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Social buffering in a eusocial invertebrate: termite soldiers reduce the lethal impact of competitor cues on workers

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1 **Title:** Social buffering in a eusocial invertebrate: termite soldiers reduce the lethal impact of
2 competitor cues on workers

3

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13

14 **Short title:** Termite soldiers rescue workers from lethal stress

15

16

17 **Abstract**

18 While the impact of predator-induced stress on prey has received considerable attention, there
19 has been far less research into the effect of competitors. Cues from aggressive competitors
20 should be particularly likely to evoke behavioral and/or physiological responses, since they may
21 be indicative of both direct (interference) and indirect (exploitative) threats. The danger posed by
22 such competitors, and the 'fear' they evoke, should be reduced at lower competitor densities and
23 by the presence of individual conspecifics specialized for defense. We assessed how
24 *Reticulitermes flavipes* termite workers and soldiers were affected by cues from conspecific
25 nestmates, conspecific non-nestmates, and the heterospecific competitor *R. virginicus*.
26 Competitor cues altered *flavipes* worker and soldier behavior, decreasing worker growth and
27 increasing their mortality. The presence of *flavipes* soldiers largely ameliorated these negative
28 impacts: adding even a single soldier (5% of *flavipes* individuals) decreased worker mortality by
29 50-80%. Although worker mortality increased with competitor density, increased soldier
30 densities did not increase the benefit to workers. The small number of soldiers required to
31 substantially alter cue-mediated interactions suggests that this caste, in addition to providing
32 direct defense, also occupies a 'keystone role' by providing homeostatic feedback to workers
33 functioning in stressful environments.

34

35 **Keywords**

36 Interference competition, risk cues, stress, nonlethal effects, eusociality, soldiers

37

38 **Introduction**

39 Organisms often react to the presence of predators, competitors, or other stressors with an
40 array of behavioral and physiological changes that reduce the probability of being injured or
41 killed. While adaptive in acutely risky situations, chronic activation of these responses can have
42 a number of negative effects (Beckerman et al. 1997, McCauley et al. 2011). Behaviorally,
43 chronic risk-induced reductions in foraging and other activities often decrease growth and
44 fecundity (Creel et al. 2009, Adamo and Baker 2011). At the population level, the cumulative
45 impact of such non-consumptive effects can equal or exceed that of direct predator-induced
46 mortality (Preisser et al. 2005).

47 The impact of predator cues on prey suggests that some organisms may respond similarly
48 to cues from dangerous heterospecific competitors. Interference competition, especially during
49 territory defense, often results in intraguild killing (Dickman et al. 2014). When interspecific
50 interactions have density-dependent outcomes, intraspecific aggregation can provide an
51 numerical advantage against competitors in a manner similar to that found in predator-prey
52 interactions (Jungwirth et al. 2015). Researchers have documented social buffering, the ability of
53 nearby conspecifics to reduce the negative impact of stressors on individuals, in a wide range of
54 vertebrate taxa (reviewed in Hennessy et al. 2009). Although this suggests that the ability to alter
55 risk responses in response to conspecifics is advantageous, a similar response has not been
56 documented in invertebrates.

57 Termites (Blattodea: Termitoidae) provide an ideal system for exploring cue-mediated
58 impacts of heterospecific competitors, the factor(s) altering their magnitude of these impacts, and
59 social buffering. These colonially-living insects communicate via chemical and vibrational cues,
60 and their almost-exclusive reliance on cellulose for nutrition prevents them from using other

61 termite species as a food source. The two dominant termite castes are workers, the primary
62 foragers and nest caretakers who are often injured or killed during interspecific interactions
63 (Shelton and Grace 1996), and soldiers, defensive specialists who provide little foraging benefit
64 (Tian and Zhou 2014). Because a single location often contains multiple termite species that
65 compete both directly and indirectly for the same habitats or food sources, foraging workers are
66 chronically exposed to risk cues (Evans et al. 2009, Li et al. 2010). The continued presence of
67 workers in such risky habitats led us to suspect that soldiers might play a 'keystone role'
68 (Modlmeier et al. 2014) by acting as social buffers whose presence reduces worker sensitivity
69 and susceptibility to stressors.

70 We assessed how *Reticulitermes flavipes* termite workers were affected by cues from
71 conspecific nestmate, conspecific non-nestmate, and heterospecific (*R. virginicus*) workers, and
72 how the presence of an *R. flavipes* soldier altered the response of *R. flavipes* workers. We
73 separated adjacent colonies using a semipermeable barrier that prevented physical contact but
74 allowed cue transmission, allowing us to isolate the impact of cues on *R. flavipes* workers. We
75 also explored how worker responses were affected by a conspecific soldier and by different
76 densities of soldiers and competitors. In addition to their direct role in colony defense, we show
77 that soldiers reduce the impact of competition stress on the relatively vulnerable worker caste.

78 **Materials and Methods**

79 ***Reticulitermes colonies***

80 We used workers and soldiers from nine field-collected *Reticulitermes flavipes* colonies
81 (A1-A6, R1-R3) in this study. Workers from one field-collected *R. virginicus* colony (A7) were
82 used as the competitor. The distribution of these congeneric species overlaps throughout North
83 America, and each is agonistic towards the other (Polizzi and Forschler 1998). We collected 'A'-

84 prefix colonies from the University of Kentucky Arboretum (Lexington KY), and ‘R’-prefix
85 from Daniel Boone National Forest (Winchester KY). We used *R. flavipes* colonies within one
86 week of their collection to minimize the impact of isolation from their original colony; they were
87 maintained in growth chambers (complete darkness at $27 \pm 1^\circ\text{C}$, $80 \pm 1\%$ RH) and provisioned
88 with pine wood mulch and fine pine wood logs. We identified termite species by a combination
89 of soldier morphology and 16S mitochondrial ribosomal gene sequencing (Szalanski et al. 2003).

90 ***Behavioral survey: R. flavipes responses to conspecifics and heterospecifics***

91 We assessed whether the presence of soldiers altered the behavioral responses of *R.*
92 *flavipes* workers to the non-lethal presence of conspecifics or a heterospecific competitor (*R.*
93 *virginicus*). Prior to the survey, *R. flavipes* workers from the same colony were individually
94 marked as follows. Workers were transferred into a 55mm Petri dish containing a moist filter
95 paper disk. As individual workers walked on the disk, the dorsal side of their head, thorax or
96 abdomen was marked with two different colors of permanent marker. To reduce the potential for
97 injury, each body part on a given individual was only marked once. Marked workers were
98 transferred into another Petri dish; workers that sustained injury during marking were discarded.

99 *Survey design:* We added color-coded *R. flavipes* workers to a 35mm Petri dish (‘test’)
100 placed at the center of a 55mm Petri dish (‘periphery’; Fig. S1). Before adding workers, we cut
101 16 evenly-spaced 1mm slits into the wall of the 35mm dish that transmitted chemical cues and
102 allowed antennal contacts, but were too narrow for damaging/lethal interactions to occur. The
103 survey began when we added *R. flavipes*, either 20 workers or 19 workers and one *R. flavipes*
104 soldier, to a test area provisioned with moistened paper disks for the termites to consume. After a
105 24-hr acclimation period, we stocked the periphery with either 40 conspecific *R. flavipes* workers
106 from the same colony or 40 heterospecific *R. virginicus* workers. This created four treatments: *R.*

107 *flavipes* with conspecific cues without soldiers ('Conspecific') and with soldiers
108 ('Conspecific+S'), with heterospecific cues without soldiers ('Heterospecific') and with soldiers
109 ('Heterospecific+S'). The Conspecific and Conspecific+S treatments tested whether the workers
110 were responding to heterospecific competitors or termite density *per se*, and whether the effect of
111 soldier presence differed for of conspecific versus heterospecific cues. We used *R. flavipes*
112 colonies A1, A2, and R1, with one petri-dish replicate per colony for each of the four treatments,
113 for a total of 12 replicates (three colonies x four treatments).

114 After adding termites into the periphery area, we covered and sealed each 55mm petri
115 dish to decrease dehydration risk. We then used a Canon VIXIA HF G20 video camera to record
116 the behavior of all *R. flavipes* workers and the soldier in each dish over the next 24 hours. All
117 three dishes were held under laboratory conditions ($25 \pm 1^\circ\text{C}$, $70 \pm 1\%$ RH) and illuminated by a
118 ceiling-mounted fluorescent lamp. While we would have preferred to record termite behavior in
119 total darkness, external lighting was necessary for our video-recording equipment. At the end of
120 the 24-hr sampling period, we analyzed the recorded footage using Observer (Noldus,
121 Wageningen, The Netherlands), a behavior analysis program. At the beginning of the survey and
122 every four hours thereafter (i.e., 0, 4, 8, 12, 16, 20, and 24 hours), we analyzed a three-minute
123 section of video for the time spent on behaviors by each marked worker and the soldier.

124 We recorded the following behaviors for each worker and for the soldier as per Korb and
125 Schmidinger (2004): locomotion, resting, feeding, grooming (both itself and another individual),
126 and vibration (rapid back-and-forward bodily movement). We also observed other behaviors
127 (e.g., trophallaxis, defecation, and moving nestmate corpses) that were too infrequent to analyze.

128 Although we marked 20 termite workers in each petri dish prior to the start of
129 observations, the markings on many workers were partially or totally rubbed off by the end.

130 Because we only analyzed data from workers whose behavior could be tracked throughout the
131 24-hour period, we observed a mean of 8.25 (range: 6-11) workers per replicate. We averaged
132 worker data to calculate the per-replicate frequency of each of the six behavioral categories
133 (summing to 100%). Replicates in the ‘soldier’ treatments used data from the single soldier per
134 replicate as the measurement of soldier behavior.

135 *Experiment I: Short-term impact of soldiers on worker survival in response to cues*
136 *from heterospecific competitors*

137 Over a two-day period, we assessed whether the presence of soldiers affected the survival
138 of *R. flavipes* workers exposed to cues produced by two different *R. virginicus* worker densities.
139 As in the behavioral survey, the test area contained either 20 *R. flavipes* workers
140 (‘Heterospecific’), or 19 workers and one nest-mate soldier (‘Heterospecific+S’); none of the
141 workers were color-coded. In both this experiment and experiment III, the first part of the
142 treatment name, i.e., ‘Heterospecific’, denotes the type of termite cue that *R. flavipes* workers
143 experienced; the second part of the treatment name, i.e., ‘+S’, indicates the presence of an *R.*
144 *flavipes* soldier with the workers. Immediately after placing *R. flavipes* in the test area, we placed
145 either 20 (1:1 ratio) or 40 (2:1 ratio) *R. virginicus* workers in the periphery area. We provided
146 termites in both the test and periphery areas with a moistened paper disk for food. The petri
147 dishes were kept in an incubator ($27 \pm 1^\circ\text{C}$, $80 \pm 1\%$ RH) in complete darkness for two days,
148 then removed and surviving workers counted. We used *R. flavipes* colonies A3, A4, and A5 in
149 order to assess the potential for colony-level differences in termite responses. There were five
150 replicates per colony for each of the four treatments (1:1 Heterospecific, 1:1 Heterospecific+S,
151 2:1 Heterospecific, 2:1 Heterospecific+S), for a total of 60 replicates (three colonies x four
152 treatments x five replicates).

153 ***Experiment II: Short-term impact of soldiers on worker survival in response to cues***
154 ***from nestmate conspecifics and non-nestmate conspecifics***

155 Over a two-day period, we assessed whether the presence of soldiers affected the survival
156 of *R. flavipes* workers exposed to cues produced by *R. flavipes* nestmates or *R. flavipes* non-
157 nestmates. It was identical in design to experiment I except for the following differences.
158 Immediately after placing *R. flavipes* workers (and, in the appropriate treatments, a single
159 soldier) in the test area, we placed either 20 *R. flavipes* nestmate workers or 20 *R. flavipes* non-
160 nestmate workers in the periphery area. This generated four treatments: nestmates (N), nestmates
161 plus soldier (N+S), non-nestmates (NN), and non-nestmates plus soldier (NN+S). We used *R.*
162 *flavipes* colonies R2, R3, and A6; workers from colony A7 were used as non-nestmate
163 conspecifics for colonies R2 and R3, and workers from colony R4 were used as non-nestmate
164 conspecifics for colony A6. There were three replicates per colony for each of the four
165 treatments, for a total of 36 replicates (three colonies x four treatments x three replicates).

166 ***Experiment III: Long-term impact of soldiers on worker feeding, growth, and survival***

167 Over a 15-day period, we assessed whether soldiers (either one or two individuals)
168 altered the feeding rate, growth rate, and survival of *R. flavipes* workers exposed to *R. virginicus*
169 cues. The test area contained either 20 *R. flavipes* workers ('Heterospecific'), 19 workers and
170 one nest-mate soldier ('Heterospecific+S'), or 18 workers and two nest-mate soldiers
171 ('Heterospecific+2S'). Immediately after placing *R. flavipes* in the test area, we placed five *R.*
172 *virginicus* workers in the periphery area. As in experiments I and II, we added another treatment
173 in which five *R. flavipes* nestmate workers ('Conspecific' treatment) were placed in the
174 periphery. Termites in both areas were provisioned with a moistened paper disk that was
175 replaced every three days. All Petri dishes were kept in an incubator as per experiment I. The 15-

176 day length was chosen to simulate chronic exposure to neighboring colonies (a situation that
177 often occurs between these two species; Polizzi and Forschler 1998).

178 Experiment III was conducted using individuals from three *R. flavipes* colonies. For
179 colony A8, there were five replicates per treatment for each of the four treatments for a total of
180 20 replicates. For colony R5, there were seven replicates per treatment (total = 28), and for
181 colony R6, there were nine replicates per treatment (total = 36).

182 We recorded worker mortality and removed dead workers each day for 15 days. While
183 dead *R. flavipes* workers were not replaced, we did replace dead *R. flavipes* soldiers and dead *R.*
184 *virginicus* workers to maintain constant conditions. At the start of the experiment and every third
185 day, surviving workers were removed from each replicate, counted, and weighed to determine
186 average worker weight. Percentage change was determined by subtracting the initial weight from
187 the current measurement, dividing by the initial weight, and multiplying by 100.

188 We provisioned *R. flavipes* workers with a paper disk that had been oven-dried at 100 °C
189 for one hour and weighed before being moistened with 100ml deionized water and placed in the
190 test area. Every third day, we replaced the partially-consumed old disk with a new disk. We
191 brushed the old disk to remove extraneous material, then dried and weighed it; paper
192 consumption was calculated using the initial and final disk dry weights. We calculated paper
193 consumption rate ('PCR'; mg paper/mg termite/day) for each three-day period as follows:
194 $((\text{paper consumed, mg})/(\text{total worker weight, mg}))/3 \text{ days}$.

195 ***Statistical analysis***

196 We analyzed the combined dataset on worker behavior using principal component
197 analysis, a standard approach (e.g., Sitvarin et al. 2016). We followed recommended guidelines
198 and retained all components whose eigenvalues exceeded 1.0 (Abdi and Williams 2010). We

199 used two-way ANOVA to test for the main effects of cue type (conspecific, heterospecific),
200 soldier presence, and their interaction on each principal component; colony was also included as
201 a blocking variable. We used a similar procedure to analyze the dataset on soldier behavior.

202 Because the data in experiment I was not normally distributed, we assessed the individual
203 effects of soldier presence, *virginicus:flavipes* ratio, and their interaction on *R. flavipes* mortality
204 by fitting a generalized linear mixed model with a quasi-binomial error distribution ('logit' link
205 function) using the penalized quasi-likelihood (PQL) 'glmmPQL' function in the MASS package
206 in R (R Development Core Team 2010). Colony was used as a random effect in the model and χ^2
207 and *P*-values were obtained by performing a Wald χ^2 test on the model using the 'Anova'
208 function in the 'car' package. The same procedure was also employed for experiment II to test
209 the individual and interactive effects of nestmate status and soldier presence on *R. flavipes*
210 mortality; *R. flavipes* source colony was a random effect.

211 A linear mixed effects modeling approach was taken to analyze PCR, % weight change,
212 and % mortality data from experiment III. Linear mixed effects models were constructed for
213 each of these response variables and treatment (i.e. Conspecific, Heterospecific, Heterospecific +
214 S, and Heterospecific + 2S) nested within colony as fixed effects and sampling day as a random
215 effect using the 'lmer' function as part of the 'lme4' package in R (R Development Core Team
216 2010). Chi-square and *P*-values were obtained for response variables as described for
217 experiments I and II via a Wald χ^2 test.

218 **Results**

219 ***Termite cues altered worker and soldier behavior***

220 Workers behaved very differently in the presence of conspecifics versus heterospecific
221 cues, and in the presence or absence of a conspecific soldier (Fig. 1, left panel). The first

222 principal component explained 43% of the variation in worker behavior, and reflected
223 differences in worker resting, walking, and vibration (Supplementary Table 1). There was a main
224 effect of both cue type ($F_{1,6} = 49.6$, $p < 0.001$) and soldier presence ($F_{1,6} = 6.5$, $p = 0.043$). Cues
225 from *R. virginicus* workers increased the amount of time *R. flavipes* workers spent moving and
226 vibrating and decreased the time they spent resting, while the presence of a *R. flavipes* soldier
227 had the opposite effect (Fig. 1, left panel); the interaction, however, was not significant ($p = 0.5$).
228 The second (21%) and third (20%) principal components reflected differences in
229 feeding/walking/other and grooming/vibration/other behaviors, respectively (Supplementary
230 Table 1), but neither component was affected by cue type or soldier presence (all $p > 0.2$).
231 Colony identity affected the first principal component ($F_{2,6} = 8.4$, $p = 0.018$), but not the second
232 or third (both $p > 0.5$).

233 Although *R. flavipes* soldiers behaved differently than workers, they had similarly strong
234 responses to heterospecific cues (Fig. 1, right panel). The first principal component captured
235 79% of the variation in soldier behavior, and reflected the fact that heterospecific cues decreased
236 soldier resting and increased walking and vibration ($F_{1,2} = 25.6$, $p = 0.037$). Colony identity did
237 not affect this response ($p = 0.56$).

238 *Soldiers decreased the impact of heterospecific competitor cues on worker mortality*

239 The mortality rate of *R. flavipes* workers increased as a function of *R. virginicus* density
240 (Fig. 2A; 1:1 ratio = 10.1 ± 3.48 [SE]; 2:1 ratio = 21.9 ± 3.56 ; $\chi^2 = 6.96$, $df = 1$, $p = 0.008$). The
241 presence of a single *R. flavipes* soldier reduced the negative impact of *R. virginicus*, decreasing
242 worker mortality in both density treatments by >80% ($\chi^2 = 19.45$, $df = 1$, $p < 0.001$). The two-
243 way interaction was not significant ($p > 0.05$).

244 *Soldiers decreased the impact of conspecific non-nestmate cues on worker mortality*

245 Non-nestmate *R. flavipes* workers increased worker mortality more than nestmate
246 workers (Fig. 2B; $\chi^2 = 18.2$, $df = 1$, $p < 0.001$). There was less mortality in the presence of
247 nestmate workers, regardless of soldier presence. In contrast, cues from non-nestmate workers
248 increased mortality 25-fold relative to nestmate workers. Workers exposed to these non-nestmate
249 cues benefitted greatly from the presence of a soldier; mortality rates were 75% lower in the
250 soldier-present treatment than in the soldier-absent treatment ($\chi^2 = 8.28$, $df = 1$, $P < 0.004$; Fig.
251 2B). The two-way interaction was not significant ($p > 0.05$).

252 *A single soldier buffered the chronic impact of competitor cues on workers*

253 In the absence of soldiers, workers exposed to heterospecific cues consumed 32% less
254 paper over the course of the experiment than did workers exposed to conspecific cues (0.075
255 versus 0.111 mg/mg worker/day, respectively; Fig. 3A; $\chi^2 = 8.11$, $df = 3$, $P = 0.044$). While the
256 presence of one *R. flavipes* soldier reduced the negative impact of *R. virginicus* workers,
257 doubling the soldier percentage from ~5% (1/19) to ~11% (2/18) of total *R. flavipes* had no
258 additional impact. Because the Heterospecific+S and Heterospecific+2S treatments had similar
259 effects on all three measured variables, we hereafter focus on the Heterospecific+S treatment.

260 Despite different feeding rates, there were no treatment-level differences in mean worker
261 weight (Fig. 3B; $\chi^2 = 1.35$, $df = 3$, $P = 0.718$). The absence of a statistically-significant difference
262 is due to the fact that (A) The rapid death of smaller workers in the Heterospecific treatments left
263 only the largest workers alive; and (B) when all of the workers in a replicate died, we excluded
264 that replicate from our statistical analyses. The impact of including 'dead' replicates is seen in
265 Figure 3B, where the Heterospecific treatment diverged sharply from the two 'S' treatments on
266 day 15. This divergence reflects the fact that in 7/21 replicates in the Heterospecific treatment

267 had 100% *R. flavipes* mortality by day 15. In contrast, none of the 63 replicates in the other
268 treatments had 100% *R. flavipes* mortality.

269 Worker mortality in the presence of conspecific cues was minimal: 6% over the 15-day
270 experiment (Fig 3C). While heterospecific cues from even a small number of *R. virginicus*
271 workers (1:4 ratio of *virginicus* to *flavipes*) increased mortality tenfold in the absence of a
272 soldier, the presence of a soldier reduced mortality from 65% (heterospecific cues without
273 soldier) to 33% (heterospecific cues with soldier; $\chi^2 = 51.41$, $df = 3$, $p < 0.001$). There was no
274 difference in mortality rates between the Heterospecific+S and Heterospecific+2S treatments.

275 **Discussion**

276 Cues from both heterospecific and non-nestmate conspecific competitors were rapidly
277 lethal to *R. flavipes* termite workers, and their impact increased as a function of competitor
278 density. Although other studies have documented lethal effects of chronic predator cue exposure
279 in invertebrates (e.g., Schmitz et al. 1997, McCauley et al. 2011), we found that even two days of
280 competitor cue exposure sharply increased worker mortality. Conspecific nestmate soldiers
281 countered this effect, and substantially decreased worker mortality in both two-day experiments
282 (Figs. 2A and 2B) and the 15-day experiment (Fig. 3). While worker mortality scaled with
283 competitor density, the ameliorating impact of soldiers was unaffected by the worker:soldier
284 ratio: a doubling of soldier densities had no impact (Fig. 3). Our results appear to provide the
285 first evidence that social buffering, the ability of nearby conspecifics to reduce the negative
286 impact of stressors on an individual (Hennessy et al. 2009), also occurs in invertebrates and
287 appears to be associated with caste identity. Given the rarity of soldiers in *R. flavipes* colonies,
288 they seem to play a 'keystone role' (Modlmeier et al. 2014) via their amelioration of antagonistic
289 cue effects on the numerically dominant worker caste.

290 The competitor-induced increase in *R. flavipes* workers' activity and vibratory behavior
291 (Fig. 1, left panel) agrees with research finding that workers from four different *Reticulitermes*
292 species vibrated/oscillated when exposed to threatening situations (Reinhard and Clément 2002),
293 and with other work showing that termite soldiers decrease the magnitude of worker
294 vibration/defensive responses (Roisin et al. 1990, Ishikawa and Miura 2012). The fact that *R.*
295 *flavipes* mortality scaled with heterospecific density (Fig. 2A; also compare these mortality
296 levels to the third-day numbers in Fig. 3c) showed that workers were responding to both the
297 presence and magnitude of the threat (as per Van Buskirk et al. 2011). Although termite
298 responses to heterospecific chemical, vibrational, and auditory cues have attracted considerable
299 attention (reviewed in Costa-Leonardo and Haifig 2014), we are unaware of other work
300 documenting that the cues themselves can prove lethal.

301 The most likely explanation for our results appears to be that the combined impact of
302 increased energy expenditure (i.e., behavioral/physiological responses) and decreased energy
303 intake (i.e., reduced feeding) lethally depleted worker resources. This interpretation is consistent
304 with data from our behavioral survey, where workers exposed to conspecific nestmate cues spent
305 approximately equal time in energetically-costly and –beneficial activities (Fig. 1, left panel; red
306 versus blue cross-sections). Competitor cues increased the ratio of costly to beneficial activities
307 and decreased the fraction of time spent resting and feeding. Similar cessation of feeding has also
308 been documented in the grasshopper *Melanoplus femurrubrum*, where exposure to spider cues
309 increases starvation risk (Schmitz et al. 1997).

310 The ability of a single termite soldier to buffer the lethal effects of competitor cues
311 suggests a previously unrecognized degree of complexity in caste relationships. While soldiers
312 play a critical role in colony defense, they are only ~2% of the individuals in *R. flavipes* colonies

313 and spend much of their time immobile (Howard and Haverty 1981, Reinhard and Clément
314 2002). We found that soldiers exposed to conspecific cues spent >80% of their time resting and
315 were never observed grooming (Fig. 1, left panel). In contrast, the numerical dominance of
316 workers makes this caste likely to first encounter threats; *R. flavipes* workers are responsible for
317 triggering soldier aggregation and defense (Hu et al. 2003). The importance of worker-derived
318 cues is indicated by the fact that soldiers from several other *Reticulitermes* species respond more
319 strongly to worker alarm cues than to the threat itself, to the point of ignoring the threat when
320 workers are absent (Reinhard and Clément 2002).

321 While worker behaviors like rapid vibration may be required to quickly alert soldiers to a
322 potential threat, these energetically-costly actions should decrease once soldiers have responded.
323 Worker alarm/defensive behavior in the termite *Nasutitermes princeps*, for instance, virtually
324 stops once soldiers arrive at a threat (Roisin et al. 1990, also see Ishikawa and Miura 2012). If
325 the cessation of alarm behavior depends on soldier presence rather than the concentration of their
326 cues, then amelioration of worker responses should be relatively insensitive to soldier density.
327 This hypothesis is consistent with the fact that doubling soldier densities had no additional
328 impact on workers (Fig. 3). In the absence of soldiers, however, the energetic cost of continued
329 alarm behavior may eventually prove lethal to the signaling workers.

330 In addition to demonstrating a strong impact of competitor cues, our work also offers
331 insight into how caste identity might affect social buffering in eusocial invertebrates. While
332 many solitary animals exhibit a negative correlation between morphological defense and the
333 magnitude of their behavioral responses to risk, the presence of specialized castes may allow this
334 tradeoff to occur at the colony level in eusocial species (Tian and Zhou 2014). While such
335 specialization provides important benefits, a high degree of inter-caste coordination is necessary

336 to ensure rapid and appropriate responses to biotic and abiotic stressors (Bignell et al. 2011). A
337 cost of this interdependence is evident in high worker mortality rates when soldiers are absent,
338 while its benefit (i.e., providing workers ‘peace of mind’) is found in the reduced impact of cues
339 from competitors. Eusocial organisms span a wide range of taxa and include species that are
340 highly-successful inhabitants of both natural and human-modified environments; it seems likely
341 that social buffering plays a similar role in many of these systems.

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352 **Literature Cited**

353 Abdi, H. and L. J. Williams. 2010. Principal component analysis. Wiley Interdisciplinary
354 Reviews: Computational Statistics **2**:433-459.

355 Adamo, S. A. and J. L. Baker. 2011. Conserved features of chronic stress across phyla: the
356 effects of long-term stress on behavior and the concentration of the neurohormone octopamine in
357 the cricket, *Gryllus texensis*. Hormones and Behavior **60**:478-483.

358 Beckerman, A., M. Uriarte, and O. Schmitz. 1997. Experimental evidence for a behavior-
359 mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of*
360 *Sciences USA* **94**:10735-10738.

361 Bignell, D. E., Y. Roisin, and N. Lo. 2011. *Biology of Termites: a Modern Synthesis*. Springer,
362 London.

363 Costa-Leonardo, A. M. and I. Haifig. 2014. Termite communication during different behavioral
364 activities. Pages 161-190 *in* G. Witzany, editor. *Biocommunication of Animals*. Springer
365 Netherlands.

366 Creel, S., J. A. Winnie, Jr., and D. Christianson. 2009. Glucocorticoid stress hormones and the
367 effect of predation risk on elk reproduction. *Proceedings of the National Academy of Sciences*
368 *USA* **106**:12388-12393.

369 Dickman, C. R., A. S. Glen, M. E. Jones, M. E. Soule, E. G. Ritchie, and A. D. Wallach. 2014.
370 Strongly interacting carnivore species: maintaining and restoring ecosystem function. Pages 301-
371 323 *in* A. S. Glen and C. R. Dickman, editors. *Carnivores of Australia: Past, Present, and Future*.
372 CSIRO Publishing, Lincoln NZ.

373 Evans, T. A., R. Inta, J. C. Lai, S. Prueger, N. W. Foo, E. W. Fu, and M. Lenz. 2009. Termites
374 eavesdrop to avoid competitors. *Proceedings of the Royal Society of London, Series B:*
375 *Biological Sciences* **276**:4035-4041.

376 Hennessy, M. B., S. Kaiser, and N. Sachser. 2009. Social buffering of the stress response:
377 diversity, mechanisms, and functions. *Frontiers in Neuroendocrinology* **30**:470-482.

378 Howard, R. and M. I. Haverty. 1981. Seasonal variation in caste proportions of field colonies of
379 *Reticulitermes flavipes* (Kollar). *Environmental Entomology* **10**:546-549.

380 Hu, X., A. Appel, and J. Traniello. 2003. Behavioral response of two subterranean termites
381 (Isoptera: Rhinotermitidae) to vibrational stimuli. *Journal of Insect Behavior* **16**:703-715.

382 Ishikawa, Y. and T. Miura. 2012. Hidden aggression in termite workers: plastic defensive
383 behaviour dependent upon social context. *Animal Behaviour* **83**:737-745.

384 Jungwirth, A., D. Josi, J. Walker, and M. Taborsky. 2015. Benefits of coloniality: communal
385 defence saves anti-predator effort in cooperative breeders. *Functional Ecology* **29**:1218-1224.

386 Korb, J. and S. Schmidinger. 2004. Help or disperse? Cooperation in termites influenced by food
387 conditions. *Behavioral Ecology and Sociobiology* **56**:89-95.

388 Li, H.-F., R.-L. Yang, and N.-Y. Su. 2010. Interspecific competition and territory defense
389 mechanisms of *Coptotermes formosanus* and *Coptotermes gestroi* (Isoptera: Rhinotermitidae).
390 *Environmental Entomology* **39**:1601-1607.

391 McCauley, S. J., L. Rowe, and M.-J. Fortin. 2011. The deadly effects of “nonlethal” predators.
392 *Ecology* **92**:2043-2048.

393 Modlmeier, A. P., C. N. Keiser, J. V. Watters, A. Sih, and J. N. Pruitt. 2014. The keystone
394 individual concept: an ecological and evolutionary overview. *Animal Behaviour* **89**:53-62.

395 Polizzi, J. and B. Forschler. 1998. Intra-and interspecific agonism in *Reticulitermes flavipes*
396 (Kollar) and *R. virginicus* (Banks) and effects of arena and group size in laboratory assays.
397 *Insectes Sociaux* **45**:43-49.

398 Preisser, E., D. Bolnick, and M. Benard. 2005. Scared to death? The effects of intimidation and
399 consumption in predator-prey interactions. *Ecology* **86**:501-509.

400 R Development Core Team. 2010. R: A language and environment for statistical computing. R
401 Foundation for Statistical Computing, Vienna, Austria.

402 Reinhard, J. and J.-L. Clément. 2002. Alarm reaction of European *Reticulitermes* termites to
403 soldier head capsule volatiles (Isoptera, Rhinotermitidae). *Journal of Insect Behavior* **15**:95-107.

404 Roisin, Y., C. Everaerts, J. M. Pasteels, and O. Bonnard. 1990. Caste-dependent reactions to
405 soldier defensive secretion and chiral alarm/recruitment pheromone in *Nasutitermes princeps*.
406 *Journal of Chemical Ecology* **16**:2865-2875.

407 Schmitz, O., A. Beckerman, and K. O'Brien. 1997. Behaviorally-mediated trophic cascades:
408 effects of predation risk on food web interactions. *Ecology* **78**:1388-1399.

409 Shelton, T. G. and J. K. Grace. 1996. Review of agonistic behaviors in the Isoptera.
410 *Sociobiology* **28**:155-176.

411 Sitvarin, M. I., S. D. Gordon, G. W. Uetz, and Ann L. Rypstra. 2016. The wolf spider *Pardosa*
412 *milvina* detects predator threat level using only vibratory cues. *Behaviour* **153**:159-173.

413 Szalanski, A. L., J. W. Austin, and C. B. Owens. 2003. Identification of *Reticulitermes* spp.
414 (Isoptera: Reticulitermatidae) from south central United States by PCR-RFLP. *Journal of*
415 *Economic Entomology* **96**:1514-1519.

416 Tian, L. and X. Zhou. 2014. The soldiers in societies: defense, regulation, and evolution.
417 *International Journal of Biological Sciences* **10**:296-308.

418 Van Buskirk, J., M. Ferrari, D. Kueng, K. Nöpflin, and N. Ritter. 2011. Prey risk assessment
419 depends on conspecific density. *Oikos* **120**:1235-1239.

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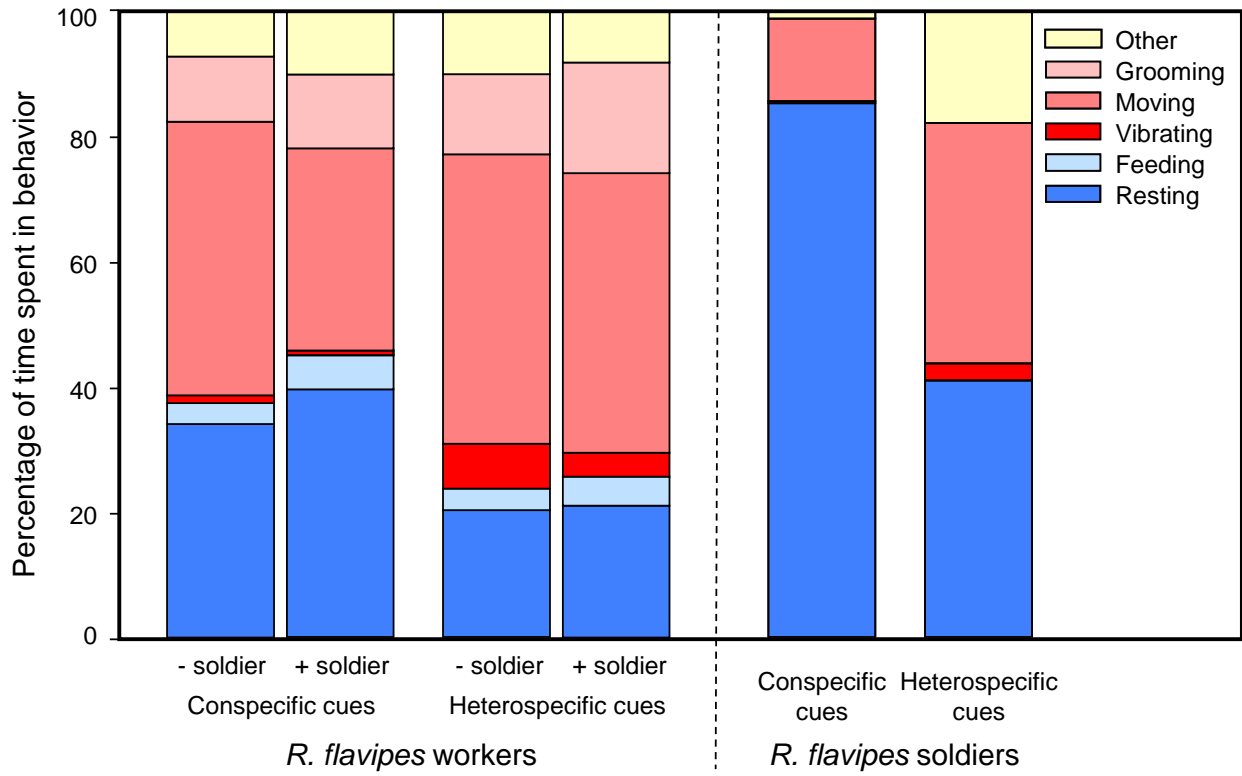
421 **Figure Legends**

422 **Figure 1.** Left panel: behavioral responses of *R. flavipes* workers to cues from conspecific
423 workers or heterospecific *R. virginicus* workers in the absence or presence of a single *R. flavipes*
424 nest-mate soldier. Right panel: behavioral responses of *R. flavipes* soldiers to cues from
425 conspecific nest-mate workers or heterospecific *R. virginicus* workers. Red bars: energetically-
426 costly activities (grooming, moving, and vibrating); blue bars: energetically-beneficial activities
427 (resting and feeding).

428 **Figure 2.** (A) Cumulative mortality of *R. flavipes* workers over two days when exposed to cues
429 from *R. virginicus* workers in the absence (yellow bars) and presence (yellow-checked bars) of a
430 single *R. flavipes* nest-mate soldier. Left pair of bars: 1:1 *virginicus:flavipes* ratio; right pair of
431 bars: 2:1 *virginicus:flavipes* ratio. (B) Cumulative mortality of *R. flavipes* workers over two days
432 when exposed to cues from *R. flavipes* workers in the absence (orange bars) and presence
433 (orange-checked bars) of a single *R. flavipes* nest-mate soldier. Left pair of bars: nestmate *R.*
434 *flavipes* workers; right pair of bars: non-nestmate *R. flavipes* workers.

435 **Figure 3.** Worker feeding rate (3A), mean percent weight change of alive workers relative to day
436 0 (3B), and percent mortality (3C) over a 15-day experimental period in the presence of cues
437 from conspecific workers (brown diamonds), *R. virginicus* workers (orange circles), or *R.*
438 *virginicus* workers and also one (yellow inverted triangles) or two (green triangles) *R. flavipes*
439 nest-mate soldiers. The large drop in percent weight change on day 15 for the Heterospecific
440 treatment is due to the fact that there was 100% mortality in 7/21 replicates; no other treatments
441 had any replicates with 100% mortality.

442 **Figure 1.**



443

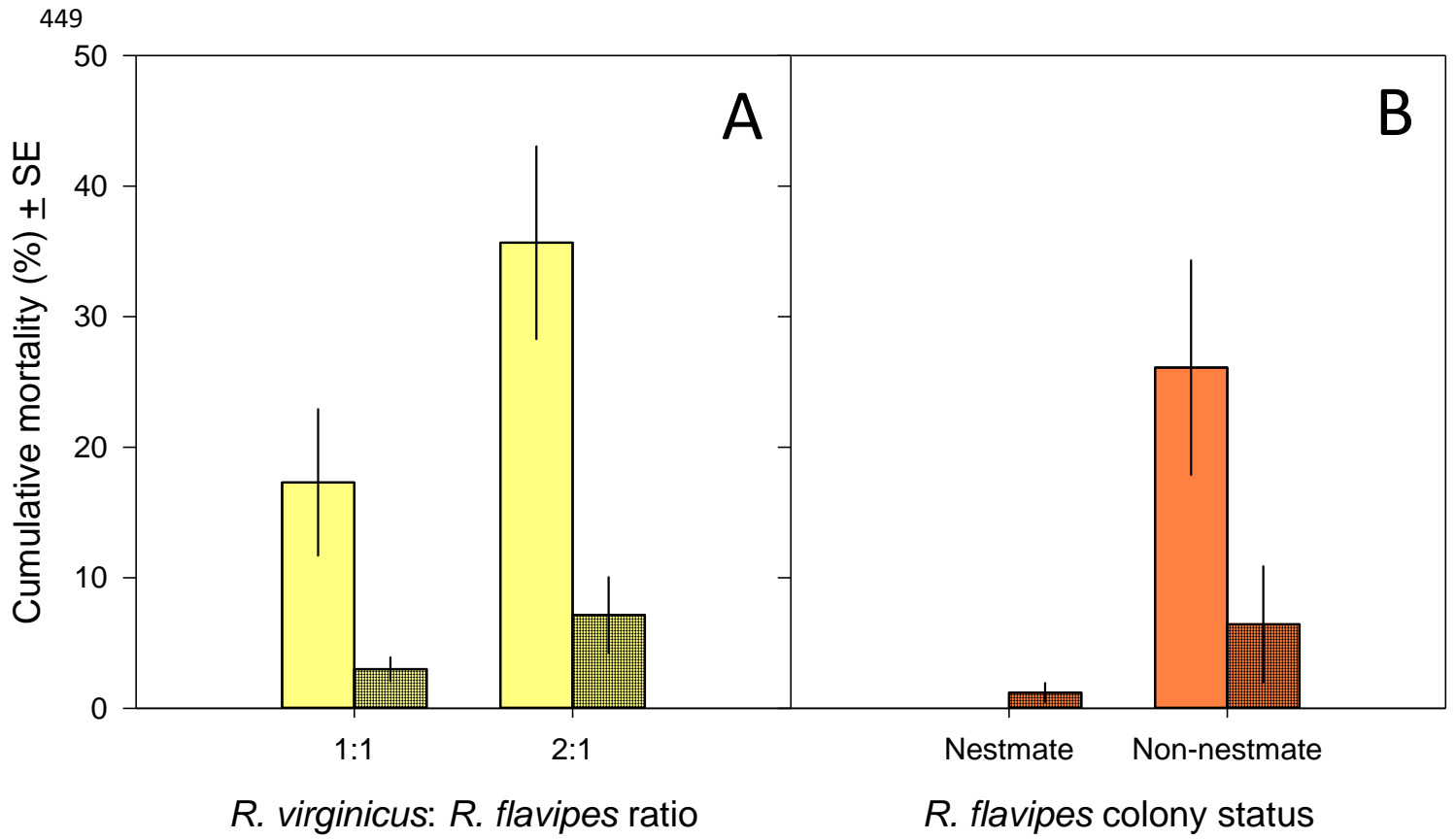
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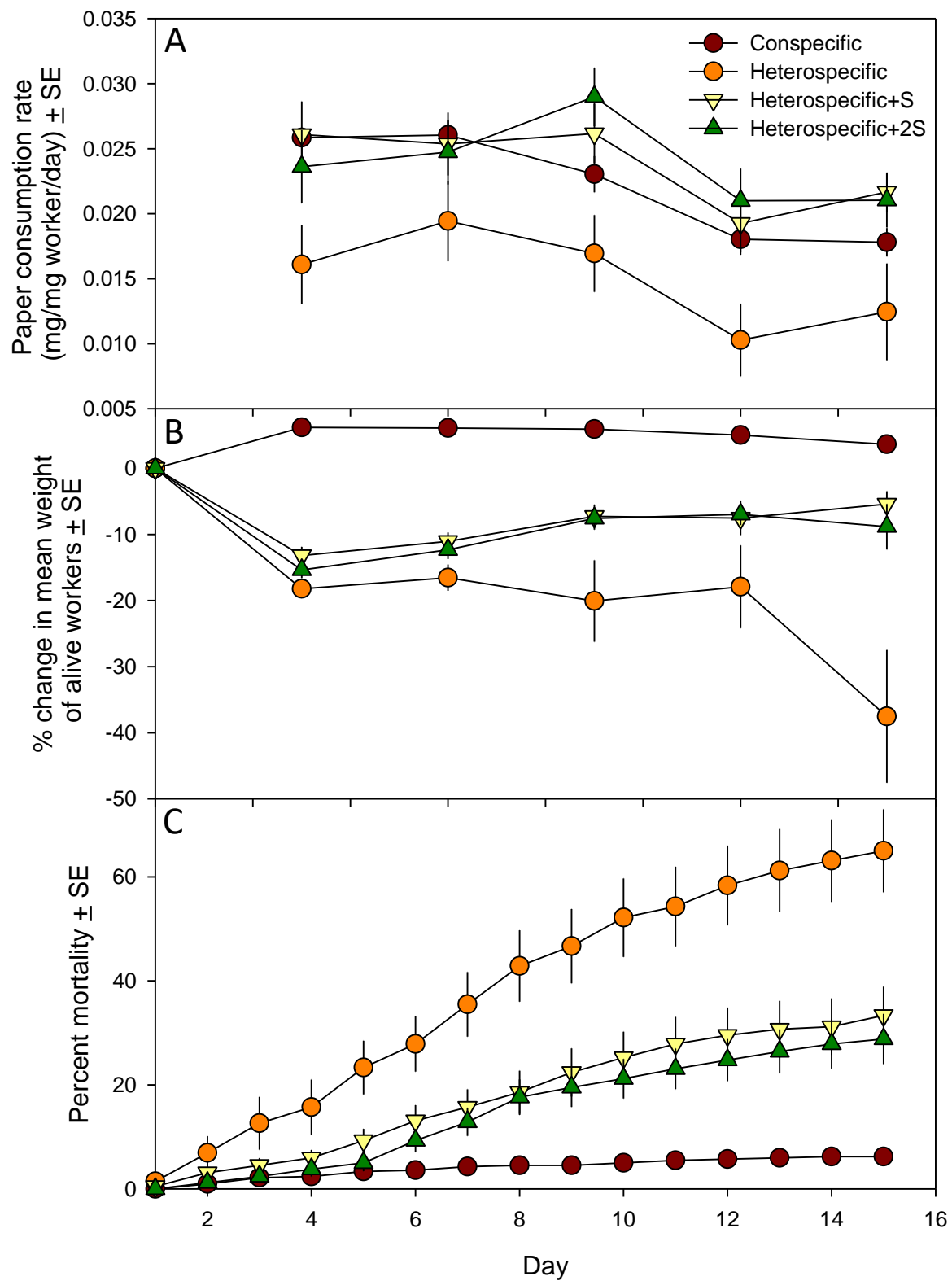
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448 **Figure 2.**



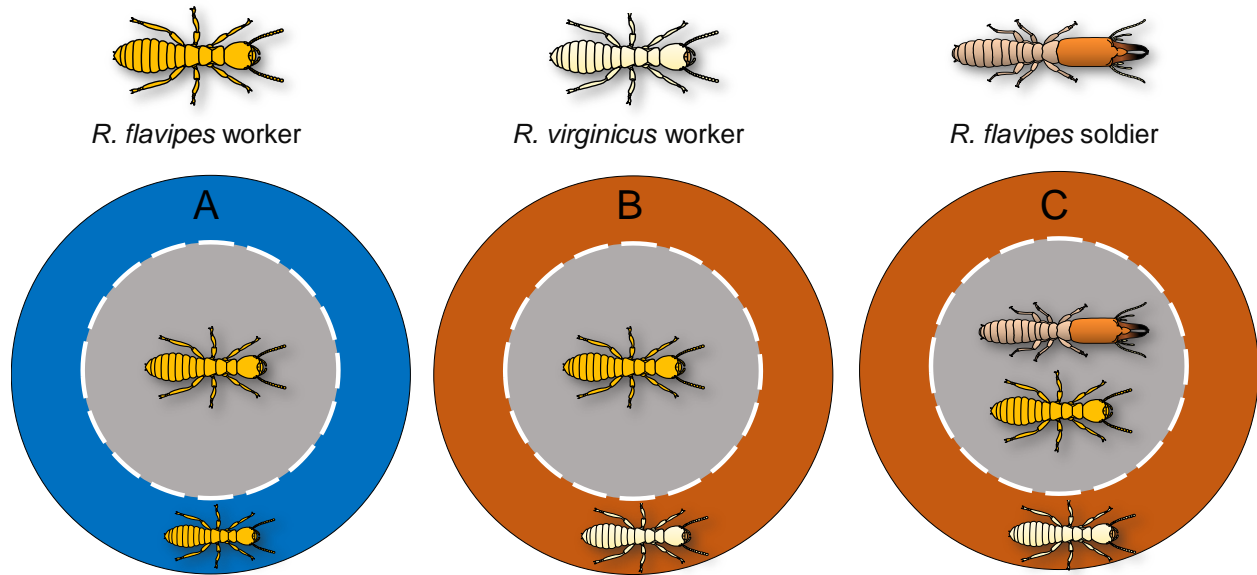
450 **Figure 3.**

451



452 **Supplementary Materials**

453 **Supplementary Figure**



454

455 **Figure S1. Schematic drawing of the experimental setup.** *Reticulitermes flavipes* workers (in
456 yellow), with/without a soldier, were confined in the inner ring (in grey). Wall of the center dish
457 was cut vertically to make 1mm-wide slits. Competition risk was perceived by *R. flavipes*
458 workers by antennation through the slits. In control (A), nestmate workers of *R. flavipes* were
459 placed in the outer ring (areas in blue), while for treatments, *R. virginicus* workers or *R. flavipes*
460 workers from other colonies (non-nestmates), the presumed competitors, were placed in the outer
461 ring (areas in orange) without (B) or with (C) the soldier caste.

462

463 **Supplementary Table**

464 **Supplementary Table 1:** Loading of behavioral variables on principal components for *R.*

465 *flavipes* workers (left portion of Table) and *R. flavipes* soldiers, and the proportion of variation

466 explained by each component. Only components with eigenvalues exceeding 1.0 are listed.

467

Behavioral variables	Worker behavior			Soldier behavior
	PC1 (42.9%)	PC2 (21.2%)	PC3 (19.5%)	PC1 (78.9%)
Resting	-0.591	-0.172	-0.012	-0.557
Feeding	-0.284	0.621	0.188	0
Vibration	0.485	0.030	0.341	0.494
Walking	0.492	0.432	0	0.481
Grooming	0.288	-0.337	-0.649	0
Other	0.104	-0.532	0.653	0.463

468