump areas where they dropped it to form waste caches. All workers performing duties inside the colonies defecated only at the two waste dumps areas, so feces do not have to be collected and carried out to these areas and other forms of waste were carried out to these places to form the wastes dumps. Thirty-three percent of the age-marked cohorts were observed collecting waste and taking it to the dump areas.

The age at which individuals first formed waste pellets was 31.2±6.5 days (X±SD, *N*= 40, range of 18-45 days). In total, 29.2% of the age-marked cohort engaged in this task (Fig 1b). Workers that started processing waste material at the dumps and forming waste pellets performed this task for about 2.5 days (range of 1-15 days) and were later observed flying outside the nest to remove the waste pellets at 34.5±5.9 days old (X±SD, *N*= 13) with a range of 29 to 59 days (Fig 1c). However, only 10% of the age-marked cohort removed waste pellets from the nest. These workers were very active, removing waste pellets during the day at a high rate (some handled 4-5 waste pellets within an hour) until all waste was removed from the nest.

Although we did not record the distance flown before waste pellets were released, total flight durations were short (X±SD = 21.7±11.1 s, N = 65 flights). The first foraging flights in the cohort of age-marked *M. beecheii* workers were performed at 41.7±7.1 days old (X±SD, *N*= 58) with a range of 22 to 63 days (Fig 1d).

The life span of the age-marked cohort was 49.0±14.0 days (X±SD, *N* = 144) with a range of 3 to 71 days (last marked-worker seen alive, Fig 1a). There was no significant difference (X² = 1.373, P= 0.354, Logrank Test) in the life span of workers which had (52.2 ± 11.6 days, N = 40) or had not (49.9±11.5 days, N= 97) formed pellets.

Discussion

Our results show that waste disposal in *M. beecheii* is subject to partitioned organization, encompassing spatial management, division of labor with age polyethism, and task partitioning. Waste management had three distinct stages connected by task partitioning with both direct and indirect transfer of waste between workers, and this pattern occurred consistently across all three study colonies. Younger workers collected waste around the nest and transferred it to the waste dump areas where it formed a cache (a case of task partitioning with indirect transfer; Ratnieks & Anderson 1999). The percentage (33%) of worker bees collecting waste and taking it to the dump areas indicates that this is a common but not universal activity in the worker age polyethism schedule.

Much of the waste in the dumps was feces, which was not transferred but deposited directly by defecating workers. Waste in the dump areas was then fashioned into discrete pellets by older workers. Most pellets, 93%, were transferred directly by a dump worker to another worker, who was on average even older, who flew from the nest to dump the pellet. The other pellets, 7%, were removed from the nest without transfer.

Waste management has not been extensively studied in social insects. However, the system found in *M. beecheii* is similar to that found in the leafcutting ant, *Atta cephalotes* Linnaeus, where the transfer of waste to disposal zones is also subject to task partitioning. In *A. cephalotes*, waste is stored in specialized chambers in the underground nest that are distinct from the chambers where the ants grow the fungus gardens that they depend on for food. Fungus garden workers deposit waste just outside the waste chambers, which they do not enter. Dedicated waste chamber workers retrieve the waste and carry it into the chamber. Thus, indirect transfer (coupled with division of labor and nest compartmentalization) enables effective isolation of hazardous waste (Hart & Ratnieks, 2001). Good hygiene is important in leafcutting ants (and other fungus growing ants) because of the presence of the vulnerable fungus on which the ants depend. In *M. beecheii*, waste may not be as hazardous. Possibly reflecting this, leafcutting ants working in waste chambers never become foragers, but in *M. beecheii* all waste dump workers eventually became foragers.

Task partitioning can have ergonomic advantages. In *M. beecheii*, de-coupling pellet making from pellet disposal allows workers to become specialized at each task (albeit only for a short period), which is likely to improve efficiency. This may be the advantage of task partitioning in this case. A potential cost of task partitioning with direct transfer is that the two task groups may become out of phase, such that members of one or other groups must wait for transfer partners. One way to reduce this problem is to introduce indirect transfer via a cache (Hart et al., 2002a). However, in *M. beecheii*, introducing
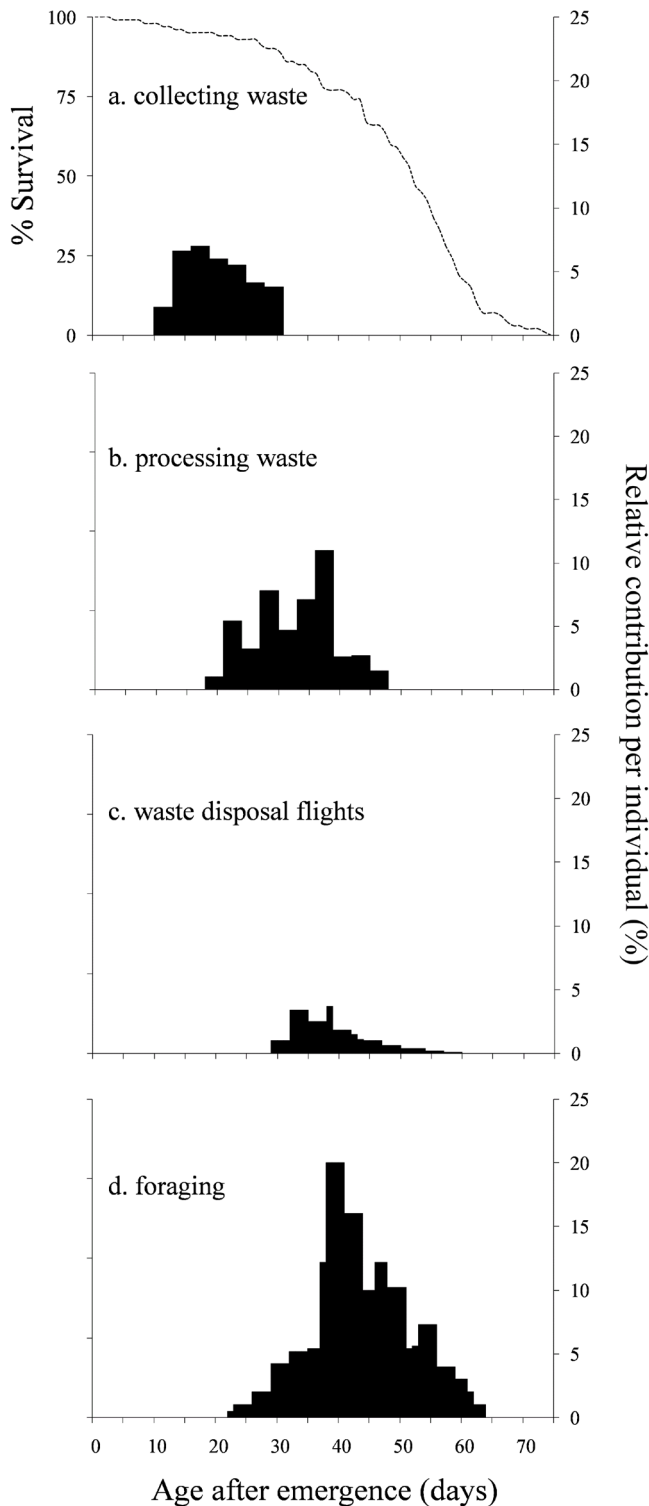

Fig 1. Survival curve (line, a) and frequency (%) of workers of *Melipona beecheii* performing tasks related with the collection of waste (a), processing (molding) the waste at dump areas (b), releasing waste outside the nest (c) and foraging (d). Age is given in days after emergence.

a further cache of waste pellets (in addition to the initial waste dump areas) is not likely to improve nest hygiene, since waste will be more distributed around the colony (increasing the area it could contaminate) and might not be collected. In order to limit the distribution of waste, workers must either transfer pellets directly to other workers who will subsequently remove the pellets from the nest or remove waste pellets themselves.

We did not record the destination of waste outside the nest, but return flights were short (mean 21.7 seconds) compared with nectar and pollen collecting flights (15.6 and 25.1 minutes respectively; Biesmeijer & Toth, 1998) and it is likely that waste was dropped at a distance comparable to other *Melipona* species (e.g. 18m in *M. compressipes* and 31m in *M. scutellaris*; Kerr & Kerr 1999). Dumping waste close to the nest is typical in stingless bees. Some species, like *Lestrimellita limao* (Smith) and *Lestrimellita niitkib* Ayala, simply drop waste in front of the nest entrance and in *Frieseomellita varia* (Lepeletier) waste is carried less than 1m from the nest (Kerr & Kerr, 1999). Carrying waste away from the nest has a number of possible advantages. Waste can reduce the space available for food storage or brood rearing and in species where waste accumulates in the nest, the thickness of waste increased with nest age (in *Trigona corvina* Cockerell a total thickness of 11-24 cm is found around the sides and base of the nest; Michener, 1974).

Additionally, waste removal may help to avoid attracting parasitic phorid flies (in *M. compressipes*, larvae and pupae of the phorid fly *Megaselia scalaris* (Loew) were found only in the waste (Kerr, 1996) and vertebrates (e.g. *Eira barbara* (Linnaeus) [Mustelidae]; Kerr & Kerr 1999), both of which can attack and destroy the colony. Waste may also provide a substrate on which potentially harmful micro-organisms can thrive.

Division of labour in waste management was age-dependent. Workers collecting waste were younger (mean age, 20.7 days old) than workers processing (31.2 days old) and releasing waste pellets (34.5 days old), and also performed brood chamber duties such as wax removal. This pattern of multi-tasking is found in other species. For example, in *M. favosa* (Sommeijer, 1984) and *Melipona bicolor* Lepeletier (Bego, 1983), young workers (<20 days) both collected waste and performed brood chamber tasks such as building and provisioning cells. In addition to temporal polyethism, the schedule of waste management tasks in *M. beecheii* exhibited a strong spatial pattern. Young workers first performed tasks within the brood chamber (waste collection), later graduating to tasks outside of the brood chamber (waste pellet forming) before finally performing tasks outside the nest (waste removal and foraging). This is an excellent example of the "conveyor belt" model (Schmid-Hempel, 1998), in which individuals move from safe tasks inside the nest to more hazardous tasks outside the nest as they age. It also mirrors the situation in honey bees, *A. mellifera* (Seeley, 1982) in which workers carry out a range of tasks that co-occur spatially in the nest. Seeley (1982) commented that stingless bees, with their distinctive nest layout, could be an important taxon to further investigate this.

Large societies, both human and insect, struggle with how to collect and dispose their waste (Doan, 1998; Dijkema et al., 2000; Bot et al., 2001; Hart & Ratnieks, 2002a). Effective waste management needs to encompass collection, processing and eventual disposal. In human society these together prevent waste building up, thereby reducing negative effects such as odor, unsightliness and disease-carrying vermin. Similar to human waste management, we found that in the nests of *M. beecheii*, waste was collected daily and taken to an intermediate processing site (the waste dump areas), where it was processed and then disposed of. This insect management system probably prevents waste accumulation and consequent attraction of "vermin" (e.g. phorid flies and predators) in the same way that human waste management does. Social organisms from widely different taxa often face similar problems for example, maintaining social hygiene and managing waste. Waste management in social insects is currently understudied and we suggest that further studies need to be carried out to investigate this potentially vital component of social life. The stingless bees provide *a priori* examples of waste management diversity and this, combined with the ease with which they can be cultured and studied in the laboratory, give them the potential to become a model group for waste management and nest hygiene in social insects studies.

Acknowledgements

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