Waste Management of the Polymorphic Ant *Camponotus aethiops* (Hymenoptera: Formicidae)

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Abstract

The division of labour is a very important trait of social insects and can be realized through morphological or age differences. The workers of most ant species undergo some changes in behavior during ageing while usually specializing on different tasks. Waste management is an important feature, which can keep the infection prevalence low. We investigated five laboratory colonies of the polymorphic ant *Camponotus aethiops* to find out if there are differences in waste management behavior among individual workers and in the response of the different morphological castes to the corpses of different sizes. We found lower activities for majors compared to the two smaller size class workers. In the case of the latter castes there were individual differences in waste management and other behavioral features. Waste management specialists made up ca. 15% of all foragers indifferent of size class, while those workers that remained generalists ca. 67%. Workers from the major caste were generalists or defence specialists. According to our results, waste management is an important trait in *C. aethiops,* although we could not identify the presence of a specialised worker caste for this task. There were, however, individuals that were more "ready" to perform these actions, thereby enhancing the efficiency of these tasks, keeping the infections prevalence low, and, this way, contributing to colony survival.

Keywords: *Camponotus aethiops,* waste management, behaviour, morphological caste, cemetery.

Introduction

The success of social insects and that of social life is due to the enhanced efficiency, mostly the outcome of the division of labour (Hölldobler and Wilson 1990). One individual can be present at one time only in one place, but the workers of a social colony can be present in many places at one time, fulfilling several tasks. This reduces the costly travelling time between different tasks, moreover, the wide range of tasks are divided among the workers, and certain labours can be also divided to different subtasks (Ratnieks and Anderson 1999). The variety of tasks can be fulfilled by different morphological castes with differing behavior (Wilson 1980, Camargo et al. 2007), nevertheless the workers of most social insects undergo some behavioral changes with ageing usually accompanied by task shifts (Hölldobler and Wilson 1990). In this way the working individuals can specialise on different roles for different periods of time, further enhancing their efficiency through practice (Oster and Wilson 1978, Ratnieks and Anderson 1999). For instance, the workers of *Temnothorax unifasciatus* usually occur in their entire lifetime on a definite location inside the nest fulfilling tasks that are in a reachable distance, but these spatial fidelity zones become bigger towards the periphery of the colony, and they expand or shrink even through the year (Sendova-Franks and Franks 1993, 1995). Sendova-Franks and Franks (1993) also observed older workers performing nursing activities and young callow workers foraging, but still a bigger part of the foraging was accomplished by a few older workers. These specialist workers usually fulfil a task with higher efficiency, as was observed in the case of *Acromyrmex versicolor*, where the majority of the experimentally placed corpses were transported by a few individuals despite the fact that they were contacted by 80% of the workers (Julian and Cahan 1999). In these colonies, the percentage of workers performing undertaking behavior was less than 30%. Beside the specialists, some individuals of *T. unifasciatus* remained generalists, being responsible for the "connection" of the different specialised groups (Sendova-Franks and Franks 1993). Similarly to this species, a within-nest generalist group was also found in *Atta sexdens*, where the minor caste and the bigger size classes showed differences in their activity (Wilson 1980, Camargo et al. 2007). In the case of *T. unifasciatus*, the workers from the edge of the nest were more active than those inside of it (Sendova-Franks and Franks 1993). The authors suggested that

young workers of *Temnothorax* actively seek for tasks and fulfil them for a longer period of time. In this way the colony can optimally allocate its workforce to tasks or subtasks unfulfilled (Anderson and Ratnieks 1999), responding with higher flexibility to the changes imposed by their variable environment.

Many tasks can be divided into different subtasks, which can be performed by more workers or castes working together (Ratnieks and Anderson 1999). Task partitioning can arise in several cases (e.g., with the growing size of colony), usually increasing the efficiency of task performing (Anderson and Ratnieks 1999, Ratnieks and Anderson 1999). Such tasks usually involve the transporting of some materials during foraging, nest excavation, colony movement, slave raids or removal of fungus gardens (Ratnieks and Anderson 1999, Anderson and Ratnieks 2000). For instance, some foragers of *Probolomyrmex dammermani,* a millipede predator, capture the prey and transport it back to the nest, while other workers process it and dump the waste nearby the nest entrance (Ratnieks and Anderson 1999). In the case of species where no division of labour occurs, task switching can quickly replenish missing workers, or when division of labour occurs without morphological differences, recruiting more workers can be achieved to perform a given task (Ratnieks and Anderson 1999). Some species have morphologically distinct worker castes with large headed majors specializing in colony defense and smaller workers performing brood tending or foraging (Oster and Wilson 1978).

On the other hand, task division supposes intensive contacts among individuals and thereby can impose many health risks by causing the fast spread of diseases (Soeprono and Rust 2004, Choe et al. 2009, Wiltz et al. 2010). Besides, social life and intensive activities can lead to the accumulation of large amounts of waste, on which many pathogens can flourish, so their removal and management is essential to the colony (Oi and Pereira 1993). Many types of waste management behaviors have evolved, among which the most important is the removal of corpses and their piling on garbage heaps (Oi and Pereira 1993, Graham 2007, Renucci et al. 2011). Imposed health risks can be in strong relation with colony size, which can influence the number of workers performing waste management activities. These behavior features are usually performed by older workers with a short life expectancy, proving the dangers of these activities (Hart and Ratnieks 2001). In the case of *Atta colombica* colonies that were infected

with the pathogen fungus *Escovopsis*, the number of active workers grew significantly in the waste chambers compared to colonies without such infection, but their number did not correlate with the rate of the infection (Bot et al. 2001). Hart and Ratnieks (2001) described *Atta* workers belonging to the smaller size class that performed only waste management and they were active only in the waste chambers, because their nestmates were aggressive towards them, so they could not leave these chambers. Contrary to leaf-cutter ants, the workers of *Myrmica rubra* performing waste management were observed all around the nest achieving other tasks as well (Graham 2007). Beside the division of labour among workers of different age, some tasks can be also divided based on morphological differences. Investigations of possible differences in waste management activities performed by workers of different size classes revealed no such differences, neither in the case of *Solenopsis invicta* (Howard and Tschinkel 1978), nor in *Pogonomyrmex badius* (Wilson et al. 1958). In both cases, the corpses of nest-mates were transported by the first workers encountering them.

In our work, we investigated the waste management activities of *Camponotus aethiops*, an another polymorphic ant species to find out if there are differences in waste management activities (1) towards corpses of different species and non-nestmate workers, (2) among workers of different size classes and (3) among different individual workers.

Materials and methods

Study species and laboratory conditions

C. aethiops (Latreille, 1798) is a Mediterranean species distributed from Spain to South Russia, but is also present in North-West Africa, Asia Minor, the Caucasus, the Middle East, Afghanistan and Kazakhstan (Radchenko 1997, Seifert 2007, Czechowski et al. 2012). It usually inhabits xerothermic grasslands and bushy regions, especially on calciferous grounds (Radchenko 1997, Seifert 2007). Nests in the ground, under stones and sometimes in fallen trees or rotten branches (Seifert 2007, Lőrinczi 2011, Stukalyuk and Radchenko 2011). It is zoo-necrophagous and aphidicolous (Seifert 2007, Stukalyuk and Radchenko 2011). Colonies are monogynous or maybe poli-

gynous with an average of 1500–2000 (up to 5000–7000) workers (Seifert 2007, Stukalyuk and Radchenko 2011). Nuptial period is unknown (Seifert 2007), but in the case of our laboratory colonies the virgin females were ready to fly off at the end of July (pers. obs.).

We collected six experimental colonies of *C. aethiops* with ca. 120–150 workers and brood from the edge of a mixed pine forest near the town of Litér (Midwest Hungary), but only five of them were used for further investigations. Here, we also collected a colony of *Aphaenogaster subterranea*, the locally most abundant species with smaller worker size. A colony of *Camponotus vagus*, a species with similar worker size, was collected from a poplar forest (*Populus alba*) near Ásotthalom (Southern Hungary). Colonies were kept in boxes of 37 cm × 26 cm × 22 cm, which were connected to a foraging arena of 60 cm × 30 cm × 15 cm with the help of a 10 cm long plastic tube. For our experiments we used plastic discs with a diameter of 10 cm, which were placed 20 cm from the entrance of the arena. Colonies were kept under laboratory conditions with 12 hours daylight (from 7 am to 7 pm) at a temperature of 22–25°C and a relative air humidity of 42–43%. Ants were fed every four days or at depletion with a commonly used artificial diet (Bhatkar and Whitcomb 1970). Colonies were moistened by spraying water on their nests and by placing a water-filled plastic tube with its opening facing downwards on a filter paper inside each arena.

Laboratory experiments

Our experiments were performed between 15–29 august 2012. A week before the experiments, we marked the foraging workers of the five colonies with enamel paint (Art Deco). Workers were classified into three size categories (minor, medium and major) and individually marked with different coloured spots on their heads, thorax and abdomen. For our experiments we used 10 corpses of freeze-killed (for 3 hours at –20°C in a Whirlpool AFG 305/G deep-freezer) workers on separate days from colonies of *A. subterranea* and *C. vagus* and from the sixth colony of a non-nest-mate *C. aethiops*, respectively. Corpses were removed from the deep-freezer with 90 minutes preceding the experiment for the development of corpse smell (Wilson et al. 1958, Howard and Tschinkel 1978, Choe and Rust 2008). Before the experiments, corpses were placed on the plastic discs inside the arena, where they were observed during a 3-hour experimental period. During the ob-

servations, the following behavioral reactions were recorded: eating food, drinking, dragging corpses, eating corpses or working on the dump site. Corpses were placed in each arena at the same time (at ca. 11 am) and they were observed three times for five minutes in two minute intervals and further until the end of the three hour observation period for five minutes in one minute intervals.

Statistical analysis

From our data table we excluded those painted individuals that were observed only one day from the 3–day observation period. For statistical analysis, we summed the waste management activities such as biting and dragging corpses and working in the waste yard (further abbreviated as WMs), and other activities such as eating food or drinking (further abbreviated as Os). The frequency of WMs was scaled to the sum of all behaviors to have a WM index for the binomial error term. We analyzed the differences in the WMs among the different size classes of *C. aethiops* workers and towards the different corpses with the help of a GLMM with binomial error term. The best model was chosen with manual model selection (ANOVA function with Chi2 test) and the smallest AIC value (Crawley 2007). In the case of the two GLMM factors the pair-wise comparisons were made with "relevel" function, afterwards we used Bonferroni-Holm correction. Since the two fixed effects (size category of workers and the factor of the separate painted individuals) were not self determined, we analyzed the behavioral differences between the painted individuals with the help of paired Wilcoxon signed rank tests. The same test was used to compare the individual

Table 1. Number and relative frequencies of individuals performing different behaviors from the three castes. WMs: waste management activities, Os: other activities.
1. táblázat: *A különböző viselkedési csoportokon belül tevékenységeket ellátó dolgozók egyedszáma és relatív gyakorisága. WMs: hulladék-kezelési tevékenységek; Os: egyéb tevékenységek).*

Caste	WMs	Os	WMs+Os
Major	4 (19.05%)	2 (9.52%)	15 (71.43%)
Medium	5 (16.13%)	5 (16.13%)	21 (67.74%)
Minor	5 (11.63%)	10 (23.26%)	28 (65.12%)

differences in the case of the different behaviors. All statistical analyses were performed in R statistical environment (R Core Team, 2012).

Results

Overall, we painted 95 workers, 21 (22.11%) of which belonged to the major, 31 (32.63%) to the medium and 43 (45.26%) to the minor caste (Table 1). Al-

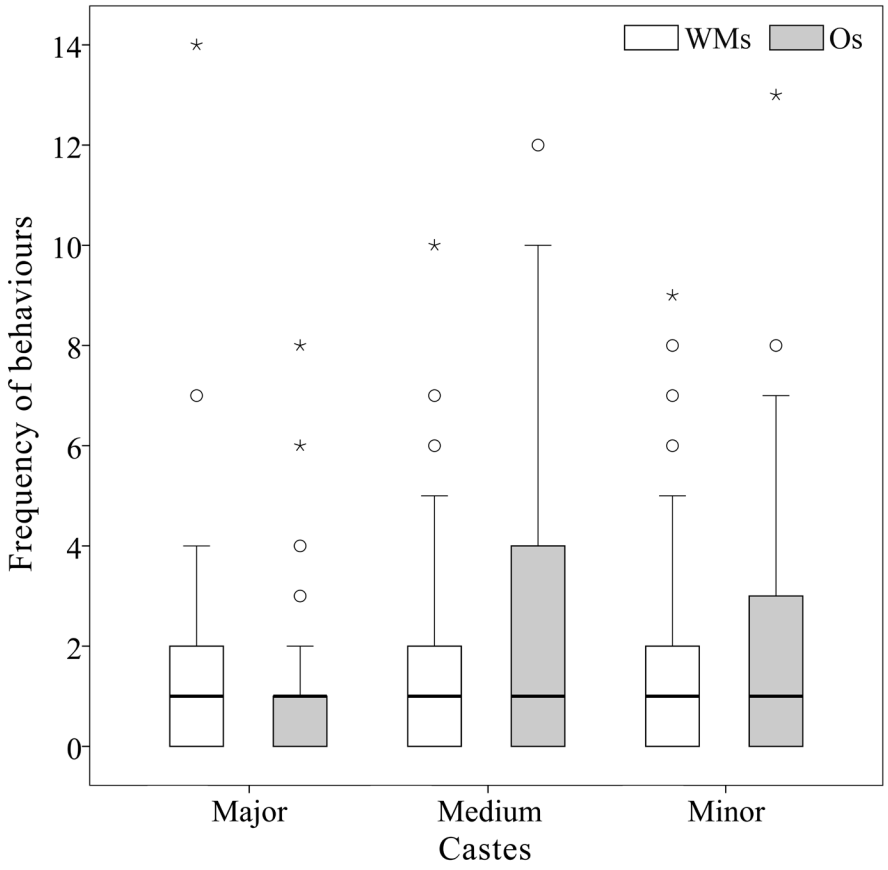

Fig. 1. Frequencies of the different behavioral classes among castes. *WMs: waste management activities, Os: other activities.*
1. ábra: *A kasztonkénti viselkedési kategóriák gyakorisága. WMs: hulladék-kezelési tevékenységek; Os: egyéb tevékenységek.*

together we observed 845 cases of the different behaviors, from which the major caste performed 152 (17.99%; 85 WMs and 67 Os), the medium 315 (37.28%; 109 WMs and 206 Os) and the minor 378 (44.73%; 127 WMs and 251 Os). On average, 4.05 WMs and 3.19 Os were performed by the major individuals, 3.52 WMs and 6.65 Os by medium individuals, and 2.95 WMs and 5.84 Os by minors.

Major workers performed significantly less WMs than the medium (GLMM $z=2.45$, $N=285$, $p=0.02$) and minor workers (GLMM $z=3.09$, $N=285$, $p<0.01$), but the difference was insignificant between workers of the two latter castes (GLMM $z=0.75$, $N=285$, $p=0.45$). Regarding the effect of the different corpses on the frequency of WMs, we found significant differences between the reactions to the corpses of *A. subterranea* and those of the two *Camponotus* species (*C. aethiops* GLMM $z=4.40$, $N=285$, $p=0.02$; *C. vagus* $z=4.30$, $N=285$, $p=0.01$), but we did not observe any difference between the reactions to the corpses of two latter species (GLMM $z=-0.08$, $N=285$, $p=0.94$). The individually painted workers showed differences in the frequency of WMs from that of the Os (paired Wilcoxon $V=7425$, $p<0.001$) and these differences remained even in the case of the medium ($V=683$, $p<0.01$) and minor ($V=1265.5$, $p<0.001$) castes, but not by the major workers ($V=615.5$, $p=0.26$) (Fig. 1).

Discussion

Workers from different morphological castes can join different activities in differing amounts. It was proved that in some species with workers of different size classes the different morphological castes may have specific activities, e.g., major workers can be defense or storage specialists, or medium workers of army ants may become carrier specialists (Hölldobler and Wilson 1990). Waste management is a very important trait for colony survival, so the colonies with greater infection prevalence may have a specialised worker caste, as was described in some *Atta* species in many studies (e.g., Wilson 1980, Ratnieks and Anderson 1999, Bot et al. 2001). In other polymorphic species, the removal of waste can be performed, independently from castes, by every individual encountering the debris, as was observed in *P. badius* and *S. invicta* (Wilson et al. 1958, Howard and Tschinkel 1978). In concordance with these latter results, we found no special-

ized morphological caste for waste management in *C. aethiops*, although we observed the medium and minor caste more active not only in corpse removal but also in other activities. Similar findings were reported for leaf-cutter ants, where the minors were observed as the most and the majors as the least active castes performing different tasks, with the medium workers somewhere between them (Wilson 1980, Camargo et al. 2007). Without specialized castes, some species may have specialized individuals that are more prone to fulfil some duties than their nestmates (Sendova-Franks and Franks 1993, Julian and Cahan 1999). These workers can complete a task with a greater efficiency and finalize it with a greater proportion (Oster and Wilson 1978, Ratnieks and Anderson 1999). Furthermore, Czechowski (2009) did not find any correlation between the corpse carrying individuals of *Formica polyctena* and those participating in other activities, so he supposed that the former are more "ready" to perform some actions than others. In *C. aethiops*, at least in the case of the minor and medium worker caste, we could distinguish individuals more prone to fulfill a given task, in our case behaviors other than WMs. In the foraging arena, around 15% of the workers performed duties in relation only with waste removal, and further 67% of workers performed other tasks beside waste management. It can be supposed that these latter individuals were generalist workers. It is not rare that some individuals can remain generalists to establish the connection among specialized individuals or castes and to fill the lack of workers at different tasks with a suboptimal performance (Wilson 1980, Sendova-Franks and Franks 1993, Camargo et al. 2007). In the case of the major workers of *C. aethiops* we could not distinguish behavioral differences among the individuals, so we can suppose that this caste is a generalist one. However, if we take into consideration that the major workers were those that rushed out firstly in case of nest disturbance, they may be specialized for nest defence (Hölldobler and Wilson 1990), fulfilling other tasks only by chance. Similarly to the retrieval of different sized food particles, the removal of debris can be size-dependent and needs cooperation, so it can lead to differences in the frequency of waste management behaviors in the case of corpses of different sizes. As expected, we did not find any difference in the reaction to the corpses of the two similar sized *Camponotus* workers, but surprisingly we found a higher frequency of behaviors to the corpses of *A. subterranea.* This can be due to the handling difficulties of smaller corpses by the workers of bigger size. The corpses were usually

dropped several times by the major workers, so it took several moments to remove them. If a removal was started by a major worker, it was usually finished by workers from a smaller size class.

In summary, we did not find any specialised castes for behaviors related to waste removal, but we could distinguish workers, at least in the medium and minor class, that were more prone to corpse removal and garbage handling. These could make up ca. 15% of all the foraging workers. Around 67% of workers seemed to be generalists, taking part in actions lacking a proper number of workers. The major size class took part in every task in similar amounts, so we can suppose that they are defence specialists and take part in other tasks in a generalist manner.

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Hulladékkezelés a polimorf *Camponotus aethiops* hangyafaj (Hymenoptera:Formicidae) esetén

A munkamegosztás a szociális rovarok egy fontos tulajdonsága, mely morfológiai és életkorbeli különbségek segítségével valósulhat meg. A legtöbb hangyafaj dolgozói átmennek korhoz kapcsolt viselkedésbeli változásokon, melynek során időszakosan specializálódhatnak különböző feladatokra. A hulladékkezelés egyike ezen fontos feladatoknak, mely alacsonyan tarthatja a fertőzési prevalenciát. Laboratóriumi körülmények között öt *Camponotus aethiops* kolónia esetén vizsgáltuk, hogy van e különbség a különböző kasztok és egyedi dolgozók között a különböző méretű tetemekkel szembeni viselkedést illetően. A többi kaszthoz képest alacsonyabb hulladékkezelési aktivitást figyeltünk meg a major dolgozók esetén. A többi kaszton belül

egyedi különbségek voltak megfigyelhetőek a hulladékkezelési és más feladatok esetén. A kereső dolgozók kb. 15%-a specializálódott időszakosan hulladékkezelésre, míg kb. 67%-a generalistaként volt jelen, kaszttól függetlenül. A major kasztba tartozó dolgozók amellett, hogy zavarás esetén mutattak nagyobb aktivitást, mint egy védelemre specializált kaszt, a többi feladat elvégzésében generalista módon vettek részt. Eredményeink alapján úgy tűnik, hogy annak ellenére, hogy nem találtunk egy csak erre a feladatra specializált dolgozói kasztot, a hulladékkezelésnek fontos szerepe van a *C. aethiops* esetén. Ezt alátámasztandó, megfigyeltünk olyan egyedeket, melyek nagyobb valószínűséggel végezték el ezen feladatot, ezáltal is növelve ennek elvégzési hatékonyságát, csökkentve ezáltal a fertőzési prevalenciát, hozzájárulva a kolónia túléléséhez.