Bring out your dead: quantifying corpse removal in

*Bombus terrestris*, an annual eusocial insect

Zoe Munday and Mark J. F. Brown*

School of Biological Sciences, Royal Holloway University of London, Egham, UK

*Corresponding author

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Correspondence: Mark J F Brown, School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, TW20 0EX, +44 7914021356. (Email: mark.brown@rhul.ac.uk).
Corpse removal is a hygienic behaviour involved in reducing the spread of parasites and
disease. It is found in social insects such as honey bees, wasps, ants and termites, insect
societies which experience high populations and dense living conditions that are ideal for the
spread of contagion. Previous studies on corpse removal have focused on perennial species
that produce thousands of workers, a life-history which may incur a greater need for hygienic
behaviours. However, whether and how corpse removal occurs in annual species of social
insect, which may experience different selection pressures for this behaviour, remains
largely unknown. Here the corpse removal behaviour of the bumblebee *Bombus terrestris*
was investigated by artificially adding larval and adult corpses into colonies. Larvae were
removed more rapidly than adults, with adult corpses eliciting significantly more antennating
and biting behaviours. Workers who removed larval corpses were significantly more
specialised than the worker population at large, but this was not the case for workers who
removed adult corpses. Workers who were previously observed spending more time inactive
were slightly, but significantly less likely to perform corpse removal. Size did not have an
effect on whether a worker removed corpses, but workers who removed larvae were
significantly larger than those who removed adult corpses. Finally, infecting larvae with the
virulent parasite *Nosema bombi* did not elicit prophylactic removal. Our results provide the
first quantification of corpse removal in an annual social insect, and set the scene for
comparative analyses of this important behaviour across social insect life-histories.

**KEY WORDS:**

*Bombus terrestris*; bumblebee; corpse removal; midden; necrophoresis; *Nosema bombi*;
social immunity.
Social insect colonies have evolved arguably the most complex societies in the animal kingdom (Wilson, 1971). Their sophisticated colonies enable ecological dominance (Wilson, 1971), but at the same time this social life comes with a range of costs. One such cost is the disposal of waste, and specifically the disposal of dead colony members (Schmid-Hempel, 1998). As in human societies, where dead individuals are identified by members of the medical profession, removed by undertakers, and buried by grave-diggers or cremated in specialised structures, perennial honey bee, ant, and termite societies have been shown to dispose of dead nestmates in a variety of ways. Honey bees remove infected or dead individuals from the hive (Visscher, 1983; Trumbo, Huang, & Robinson, 1997; Trumbo & Robinson, 1997; Breed, Williams, & Queral, 2002), and detect and remove dead or diseased brood (Rothenbuhler, 1964; Spivak & Gilliam, 1993). Similar behaviours have been found in ants, where workers remove dead workers (Julian & Cahan, 1999; Arathi, Burns, & Spivak, 2000; Bot, Currie, Hart, & Boomsma, 2001; Choe, Millar, & Rust, 2009; Diez, Deneubourg, & Detrain, 2012; Diez, Borgne, Lejeune, & Detrain, 2013; Diez, Lejeune, & Detrain, 2014) and pupae (Qiu, Lu, Shi, Tu, Lin, & He, 2015). Termites either remove (Renucci, Tirard, & Provost, 2011), isolate (Ulyshen & Shelton, 2012), or bury (Chouvenc, Robert, Sémon, & Bordereau, 2012) the dead members of their colony. In both ants and honey bees, such behaviours are often conducted by a set of workers, who have been primarily allocated to the task of corpse removal, the so-called ‘undertakers’ (Rothenbuhler, 1964; Julian & Cahan, 1999). The removal and isolation of dead nestmates is associated with the potential threat of contamination and disease from decaying corpses, which is a particular issue in the densely-populated nests of perennial social insects. The evolution of such corpse-removal behaviour and task allocation is thus presumably a balance between the costs of not removing corpses and the costs of doing so, modified by the benefits gained from corpse disposal. Previous work has focused on large, complex, perennial societies (see above), which usually have large forces of relatively inactive, reserve workers, and here the benefits of removing corpses clearly outweigh the costs of doing so, or the costs of not disposing of corpses at all.
The costs and benefits of corpse removal are likely to vary with colony size and longevity. For example, the costs of not removing corpses from the colony are likely to increase with colony longevity, as the longer a colony lives the more such waste will build up. In contrast, the costs of corpse removal, in terms of both the actual energetic removal cost and the allocation of workers to this task, are likely to be relatively lower in large colonies, where large groups of reserve workers exist. Given this, understanding whether corpse removal occurs in small annual eusocial colonies, and, if so, how it is done, may provide insight into the costs and benefits of such behaviour.

Bumblebees provide an ideal model system to address such questions. Colonies have an annual life-cycle, existing for a few months from foundation to senescence, and generally consist of tens to a few hundreds of workers. While corpse removal has been observed (Sladen, 1912) or assumed (Jandt & Dornhaus, 2014) in previous studies, such behaviour has not been systematically studied or quantified. Here we use controlled laboratory experiments to address the following questions: 1) do bumblebees exhibit consistent corpse removal? 2) does the type of corpse influence removal behaviour? 3) can we predict, based on size or behavioural profile, what individuals are responsible for corpse removal? 4) can bumblebees perform prophylactic removal of diseased brood?

MATERIALS AND METHODS

Study colonies

Three colonies of Bombus terrestris audax were ordered from BioBest (Belgium). These colonies were labelled A, B and C. The colonies were transferred into new plastic observation boxes (29.5 x 23 x 14 cm) and 40 workers were randomly selected, removed and allocated an individual number tag that was glued to their thorax; all other workers were
removed. Different coloured tags were used for each of the colonies. During worker removal, while the brood was unoccupied, a larval clump was removed and 10 approximately equal-sized larvae were extracted from each colony (Colony A: mean length ± SD = 3.8 ± 0.34 mm, N = 10; Colony B: X ± SD = 4.8 ± 0.85 mm, N = 10; Colony C: X ± SD = 5.5 ± 0.47 mm, N = 10). The larvae were stored in individually labelled Eppendorf tubes corresponding to that colony and frozen prior to experiments. The remaining adults that were not allocated a number tag were freeze-killed and 10 of approximate equal size (Colony A: mean thorax width ± SD = 11.4 ± 0.51 mm, N = 10; Colony B: X ± SD = 11.4 ± 0.51 mm, N = 10; Colony C: X ± SD = 11.9 ± 0.31 mm, N = 10) were removed from each colony and stored ready for experiments. After the brood and queens were transferred into the observation boxes the newly tagged adults were re-introduced to their original colony. The observation boxes were attached to their own individual foraging arena (104 x 79 x 52 cm) with a plastic tunnel (22 x 3.5 x 3.5 cm). These arenas were supplied *ad libitum* with nectar in plastic dispensers, and false flowers made from cardboard and pipe-cleaners that replicated the anther of a flower to which ground pollen was applied by hand. The colonies were given several days to enable a regular foraging pattern to be established; this was identified by foragers venturing into the foraging arena, drinking nectar or collecting pollen and returning straight back to the colony box. Nectar was provided in dispensers that were connected to colony boxes overnight and pollen added to the nest to ensure larvae were fed if pollen was not foraged from the arenas. Throughout the course of observations newly emerged bees were tagged with a new number tag with the colour corresponding to that colony. A maximum of ~60 bees were tagged from each colony, after this limit was reached un-tagged bees were then removed and frozen; this enabled accurate in-colony observations.

*Behavioural observations*

*Scan sampling to create an individual-level behavioural profile prior to experimental trials*
Each colony was observed for approximately 30 minutes every morning over 2 weeks. The behaviour of each worker was recorded and allocated to a behaviourally-defined ‘task’ (Table 1). These data were inputted by date and time to create a unique behavioural profile for each individual bee.

Corpse removal trials

The time taken for larvae or adult corpses to defrost was kept constant across experimental trials, as the odour profile of the corpse may change with defrosting time (Diez, Moquet, & Detrain, 2013). For each trial, once the corpse was defrosted it was added back into its original colony onto an area of brood where no bees were within 2 cm. Once the corpse was added a timer was set. Focal animal sampling was used to identify the behaviour displayed towards each corpse by the interacting worker or workers (see Table 1). The tag numbers of the workers who performed the interactions and the times at which these interactions occurred were recorded. Observations stopped when this behaviour resulted in the corpse being deposited in (i) a refuse area within the nest or (ii) the foraging arena, if no further interactions were made for 2 minutes, or if the corpse was lost from view. Individual larval corpses were added into one colony at a time and observed. Adult corpses were then added into each colony and observed. This process was repeated until behavioural observations had been completed for 10 larval corpses and 10 adult corpses per colony. We conducted experimental replicates over a series of successive days, separating repeats of corpse-type in individual colonies by approximately 24 h, making short-term reinforcement or specialisation unlikely.

Experiments to test prophylactic removal

*No* *sema* *bo* *mbi* is a virulent pathogen of bumblebees (Otti & Schmid-Hempel, 2007; Otti & Schmid-Hempel, 2008; Rutrecht & Brown, 2009) that is most infective to larvae (Rutrecht,
Klee, & Brown, 2007). After eclosion, infected individuals (a proportion of which have crippled wings and thus never leave the nest; Rutrecht & Brown, 2009) shed spores within the nest, leading to an increase in the prevalence, and presumably impact of the parasite over the colony lifecycle (Rutrecht & Brown, 2008). Removal of such infected larvae could be used to control the parasite, and thus this provides an excellent system in which to test for prophylactic brood removal.

Preparation of inoculum

The inoculum was prepared by dissecting the abdomens and extracting the guts of four B. terrestris males that had been infected with N. bombi. The gut contents from each male bee were placed in an individual Eppendorf tube together with 250 µl of ammonium chloride. This was then crushed using a blunt pipette tip until the solution was mixed and spores of N. bombi were suspended. Presence of N. bombi was confirmed for each bee by observing 5µl of each inoculum under a phase contrast microscope at x400 magnification and scanning for spores. Tubes containing spores were then stored on ice to prevent spores from germinating. To prepare purified inocula, tubes were spun in a balanced cold centrifuge at 4°C, 5000 rpm for 10 minutes. Supernatant was removed from each of the tubes using a pipette, taking care not to dislodge the pellet that had formed, and checked for spores. No spores were found so the effluent was discarded. 250 µl of ammonium chloride was added to each of the Eppendorf tubes, which were then spun down again following the same protocol as above. The supernatant was again removed from the Eppendorf tubes, and 250 µl ammonium chloride was added, vortexed to dissolve the pellet and stored on ice. To determine the concentration of inoculum 15 µl was taken from each sample and spores were quantified using a Neubauer haemocytometer. Eppendorf tubes containing inoculum were stored in a freezer at -80 °C for 24 h before use.
Preparation of micro-colonies and exposure of larvae to *N. bombi*

Micro-colonies were established using three additional *B. terrestris* colonies from Biobest.

Clumps of brood made up of approximately 10 larvae were carefully extracted from the colonies using rounded-end forceps so as not to break the wax cocoon. Each brood clump was used to make one micro-colony. Three workers were taken from the original colony that brood was extracted from and added to the corresponding micro-colony. Each worker had a unique number tag attached to its thorax, with colour corresponding to whether it was in a control (white tag) or treatment (green tag) micro-colony. Three control and three treatment replicate groups were produced from each original colony, for a total of nine control and nine treatment micro-colonies. Individual larvae within control groups were fed 4 µl of a diet we call worker mix, made from 10 ml of 50% sugar water and 6 g pollen; if the mixture was too viscous more sugar water was added. The treatment inoculum fed to larvae was made by combining 2 µl of worker mix with 2 µl of an inoculum that contained 53 400 spores of *N. bombi*.

**Micro-colony observations**

Each micro-colony was scan-sampled on a daily basis for 5 minutes, recording the behaviours performed by workers, such as brood care and grooming (see Table 1). This was carried out over 10 days or until all larvae had eclosed.

**Larval dissection and screening for *N. bombi***

Larvae that had been discarded from the brood by workers in the micro colony were removed and frozen in individual Eppendorf tubes, labelled with the name of the colony and the date, and frozen in a -80 °C freezer. For dissections the larvae were individually defrosted until soft, and the entire gut was removed using sterilised forceps and placed in an
Eppendorf tube. 150 µl of 0.9% Ringer solution was added and the gut was then mashed with a blunt pipette tip. Using a Blaubrand ® Intramark microcapillary tube, approximately 5 µl of the homogenate was extracted and deposited on a glass slide. This was repeated 3 times on the same slide. The samples were then scanned using a phase contrast microscope at x400 magnification for presence/absence of *N. bombi* spores.

Worker dissections and screening for *N. bombi*

Adults that had eclosed from the micro-colonies were each placed into individual plastic vials. A microcapillary tube was used to extract the faeces of each bee once they had defecated, and deposited onto a glass slide. Presence/absence of *N. bombi* was recorded by scanning slides for spores using a phase contrast microscope at x400 magnification. Once workers were screened they were frozen in a -25 °C freezer, then individually placed into Eppendorf tubes and kept in a -80 °C freezer. Workers that had died before being able to be screened were placed into individual Eppendorf tubes and stored in a freezer at -25 °C prior to dissection. Workers were removed and defrosted, following which the abdomen of each bee was dissected and prepared following the same procedure as for larvae (see Larval dissection). Presence/absence of *N. bombi* were recorded.

Data analysis

The behavioural profile of each bee in the larval and adult corpse removal experiments was characterised using Simpsons Diversity Index. Statistical analyses were conducted using IBM SPSS statistics 21. Chi-square tests were used to look for differences across colonies, and across corpse types in the likelihood of corpse removal taking place, and to examine potential differences in the number of inoculated and control larvae discarded in the *Nosema* experiment. General linear models, with colony as a random variable, were used to examine the temporal patterns of corpse removal, the relationship between behavioural diversity and
corpse removal, size and corpse removal, and behavioural responses to inoculated larvae. A binary logistic regression was used to determine whether behavioural profile predicted the corpse removal behaviour of workers.

RESULTS

Worker response to adult vs larval corpses

Corpse removal behaviour occurred in all colonies. Interactions between workers and corpses ranged from brief antennation to picking up the corpse and flying with it out of the colony into the foraging arena before discarding it. A total of 33 bees were observed performing complete corpse removal behaviour across the 60 trials (defined as actively picking up a corpse from the brood area where it was laid, and disposing of the corpse in a midden pile in the nest or in the foraging arena). An additional 5 corpses (3 larvae, 2 adult) were completely disposed of, but these disposals involved multiple individuals who each carried the corpse only part of the way between the brood and a refuse area. In the remaining trials, corpses were either lost from sight (after being taken underneath the brood), or workers ceased interacting with them.

Only a small number of bees interacted with a corpse in more than one larval trial (Larvae: Colony A = 2/11 bees, Colony B = 5/25, Colony C = 3/19; where the denominator is the total number of bees from a colony that interacted with larvae across trials). In contrast, and presumably due to the large number of workers that interacted with corpses in trials with adults (Adults: $X \pm SD = 12 \pm 5.3$ across all 30 trials; Larvae: $X \pm SD = 2 \pm 1.5$ across all 30 trials), most bees that interacted with adult corpses did so in more than one trial (Colony A = 33/48, Colony B = 41/66, Colony C = 39/61). There was no difference across colonies in the likelihood of a complete corpse removal event (as defined above) occurring in a trial for either larvae ($X^2_2 = 1.071, P = 0.585$) or workers ($X^2_2 = 5.700, P = 0.058$). However, larval
corpses were significantly more likely to be disposed of within a trial than adult corpses ($X^2_{1} = 20.742, P < 0.001$; Figure 1). Interestingly, only larval corpses were flown into and discarded in the foraging arena by workers, with 20% ($N = 6$) of larval corpses being discarded of in this way.

There was no effect of either corpse-type ($F_{1,54} = 0.009, P = 0.931$), colony ($F_{2,54} = 0.426, P = 0.701$), or their interaction ($F_{2,54} = 1.299, P = 0.281$) on the length of time between the start of a trial and when the corpse was first encountered by a worker (Table 2). In contrast, larval corpses were picked up for disposal much more rapidly than adult corpses ($F_{1,47} = 208.528, P = 0.004$; Table 2). There was no effect of either colony ($F_{2,47} = 1.483, P = 0.403$) or the colony-corpse interaction ($F_{2,47} = 0.381, P = 0.685$) on the delay to a corpse being picked up for disposal. Adult corpses were not ignored prior to being picked up for disposal – in fact, they received significantly more interactions (antennations and biting events) from workers prior to being picked up than did larval corpses ($F_{1,47} = 346.097, P = 0.003$; Table 2). Again, there was no effect of either colony ($F_{2,47} = 2.345, P = 0.299$) or the colony-corpse interaction ($F_{2,47} = 0.494, P = 0.614$) on this metric. Surprisingly, despite the difference in size of larval corpses (mean length = $4.7 \pm 0.17$ mm) and adult corpses (mean length = $11.6 \pm 0.09$ mm), there was no difference in the length of time for complete removal between the two types of corpses ($F_{1,26} = 0.016, P = 0.909$; Table 2). While there was similarly no effect of experimental colony ($F_{2,26} = 0.180, P = 0.847$), there was a significant interaction between colony and corpse-type ($F_{2,26} = 3.553, P = 0.043$), largely driven by colony C taking longer to dispose of larvae ($313.9 \pm 92.45$ s) than adult corpses ($81.0 \pm 40.95$ s).

Are bees performing corpse removal behavioural specialists?

Workers who removed larval corpses were significantly more specialised individuals, based on their behavioural profile prior to experimental trials, than those who did not (Simpson’s...
Index mean ± SE: removal workers = 0.420 ± 0.046, non-removal workers = 0.304 ± 0.016; ANOVA: \( F_{1,218} = 35.10, P = 0.012 \); see supplementary data file for behavioural profile. This was not true for workers who removed adult corpses (Simpson’s Index mean ± SE: removal workers = 0.114 ± 0.014, non-removal workers = 0.116 ± 0.005; ANOVA: \( F_{1,218} = 0.05, P = 0.845 \)). There were no effects of colony in any of these analyses.

Does behavioural profile predict propensity to remove corpses?

Neither colony of origin nor time spent performing different behaviours prior to experimental trials were able to predict whether bees were likely to remove a larval corpse (the model contained no significant predictor variables). In contrast, workers who spent more time in an inactive state (that is, neither foraging, conducting brood-care, nest maintenance, or guarding) were slightly, but significantly less likely to remove adult corpses (Wald = 4.127, \( P = 0.042 \), \( \text{Exp}(B) = 0.957 \)).

Size of workers that perform corpse removal behaviour

Workers who removed larval corpses did not differ in size from their sister workers (thorax width X ±SE = 4.59 ± 0.103 mm vs. 4.43 ± 0.034 mm; \( F_{1,177} = 2.659, P = 0.105 \)). Neither colony (\( F_{2,177} = 0.794, P = 0.454 \)), nor the interaction between corpse removal and colony had significant effects on worker size (\( F_{2,177} < 0.001, P = 1.000 \)). The same lack of pattern was seen for workers who removed adult corpses (thorax width X ±SE = 4.36 ± 0.103 mm vs. 4.46 ± 0.034 mm; corpse removal - \( F_{1,177} = 1.604, P = 0.324 \), Colony – \( F_{2,177} = 1.927, P = 0.342 \), CR x C – \( F_{2,177} = 0.535, P = 0.586 \)). However, workers who removed larvae were significantly larger than those who removed adult corpses (Fig. 2; X ±SE = 4.58 ± 0.113 mm vs. 4.30 ± 0.101 mm; \( F_{1,22} = 23.675, P = 0.035 \); again, there were no effects of colony or the interaction (Colony – \( F_{2,22} = 4.421, P = 0.184 \), CR x C – \( F_{2,22} = 0.103, P = 0.902 \)).
Worker responses to larvae inoculated with *N. bombi* in microcolonies

There was no effect of inoculation on the behaviour shown to larvae (MANOVA - Parasite treatment: $F_{4,9} = 1.138, P = 0.486$; Colony: $F_{10,18} = 2.087, P = 0.118$; Parasite x Colony: $F_{10,18} = 0.845, P = 0.638$)

Proportion of *N. bombi* inoculated larvae discarded

Fourteen larvae were discarded by workers, five in total from the *N. bombi* inoculated microcolonies, and nine from the control micro-colonies. There was no significant difference between the two groups in the proportion of larvae discarded ($X^2_1 = 1.504, P = 0.220$). Out of the five larvae discarded by workers in *Nosema*-inoculated micro-colonies, 40% carried *Nosema* infections, as detected by dissection. As expected, none of the discarded larvae from control micro-colonies were infected. Across the whole experiment, 47 larvae pupated and hatched out as callow workers. 64% of callows hatched from *Nosema*-inoculated larvae carried a *Nosema* infection (21/33) and 19% of these emerged with deformed wings (4/21) while, as expected, none of the callows from control colonies were found to be infected ($N = 14$)

DISCUSSION

Adult workers of the annual social insect, *B. terrestris*, respond to both larval and adult corpses with a suite of behaviours culminating in corpse removal. These behavioural responses vary with corpse type, with larval corpses being dealt with significantly more quickly than adult corpses. While there is evidence that workers who removed larval corpses had an overall more specialised behavioural profile than those who did not, neither worker size nor specific past behaviours predicted the occurrence of corpse removal behaviour.
Finally, we found no evidence for prophylactic removal responses towards larvae that had been inoculated with a virulent and crippling pathogen.

Bumblebee workers responded to the presence of a larval corpse by rapidly picking it up and either walking it to a midden within the nestbox (middens had already been established by colonies before they were attached to their foraging arena) or, in a fifth of cases, flying it out of the nestbox and dropping it in the foraging arena. This latter mode of larval removal has been observed in the wild (D. Goulson, pers. comm.) and seems likely to be the most natural mode of larval corpse removal. As such, it is similar to brood and adult disposal in ants, honey bees, and termites (e.g., Rothenbuhler, 1964; Julian & Cahan 1999; Renucci, Tirard, & Provost, 2011). This suggests that the costs of contamination and disease posed by dead larvae are high enough to have selected for corpse removal behaviour in this annual social insect, despite its small colony size and short life-cycle. However, it should be noted that our results cannot prove that the corpse removal seen in these experiments is an example of necrophoresis, sensu stricto. It is equally plausible that corpse removal took place as part of a broader suite of cleaning behaviours, with corpses simply being recognised as waste items. Nevertheless, even if this is the case, the cost of leaving corpses in the nest must have contributed to the selective pressure for cleaning behaviour in general. Consequently, their removal indicates that the costs of not removing them are higher than the costs of leaving them in the nest. A formal categorisation of bumblebee corpse removal as necrophoretic behaviour would require further experiments using inert controls.

The response of workers to dead adult corpses was less direct, with adult corpses being antennated and bitten by multiple workers prior to any attempts to remove them from the brood area. The larger size of adult corpses and smaller size of workers who dealt with them made it more difficult for them to be carried, and few adult corpses were placed in the in-nest midden areas by the end of our trial periods. No worker flew with an adult corpse, again perhaps due to the corpse-worker size ratio, suggesting that in the wild adults who die within the nest are either disposed of within the nest area, or walked out of the nest entrance. A
previous experimental study that placed adult corpses in the nests of *B. impatiens* found that less than 50% were removed in a 24-h period, although it is unclear from this study whether corpses were taken into the foraging arena, deposited in nest-box midden piles, or were hidden somewhere in the nest (Jandt & Dornhaus, 2014). We frequently observe dismembered workers in the nest boxes of bumblebees (MJF Brown, pers. obs.), which, together with the biting behaviour we observed in this study, suggests that the response of bumblebees to adult corpses may differ significantly from that elicited by larval corpses. A number of possible reasons for these differences exist. For example, adults may take longer to decompose than larvae, and thus take longer to appear chemically dead to nest-mates. As we do not know which chemicals signal death in bumble bees, we were not able to investigate this. A second issue is that it is unclear what proportion of adult bumblebees naturally die within the nest, as opposed to outside it. If most adult bees die outside the nest, then behaviours for removing adult corpses would be under weaker selection. However, in our personal experience (20 years of working with bumblebee colonies) at least some dead workers are present in every bumblebee nest we have worked with, whether they were connected to indoor foraging arenas or to the outside world (MJF Brown pers. obs.). Longer-term observational, experimental, and chemo-ecological studies are needed to address these questions.

Our behavioural observations of workers prior to the corpse removal experiments showed that they performed multiple tasks. However, workers nevertheless varied in their degree of specialisation (that is, the degree to which their behaviour was dominated by one task), and this was related to the likelihood of them subsequently removing larval corpses. Workers that removed larval corpses had a more specialised behavioural profile than their sisters who did not interact with or remove corpses. The most obvious explanation for this is that workers who concentrated on brood care were more likely to encounter the experimental corpses. However, this explanation was not supported by the logistic regression analysis, which found that only time spent inactive was associated, and that negatively, with the likelihood of
performing corpse removal. One reason for this might be that workers conduct brood-care only towards larvae, but brood patches in *B. terrestris* colonies contain both larvae and pupae, and thus the population of workers in the brood area is not solely made up of animals involved in brood-care.

In contrast to studies in ants and honey bees (Trumbo, Huang, & Robinson, 1997; Julian & Cahan, 1999), we found no evidence for a specialised group of ‘undertakers’. In our larval experiments, it was usually the first worker who encountered the larva who removed it, and the identity of this individual varied from trial to trial. In the adult corpse experiments, corpses were encountered by a large proportion of the colony’s workforce prior to their removal, and there was no evidence that behavioural specialisation was associated with the likelihood of removing adult corpses. We suggest that, for larval corpses, spatial location of workers determines who removes them through a process of task allocation (as per the ‘foraging for work’ algorithm first suggested by Franks & Tofts, 1994; Gordon, 2016). Further experiments could test this by determining the spatial fidelity zones of individual workers (Sendova-Franks & Franks, 1995) prior to the experimental addition of corpses. In contrast, for adult corpses, while workers clearly recognised corpses (responding with biting and antennation behaviour), they may vary in their response threshold in terms of actually moving a corpse.

Task allocation in bumblebees is related to body size, with larger workers generally performing tasks that involve leaving the nest (such as foraging) and smaller workers performing in-nest tasks (such as brood care)(Free, 1955; Morse, 1978; Goulson, 2009).

However, we found no relationship between body size and whether a worker removed larval or adult corpses. Surprisingly, workers who removed larval corpses were larger than their sisters who removed adult corpses. Given the size difference between the two corpse types, we would have predicted exactly the opposite relationship.

Even though size was not found to play a part in whether a worker removed corpses, this does not completely rule out a role for morphology. For example, activation of special sense receptors in the olfactory epithelium known as trace-amine associated receptors (TAAR’s)
may be associated with necrophobic behaviours (Hussain, Saraiva, Ferrero, Ahuja, & Krishna et al., 2013; Li & Liberles, 2015; Wisman & Shrira, 2015). If workers vary in the amount of these receptors, they may also vary in their response threshold to corpses, which may explain the response we observed to adult corpses. This would be an intriguing line of investigation for future studies.

Prophylactic necrophoresis – the removal of diseased individuals prior to their death – has been demonstrated in honey bees, where workers remove diseased brood and adults (Rothenbuhler, 1964; Baracchi, Fadd, & Turillazzi, 2012). However, our experiments found no evidence to support the existence of such behaviour in bumblebees. Workers did not behave differently towards larvae inoculated with spores of the virulent parasite Nosema bombi, and these larvae were not discarded at a higher rate than healthy larvae. This is surprising, as our inoculations were successful at causing infections, and the removal of infected larvae would impede the intra-colony epidemic of a parasite (Rutrecht & Brown, 2008), that, unchecked, can devastate colony fitness (Otti & Schmid-Hempel, 2007; Otti & Schmid-Hempel, 2008; Rutrecht & Brown, 2009). The absence of a chemical cue for infection may explain the absence of prophylactic necrophoresis against diseased individuals. Indeed, selection on the pathogen to avoid stimulating its removal from the colony should be considerably stronger than selection on workers to detect its presence, as infected colonies still have some fitness (Rutrecht & Brown, 2009), whereas if the pathogen is removed its fitness is zero.

We have taken the first steps towards quantifying corpse removal, and possibly necrophoresis, in annual social insects. Our results suggest that the costs that drive such behaviour in large, perennial social insect colonies may also be sufficient to produce it in their smaller, annual analogues. Whether these costs are specific to the evolution of necrophoresis in bumblebees, or relate more generally to the evolution of waste removal, remains unclear. Regardless, there are nevertheless clear differences in the features of corpse removal between annual and perennial systems, both in terms of the degree of
specialisation of workers who handle and remove corpses, and the presence or absence of prophylactic necrophoresis. Further studies in bumblebees, and in other annual social insects, focusing on the disposal of adult corpses will enhance our comparative understanding of waste management and disease control in complex animal societies.

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REFERENCES


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Table 1. Description of the behaviours observed and recorded throughout the experiments.

<table>
<thead>
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<th>Behaviour</th>
<th>Description</th>
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<tbody>
<tr>
<td>Antennation</td>
<td>Touching the corpse with the antennae</td>
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<tr>
<td>Picking up</td>
<td>Grasping the corpse with mandibles or mouth parts and lifting (before transport)</td>
</tr>
<tr>
<td>Nudging</td>
<td>Gently pushing or touching a corpse with the head</td>
</tr>
<tr>
<td>Transporting corpse</td>
<td>Picking up and carrying corpse over the brood patch to deposit in another area of the nest, ultimately a refuse pile or foraging arena</td>
</tr>
<tr>
<td>Tugging</td>
<td>Grasping corpse and repetitively pulling to move a short distance, usually associated with large adult corpses that are heavier and harder to move</td>
</tr>
<tr>
<td>Dragging</td>
<td>Grasping corpse and pulling corpse a distance greater than 5mm</td>
</tr>
<tr>
<td>Attempted flying with corpse</td>
<td>Bee picks up corpse in mandibles and lifts off the surface for a short time but often lands again or tumbles</td>
</tr>
<tr>
<td>Flying with corpse</td>
<td>Bee picks up corpse in mandibles and lifts off surface. Marked as successful when worker reaches entrance nest exit into foraging arena with corpse still in mouth</td>
</tr>
<tr>
<td>Grooming</td>
<td>After handling or moving a corpse, bees clean themselves by running their middle and back legs over areas of their body that made contact with the corpse</td>
</tr>
<tr>
<td>Conflicting corpse-removers</td>
<td>Where two bees both attempt to move a corpse at the same time in opposite directions. Conflict starts when both bees grasp the corpse and attempt to move it</td>
</tr>
<tr>
<td>Brood care</td>
<td>Warming brood, feeding brood, making feeding holes and biting wax around brood cells</td>
</tr>
<tr>
<td>Nest maintenance</td>
<td>Moulding wax, moulding wax roof over brood, inspecting wax pots and moving debris</td>
</tr>
<tr>
<td>Activity</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Foraging</td>
<td>Bee seen entering the foraging arena, drinking nectar/eating pollen and returning back to the nest to deposit nectar in pot</td>
</tr>
<tr>
<td>Undertaking</td>
<td>Carrying a dead larvae or dead adult worker to a refuse pile or into foraging arena</td>
</tr>
<tr>
<td>No activity</td>
<td>Bee is stationary and not obviously conducting any of the behaviours described above</td>
</tr>
</tbody>
</table>
Table 2. Summary of behavioural interactions between adult bees and larval and adult corpses. Data shown are mean ± standard error

<table>
<thead>
<tr>
<th></th>
<th>Larval corpse</th>
<th>Adult corpse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to encounter (s)</td>
<td>15.2 ± 3.09</td>
<td>14.6 ± 4.42</td>
</tr>
<tr>
<td>Interactions with corpse prior to pick-up</td>
<td>1.5 ± 0.25</td>
<td>20.7 ± 1.60</td>
</tr>
<tr>
<td>Time to pick-up (s)</td>
<td>49.4 ± 8.49</td>
<td>716 ± 8.12</td>
</tr>
<tr>
<td>Time for complete removal (s)</td>
<td>177.8 ± 39.35</td>
<td>175.6 ± 65.80</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. The number of trials in which corpse removal was successful for larvae or adults, across colonies.

Figure 2. The size (mean thorax width ± standard deviation, mm) of workers who removed adult or larval corpses.

Supplementary data file

This excel spreadsheet contains the behavioural profile data used to calculate the index of behavioural specialisation. The data are given as percentage of total observed time for each animal.
Figure 1

![Bar chart showing successful trials for Colony A, Colony B, and Colony C. The chart compares Larva and Adult phases.](image)
Figure 2

![Figure 2: Mean thorax width comparison between Adult and Larva stages.](image)