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Bring out your dead: quantifying corpse removal in

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Bombus terrestris, an annual eusocial insect

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16 Corpse removal is a hygienic behaviour involved in reducing the spread of parasites and
17 disease. It is found in social insects such as honey bees, wasps, ants and termites, insect
18 societies which experience high populations and dense living conditions that are ideal for the
19 spread of contagion. Previous studies on corpse removal have focused on perennial species
20 that produce thousands of workers, a life-history which may incur a greater need for hygienic
21 behaviours. However, whether and how corpse removal occurs in annual species of social
22 insect, which may experience different selection pressures for this behaviour, remains
23 largely unknown. Here the corpse removal behaviour of the bumblebee *Bombus terrestris*
24 was investigated by artificially adding larval and adult corpses into colonies. Larvae were
25 removed more rapidly than adults, with adult corpses eliciting significantly more antennating
26 and biting behaviours. Workers who removed larval corpses were significantly more
27 specialised than the worker population at large, but this was not the case for workers who
28 removed adult corpses. Workers who were previously observed spending more time inactive
29 were slightly, but significantly less likely to perform corpse removal. Size did not have an
30 effect on whether a worker removed corpses, but workers who removed larvae were
31 significantly larger than those who removed adult corpses. Finally, infecting larvae with the
32 virulent parasite *Nosema bombi* did not elicit prophylactic removal. Our results provide the
33 first quantification of corpse removal in an annual social insect, and set the scene for
34 comparative analyses of this important behaviour across social insect life-histories.

35 **KEY WORDS:**

36 *Bombus terrestris*; bumblebee; corpse removal; midden; necrophoresis; *Nosema bombi*;
37 social immunity.

38

39 Social insect colonies have evolved arguably the most complex societies in the animal
40 kingdom (Wilson, 1971). Their sophisticated colonies enable ecological dominance (Wilson,
41 1971), but at the same time this social life comes with a range of costs. One such cost is the
42 disposal of waste, and specifically the disposal of dead colony members (Schmid-Hempel,
43 1998). As in human societies, where dead individuals are identified by members of the
44 medical profession, removed by undertakers, and buried by grave-diggers or cremated in
45 specialised structures, perennial honey bee, ant, and termite societies have been shown to
46 dispose of dead nestmates in a variety of ways. Honey bees remove infected or dead
47 individuals from the hive (Visscher, 1983; Trumbo, Huang, & Robinson, 1997; Trumbo &
48 Robinson, 1997; Breed, Williams, & Queral, 2002), and detect and remove dead or diseased
49 brood (Rothenbuhler, 1964; Spivak & Gilliam, 1993). Similar behaviours have been found in
50 ants, where workers remove dead workers (Julian & Cahan, 1999; Arathi, Burns, & Spivak,
51 2000; Bot, Currie, Hart, & Boomsma, 2001; Choe, Millar, & Rust, 2009; Diez, Deneubourg, &
52 Detrain, 2012; Diez, Borgne, Lejeune, & Detrain, 2013; Diez, Lejeune, & Detrain, 2014) and
53 pupae (Qiu, Lu, Shi, Tu, Lin, & He, 2015). Termites either remove (Renucci, Tirard, &
54 Provost, 2011), isolate (Ulyshen & Shelton, 2012), or bury (Chouvenc, Robert, Sémon, &
55 Bordereau, 2012) the dead members of their colony. In both ants and honey bees, such
56 behaviours are often conducted by a set of workers, who have been primarily allocated to
57 the task of corpse removal, the so-called 'undertakers' (Rothenbuhler, 1964; Julian & Cahan,
58 1999). The removal and isolation of dead nestmates is associated with the potential threat of
59 contamination and disease from decaying corpses, which is a particular issue in the densely-
60 populated nests of perennial social insects. The evolution of such corpse-removal behaviour
61 and task allocation is thus presumably a balance between the costs of not removing corpses
62 and the costs of doing so, modified by the benefits gained from corpse disposal. Previous
63 work has focused on large, complex, perennial societies (see above), which usually have
64 large forces of relatively inactive, reserve workers, and here the benefits of removing
65 corpses clearly outweigh the costs of doing so, or the costs of not disposing of corpses at all.

66

67 The costs and benefits of corpse removal are likely to vary with colony size and longevity.
68 For example, the costs of not removing corpses from the colony are likely to increase with
69 colony longevity, as the longer a colony lives the more such waste will build up. In contrast,
70 the costs of corpse removal, in terms of both the actual energetic removal cost and the
71 allocation of workers to this task, are likely to be relatively lower in large colonies, where
72 large groups of reserve workers exist. Given this, understanding whether corpse removal
73 occurs in small annual eusocial colonies, and, if so, how it is done, may provide insight into
74 the costs and benefits of such behaviour.

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

84

85 MATERIALS AND METHODS

86 *Study colonies*

87 Three colonies of *Bombus terrestris audax* were ordered from BioBest (Belgium). These
88 colonies were labelled A, B and C. The colonies were transferred into new plastic
89 observation boxes (29.5 x 23 x 14 cm) and 40 workers were randomly selected, removed
90 and allocated an individual number tag that was glued to their thorax; all other workers were

91 removed. Different coloured tags were used for each of the colonies. During worker removal,
92 while the brood was unoccupied, a larval clump was removed and 10 approximately equal-
93 sized larvae were extracted from each colony (Colony A: mean length \pm SD = 3.8 ± 0.34
94 mm, $N = 10$; Colony B: $X \pm$ SD = 4.8 ± 0.85 mm, $N = 10$; Colony C: $X \pm$ SD = 5.5 ± 0.47 mm,
95 $N = 10$). The larvae were stored in individually labelled Eppendorf tubes corresponding to
96 that colony and frozen prior to experiments. The remaining adults that were not allocated a
97 number tag were freeze-killed and 10 of approximate equal size (Colony A: mean thorax
98 width \pm SD = 11.4 ± 0.51 mm, $N = 10$; Colony B: $X \pm$ SD = 11.4 ± 0.51 mm, $N = 10$; Colony
99 C: $X \pm$ SD = 11.9 ± 0.31 mm, $N = 10$) were removed from each colony and stored ready for
100 experiments. After the brood and queens were transferred into the observation boxes the
101 newly tagged adults were re-introduced to their original colony. The observation boxes were
102 attached to their own individual foraging arena (104 x 79 x 52 cm) with a plastic tunnel (22 x
103 3.5 x 3.5 cm). These arenas were supplied *ad libitum* with nectar in plastic dispensers, and
104 false flowers made from cardboard and pipe-cleaners that replicated the anther of a flower to
105 which ground pollen was applied by hand. The colonies were given several days to enable a
106 regular foraging pattern to be established; this was identified by foragers venturing into the
107 foraging arena, drinking nectar or collecting pollen and returning straight back to the colony
108 box. Nectar was provided in dispensers that were connected to colony boxes overnight and
109 pollen added to the nest to ensure larvae were fed if pollen was not foraged from the arenas.
110 Throughout the course of observations newly emerged bees were tagged with a new
111 number tag with the colour corresponding to that colony. A maximum of ~60 bees were
112 tagged from each colony, after this limit was reached un-tagged bees were then removed
113 and frozen; this enabled accurate in-colony observations.

114

115 *Behavioural observations*

116 *Scan sampling to create an individual-level behavioural profile prior to experimental trials*

117 Each colony was observed for approximately 30 minutes every morning over 2 weeks. The
118 behaviour of each worker was recorded and allocated to a behaviourally-defined 'task'
119 (Table 1). These data were inputted by date and time to create a unique behavioural profile
120 for each individual bee.

121

122 *Corpse removal trials*

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

139

140 *Experiments to test prophylactic removal*

141 *Nosema bombi* is a virulent pathogen of bumblebees (Otti & Schmid-Hempel, 2007; Otti &
142 Schmid-Hempel, 2008; Rutrecht & Brown, 2009) that is most infective to larvae (Rutrecht,

143 Klee, & Brown, 2007). After eclosion, infected individuals (a proportion of which have
144 crippled wings and thus never leave the nest; Rutrecht & Brown, 2009) shed spores within
145 the nest, leading to an increase in the prevalence, and presumably impact of the parasite
146 over the colony lifecycle (Rutrecht & Brown, 2008). Removal of such infected larvae could be
147 used to control the parasite, and thus this provides an excellent system in which to test for
148 prophylactic brood removal.

149

150 *Preparation of inoculum*

151 The inoculum was prepared by dissecting the abdomens and extracting the guts of four *B.*
152 *terrestris* males that had been infected with *N. bombi*. The gut contents from each male bee
153 were placed in an individual Eppendorf tube together with 250 µl of ammonium chloride. This
154 was then crushed using a blunt pipette tip until the solution was mixed and spores of *N.*
155 *bombi* were suspended. Presence of *N. bombi* was confirmed for each bee by observing 5µl
156 of each inoculum under a phase contrast microscope at x400 magnification and scanning for
157 spores. Tubes containing spores were then stored on ice to prevent spores from
158 germinating. To prepare purified inocula, tubes were spun in a balanced cold centrifuge at 4
159 °C, 5000 rpm for 10 minutes. Supernatant was removed from each of the tubes using a
160 pipette, taking care not to dislodge the pellet that had formed, and checked for spores. No
161 spores were found so the effluent was discarded. 250 µl of ammonium chloride was added
162 to each of the Eppendorf tubes, which were then spun down again following the same
163 protocol as above. The supernatant was again removed from the Eppendorf tubes, and 250
164 µl ammonium chloride was added, vortexed to dissolve the pellet and stored on ice. To
165 determine the concentration of inoculum 15 µl was taken from each sample and spores were
166 quantified using a Neubauer haemocytometer. Eppendorf tubes containing inoculum were
167 stored in a freezer at -80 °C for 24 h before use.

168

169 *Preparation of micro-colonies and exposure of larvae to N. bombi*

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

183

184 *Micro-colony observations*

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

188

189 *Larval dissection and screening for N. bombi*

190 Larvae that had been discarded from the brood by workers in the micro colony were
191 removed and frozen in individual Eppendorf tubes, labelled with the name of the colony and
192 the date, and frozen in a -80 °C freezer. For dissections the larvae were individually
193 defrosted until soft, and the entire gut was removed using sterilised forceps and placed in an

194 Eppendorf tube. 150 µl of 0.9% Ringer solution was added and the gut was then mashed
195 with a blunt pipette tip. Using a Blaubrand ® Intramark microcapillary tube, approximately 5
196 µl of the homogenate was extracted and deposited on a glass slide. This was repeated 3
197 times on the same slide. The samples were then scanned using a phase contrast
198 microscope at x400 magnification for presence/absence of *N. bombi* spores.

199

200 *Worker dissections and screening for N. bombi*

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

211

212 *Data analysis*

213 The behavioural profile of each bee in the larval and adult corpse removal experiments was
214 characterised using Simpsons Diversity Index. Statistical analyses were conducted using
215 IBM SPSS statistics 21. Chi-square tests were used to look for differences across colonies,
216 and across corpse types in the likelihood of corpse removal taking place, and to examine
217 potential differences in the number of inoculated and control larvae discarded in the *Nosema*
218 experiment. General linear models, with colony as a random variable, were used to examine
219 the temporal patterns of corpse removal, the relationship between behavioural diversity and

220 corpse removal, size and corpse removal, and behavioural responses to inoculated larvae. A
221 binary logistic regression was used to determine whether behavioural profile predicted the
222 corpse removal behaviour of workers.

223

224 RESULTS

225 *Worker response to adult vs larval corpses*

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

236

237 Only a small number of bees interacted with a corpse in more than one larval trial (Larvae:
238 Colony A = 2/11 bees, Colony B = 5/25, Colony C = 3/19; where the denominator is the total
239 number of bees from a colony that interacted with larvae across trials). In contrast, and
240 presumably due to the large number of workers that interacted with corpses in trials with
241 adults (Adults: $X \pm SD = 12 \pm 5.3$ across all 30 trials; Larvae: $X \pm SD = 2 \pm 1.5$ across all 30
242 trials), most bees that interacted with adult corpses did so in more than one trial (Colony A =
243 33/48, Colony B = 41/66, Colony C = 39/61). There was no difference across colonies in the
244 likelihood of a complete corpse removal event (as defined above) occurring in a trial for
245 either larvae ($X^2_2 = 1.071$, $P = 0.585$) or workers ($X^2_2 = 5.700$, $P = 0.058$). However, larval

246 corpses were significantly more likely to be disposed of within a trial than adult corpses (χ^2_1
247 = 20.742, $P < 0.001$; Figure 1). Interestingly, only larval corpses were flown into and
248 discarded in the foraging arena by workers, with 20% ($N = 6$) of larval corpses being
249 discarded of in this way.

250

251 There was no effect of either corpse-type ($F_{1,54} = 0.009$, $P = 0.931$), colony ($F_{2,54} = 0.426$, $P =$
252 0.701), or their interaction ($F_{2,54} = 1.299$, $P = 0.281$) on the length of time between the start of
253 a trial and when the corpse was first encountered by a worker (Table 2). In contrast, larval
254 corpses were picked up for disposal much more rapidly than adult corpses ($F_{1,47} = 208.528$,
255 $P = 0.004$; Table 2). There was no effect of either colony ($F_{2,47} = 1.483$, $P = 0.403$) or the
256 colony-corpse interaction ($F_{2,47} = 0.381$, $P = 0.685$) on the delay to a corpse being picked up
257 for disposal. Adult corpses were not ignored prior to being picked up for disposal – in fact,
258 they received significantly more interactions (antennations and biting events) from workers
259 prior to being picked up than did larval corpses ($F_{1,47} = 346.097$, $P = 0.003$; Table 2). Again,
260 there was no effect of either colony ($F_{2,47} = 2.345$, $P = 0.299$) or the colony-corpse interaction
261 ($F_{2,47} = 0.494$, $P = 0.614$) on this metric. Surprisingly, despite the difference in size of larval
262 corpses (mean length = 4.7 ± 0.17 mm) and adult corpses (mean length = 11.6 ± 0.09 mm),
263 there was no difference in the length of time for complete removal between the two types of
264 corpses ($F_{1,26} = 0.016$, $P = 0.909$; Table 2). While there was similarly no effect of
265 experimental colony ($F_{2,26} = 0.180$, $P = 0.847$), there was a significant interaction between
266 colony and corpse-type ($F_{2,26} = 3.553$, $P = 0.043$), largely driven by colony C taking longer to
267 dispose of larvae (313.9 ± 92.45 s) than adult corpses (81.0 ± 40.95 s).

268

269 *Are bees performing corpse removal behavioural specialists?*

270 Workers who removed larval corpses were significantly more specialised individuals, based
271 on their behavioural profile prior to experimental trials, than those who did not (Simpson's

272 Index mean \pm SE: removal workers = 0.420 ± 0.046 , non-removal workers = 0.304 ± 0.016 ;
273 ANOVA: $F_{1,218} = 35.10$, $P = 0.012$; see supplementary data file for behavioural profile). This
274 was not true for workers who removed adult corpses (Simpson's Index mean \pm SE: removal
275 workers = 0.114 ± 0.014 , non-removal workers = 0.116 ± 0.005 ; ANOVA: $F_{1,218} = 0.05$, $P =$
276 0.845). There were no effects of colony in any of these analyses.

277

278 *Does behavioural profile predict propensity to remove corpses?*

279 Neither colony of origin nor time spent performing different behaviours prior to experimental
280 trials were able to predict whether bees were likely to remove a larval corpse (the model
281 contained no significant predictor variables). In contrast, workers who spent more time in an
282 inactive state (that is, neither foraging, conducting brood-care, nest maintenance, or
283 guarding) were slightly, but significantly less likely to remove adult corpses (Wald = 4.127, P
284 = 0.042, Exp(B) = 0.957).

285

286 *Size of workers that perform corpse removal behaviour*

287 Workers who removed larval corpses did not differ in size from their sister workers (thorax
288 width $X \pm SE = 4.59 \pm 0.103$ mm vs. 4.43 ± 0.034 mm; $F_{1,177} = 2.659$, $P = 0.105$). Neither
289 colony ($F_{2,177} = 0.794$, $P = 0.454$), nor the interaction between corpse removal and colony
290 had significant effects on worker size ($F_{2,177} < 0.001$, $P = 1.000$). The same lack of pattern
291 was seen for workers who removed adult corpses (thorax width $X \pm SE = 4.36 \pm 0.103$ mm
292 vs. 4.46 ± 0.034 mm; corpse removal - $F_{1,177} = 1.604$, $P = 0.324$, Colony - $F_{2,177} = 1.927$, $P =$
293 0.342 , CR x C - $F_{2,177} = 0.535$, $P = 0.586$). However, workers who removed larvae were
294 significantly larger than those who removed adult corpses (Fig. 2; $X \pm SE = 4.58 \pm 0.113$ mm
295 vs. 4.30 ± 0.101 mm; $F_{1,22} = 23.675$, $P = 0.035$); again, there were no effects of colony or the
296 interaction (Colony - $F_{2,22} = 4.421$, $P = 0.184$, CR x C - $F_{2,22} = 0.103$, $P = 0.902$).

297

298 *Worker responses to larvae inoculated with N. bombi in microcolonies*

299 There was no effect of inoculation on the behaviour shown to larvae (MANOVA - Parasite
300 treatment: $F_{4,9} = 1.138$, $P = 0.486$; Colony: $F_{10,18} = 2.087$, $P = 0.118$; Parasite x Colony: $F_{10,18}$
301 $= 0.845$, $P = 0.638$)

302

303 *Proportion of N. bombi inoculated larvae discarded*

304 Fourteen larvae were discarded by workers, five in total from the *N. bombi* inoculated micro-
305 colonies, and nine from the control micro-colonies. There was no significant difference
306 between the two groups in the proportion of larvae discarded ($\chi^2_1 = 1.504$, $P = 0.220$). Out of
307 the five larvae discarded by workers in *Nosema*-inoculated micro-colonies, 40% carried
308 *Nosema* infections, as detected by dissection. As expected, none of the discarded larvae
309 from control micro-colonies were infected. Across the whole experiment, 47 larvae pupated
310 and hatched out as callow workers. 64% of callows hatched from *Nosema*-inoculated larvae
311 carried a *Nosema* infection (21/33) and 19% of these emerged with deformed wings (4/21)
312 while, as expected, none of the callows from control colonies were found to be infected (N =
313 14)

314

315 DISCUSSION

316 Adult workers of the annual social insect, *B. terrestris*, respond to both larval and adult
317 corpses with a suite of behaviours culminating in corpse removal. These behavioural
318 responses vary with corpse type, with larval corpses being dealt with significantly more
319 quickly than adult corpses. While there is evidence that workers who removed larval corpses
320 had an overall more specialised behavioural profile than those who did not, neither worker
321 size nor specific past behaviours predicted the occurrence of corpse removal behaviour.

322 Finally, we found no evidence for prophylactic removal responses towards larvae that had
323 been inoculated with a virulent and crippling pathogen.

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

342 The response of workers to dead adult corpses was less direct, with adult corpses being
343 antennated and bitten by multiple workers prior to any attempts to remove them from the
344 brood area. The larger size of adult corpses and smaller size of workers who dealt with them
345 made it more difficult for them to be carried, and few adult corpses were placed in the in-nest
346 midden areas by the end of our trial periods. No worker flew with an adult corpse, again
347 perhaps due to the corpse-worker size ratio, suggesting that in the wild adults who die within
348 the nest are either disposed of within the nest area, or walked out of the nest entrance. A

349 previous experimental study that placed adult corpses in the nests of *B. impatiens* found that
350 less than 50% were removed in a 24-h period, although it is unclear from this study whether
351 corpses were taken into the foraging arena, deposited in nest-box midden piles, or were
352 hidden somewhere in the nest (Jandt & Dornhaus, 2014). We frequently observe
353 dismembered workers in the nest boxes of bumblebees (MJF Brown, pers. obs.), which,
354 together with the biting behaviour we observed in this study, suggests that the response of
355 bumblebees to adult corpses may differ significantly from that elicited by larval corpses. A
356 number of possible reasons for these differences exist. For example, adults may take longer
357 to decompose than larvae, and thus take longer to appear chemically dead to nest-mates.
358 As we do not know which chemicals signal death in bumble bees, we were not able to
359 investigate this. A second issue is that it is unclear what proportion of adult bumblebees
360 naturally die within the nest, as opposed to outside it. If most adult bees die outside the nest,
361 then behaviours for removing adult corpses would be under weaker selection. However, in
362 our personal experience (20 years of working with bumblebee colonies) at least some dead
363 workers are present in every bumblebee nest we have worked with, whether they were
364 connected to indoor foraging arenas or to the outside world (MJF Brown pers. obs.). Longer-
365 term observational, experimental, and chemo-ecological studies are needed to address
366 these questions.

367 Our behavioural observations of workers prior to the corpse removal experiments showed
368 that they performed multiple tasks. However, workers nevertheless varied in their degree of
369 specialisation (that is, the degree to which their behaviour was dominated by one task), and
370 this was related to the likelihood of them subsequently removing larval corpses. Workers that
371 removed larval corpses had a more specialised behavioural profile than their sisters who did
372 not interact with or remove corpses. The most obvious explanation for this is that workers
373 who concentrated on brood care were more likely to encounter the experimental corpses.
374 However, this explanation was not supported by the logistic regression analysis, which found
375 that only time spent inactive was associated, and that negatively, with the likelihood of

376 performing corpse removal. One reason for this might be that workers conduct brood-care
377 only towards larvae, but brood patches in *B. terrestris* colonies contain both larvae and
378 pupae, and thus the population of workers in the brood area is not solely made up of animals
379 involved in brood-care.

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

393 Task allocation in bumblebees is related to body size, with larger workers generally
394 performing tasks that involve leaving the nest (such as foraging) and smaller workers
395 performing in-nest tasks (such as brood care)(Free, 1955; Morse, 1978; Goulson, 2009).
396 However, we found no relationship between body size and whether a worker removed larval
397 or adult corpses. Surprisingly, workers who removed larval corpses were larger than their
398 sisters who removed adult corpses. Given the size difference between the two corpse types,
399 we would have predicted exactly the opposite relationship.

400 Even though size was not found to play a part in whether a worker removed corpses, this
401 does not completely rule out a role for morphology. For example, activation of special sense
402 receptors in the olfactory epithelium known as trace-amine associated receptors (TAAR’s)

403 may be associated with necrophobic behaviours (Hussain, Saraiva, Ferrero, Ahuja, &
404 Krishna et al., 2013; Li & Liberles, 2015; Wisman & Shrira, 2015). If workers vary in the
405 amount of these receptors, they may also vary in their response threshold to corpses, which
406 may explain the response we observed to adult corpses. This would be an intriguing line of
407 investigation for future studies.

408 Prophylactic necrophoresis – the removal of diseased individuals prior to their death – has
409 been demonstrated in honey bees, where workers remove diseased brood and adults
410 (Rothenbuhler, 1964; Baracchi, Fadd, & Turillazzi, 2012). However, our experiments found
411 no evidence to support the existence of such behaviour in bumblebees. Workers did not
412 behave differently towards larvae inoculated with spores of the virulent parasite *Nosema*
413 *bombi*, and these larvae were not discarded at a higher rate than healthy larvae. This is
414 surprising, as our inoculations were successful at causing infections, and the removal of
415 infected larvae would impede the intra-colony epidemic of a parasite (Rutrecht & Brown,
416 2008), that, unchecked, can devastate colony fitness (Otti & Schmid-Hempel, 2007; Otti &
417 Schmid-Hempel, 2008; Rutrecht & Brown, 2009). The absence of a chemical cue for
418 infection may explain the absence of prophylactic necrophoresis against diseased
419 individuals. Indeed, selection on the pathogen to avoid stimulating its removal from the
420 colony should be considerably stronger than selection on workers to detect its presence, as
421 infected colonies still have some fitness (Rutrecht & Brown, 2009), whereas if the pathogen
422 is removed its fitness is zero.

423 We have taken the first steps towards quantifying corpse removal, and possibly
424 necrophoresis, in annual social insects. Our results suggest that the costs that drive such
425 behaviour in large, perennial social insect colonies may also be sufficient to produce it in
426 their smaller, annual analogues. Whether these costs are specific to the evolution of
427 necrophoresis in bumblebees, or relate more generally to the evolution of waste removal,
428 remains unclear. Regardless, there are nevertheless clear differences in the features of
429 corpse removal between annual and perennial systems, both in terms of the degree of

430 specialisation of workers who handle and remove corpses, and the presence or absence of
431 prophylactic necrophoresis. Further studies in bumblebees, and in other annual social
432 insects, focusing on the disposal of adult corpses will enhance our comparative
433 understanding of waste management and disease control in complex animal societies.

434

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440

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

Behaviour	Description
Antennation	Touching the corpse with the antennae
Picking up	Grasping the corpse with mandibles or mouth parts and lifting (before transport)
Nudging	Gently pushing or touching a corpse with the head
Transporting corpse	Picking up and carrying corpse over the brood patch to deposit in another area of the nest, ultimately a refuse pile or foraging arena
Tugging	Grasping corpse and repetitively pulling to move a short distance, usually associated with large adult corpses that are heavier and harder to move
Dragging	Grasping corpse and pulling corpse a distance greater than 5mm
Attempted flying with corpse	Bee picks up corpse in mandibles and lifts off the surface for a short time but often lands again or tumbles
Flying with corpse	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
Grooming	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
Conflicting corpse-removers	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
Brood care	Warming brood, feeding brood, making feeding holes and biting wax around brood cells
Nest maintenance	Moulding wax, moulding wax roof over brood, inspecting wax pots and moving debris

Foraging	Bee seen entering the foraging arena, drinking nectar/eating pollen and returning back to the nest to deposit nectar in pot
Undertaking	Carrying a dead larvae or dead adult worker to a refuse pile or into foraging arena
No activity	Bee is stationary and not obviously conducting any of the behaviours described above

521

522

523 Table 2. Summary of behavioural interactions between adult bees and larval and adult
524 corpses. Data shown are mean \pm standard error

	Larval corpse	Adult corpse
Time to encounter (s)	15.2 \pm 3.09	14.6 \pm 4.42
Interactions with corpse prior to pick-up	1.5 \pm 0.25	20.7 \pm 1.60
Time to pick-up (s)	49.4 \pm 8.49	716 \pm 8.12
Time for complete removal (s)	177.8 \pm 39.35	175.6 \pm 65.80

525

526

527 **Figure legends**

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

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

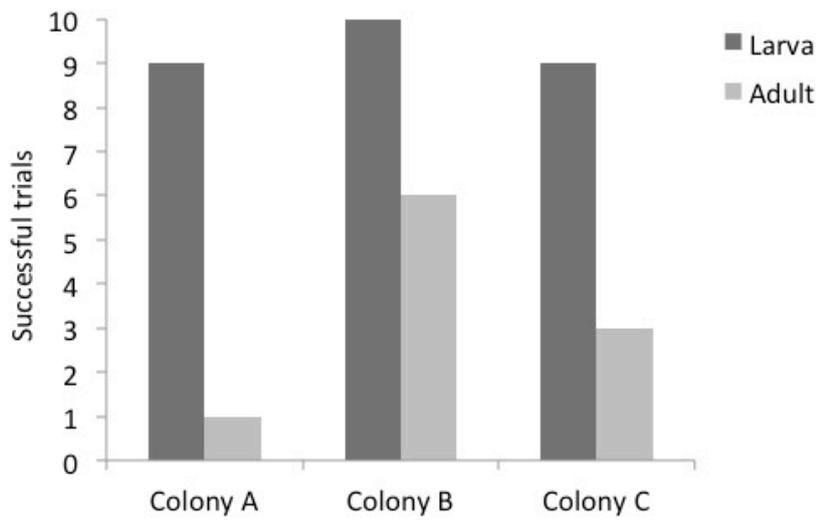
532

533 **Supplementary data file**

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

537

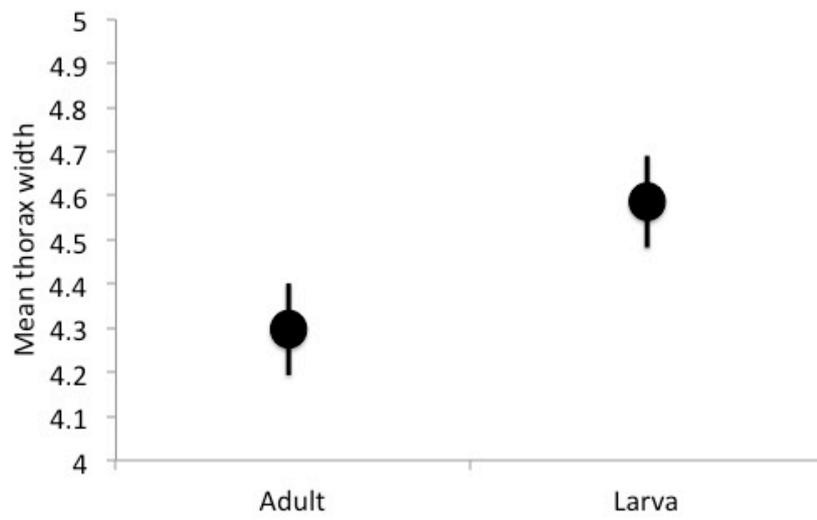
538 **Figure 1**



539

540

541 **Figure 2**



542