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1   **Title:** Social buffering in a eusocial invertebrate: termite soldiers reduce the lethal impact of

2   competitor cues on workers

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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

37

Introduction

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

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

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

termite species as a food source. The two dominant termite castes are workers, the primary

foragers and nest caretakers who are often injured or killed during interspecific interactions

(Shelton and Grace 1996), and soldiers, defensive specialists who provide little foraging benefit

(Tian and Zhou 2014). Because a single location often contains multiple termite species that

compete both directly and indirectly for the same habitats or food sources, foraging workers are

chronically exposed to risk cues (Evans et al. 2009, Li et al. 2010). The continued presence of

workers in such risky habitats led us to suspect that soldiers might play a 'keystone role'

(Modlmeier et al. 2014) by acting as social buffers whose presence reduces worker sensitivity

and susceptibility to stressors.

  We assessed how *Reticulitermes flavipes* termite workers were affected by cues from

conspecific nestmate, conspecific non-nestmate, and heterospecific (*R. virginicus*) workers*,* and

how the presence of an *R. flavipes* soldier altered the response of *R. flavipes* workers. We

separated adjacent colonies using a semipermeable barrier that prevented physical contact but

allowed cue transmission, allowing us to isolate the impact of cues on *R. flavipes* workers. We

also explored how worker responses were affected by a conspecific soldier and by different

densities of soldiers and competitors. In addition to their direct role in colony defense, we show

that soldiers reduce the impact of competition stress on the relatively vulnerable worker caste.

  Materials and Methods

  Reticulitermes colonies

  We used workers and soldiers from nine field-collected *Reticulitermes flavipes* colonies

(A1-A6, R1-R3) in this study. Workers from one field-collected *R. virginicus* colony (A7) were

used as the competitor. The distribution of these congeneric species overlaps throughout North

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 ± 1ºC, 80 ± 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 ± 1ºC, $70\pm 1\%$ 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 ± 1℃, $80 \pm 1\%$ 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 ℃

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   ((paper consumed, mg)/(total worker weight, mg))/3 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.

342   **Acknowledgements**

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420

Figure Legends

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

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

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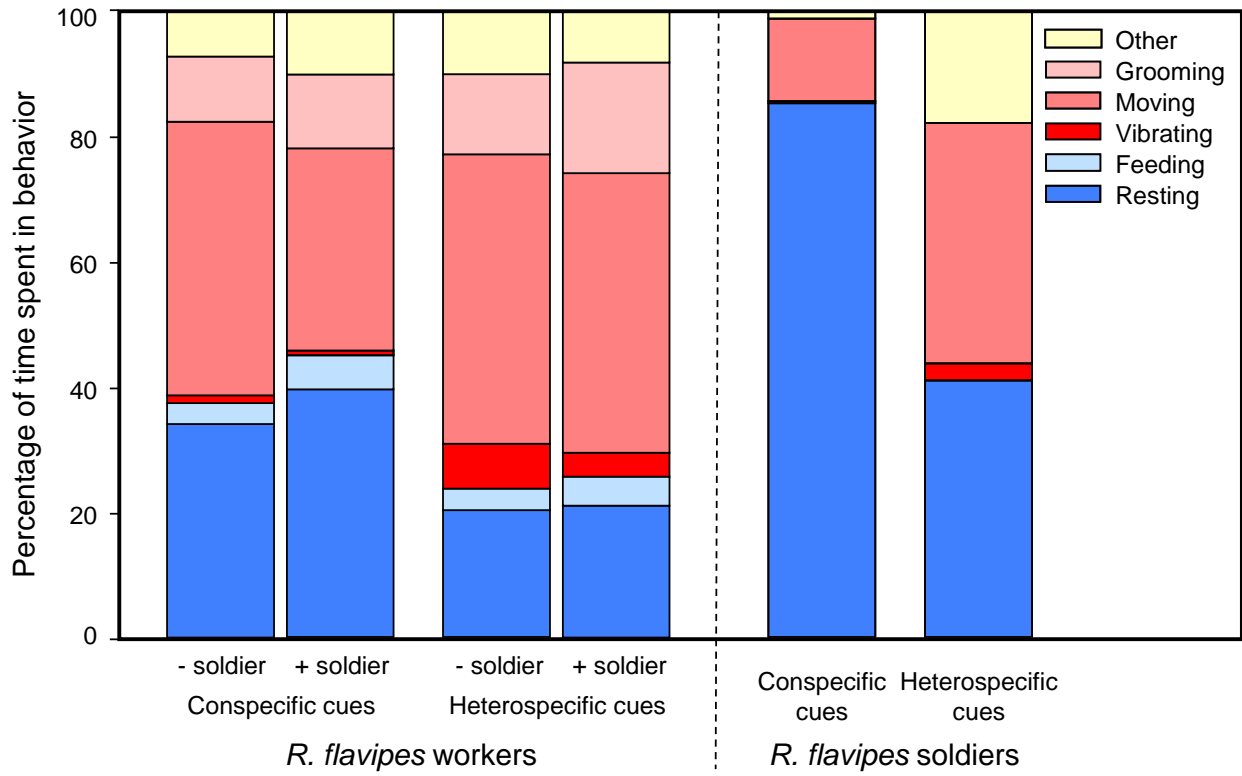
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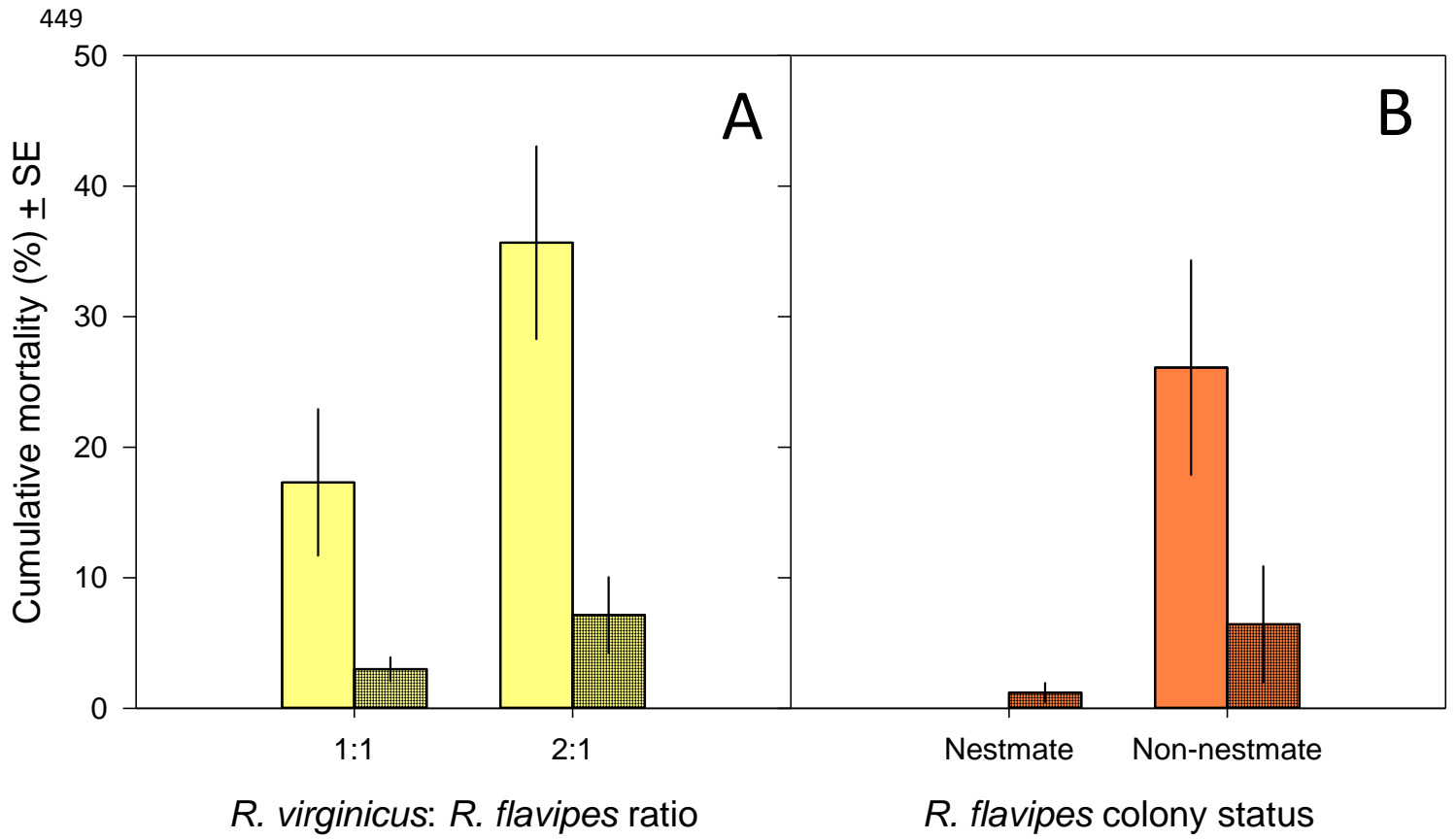
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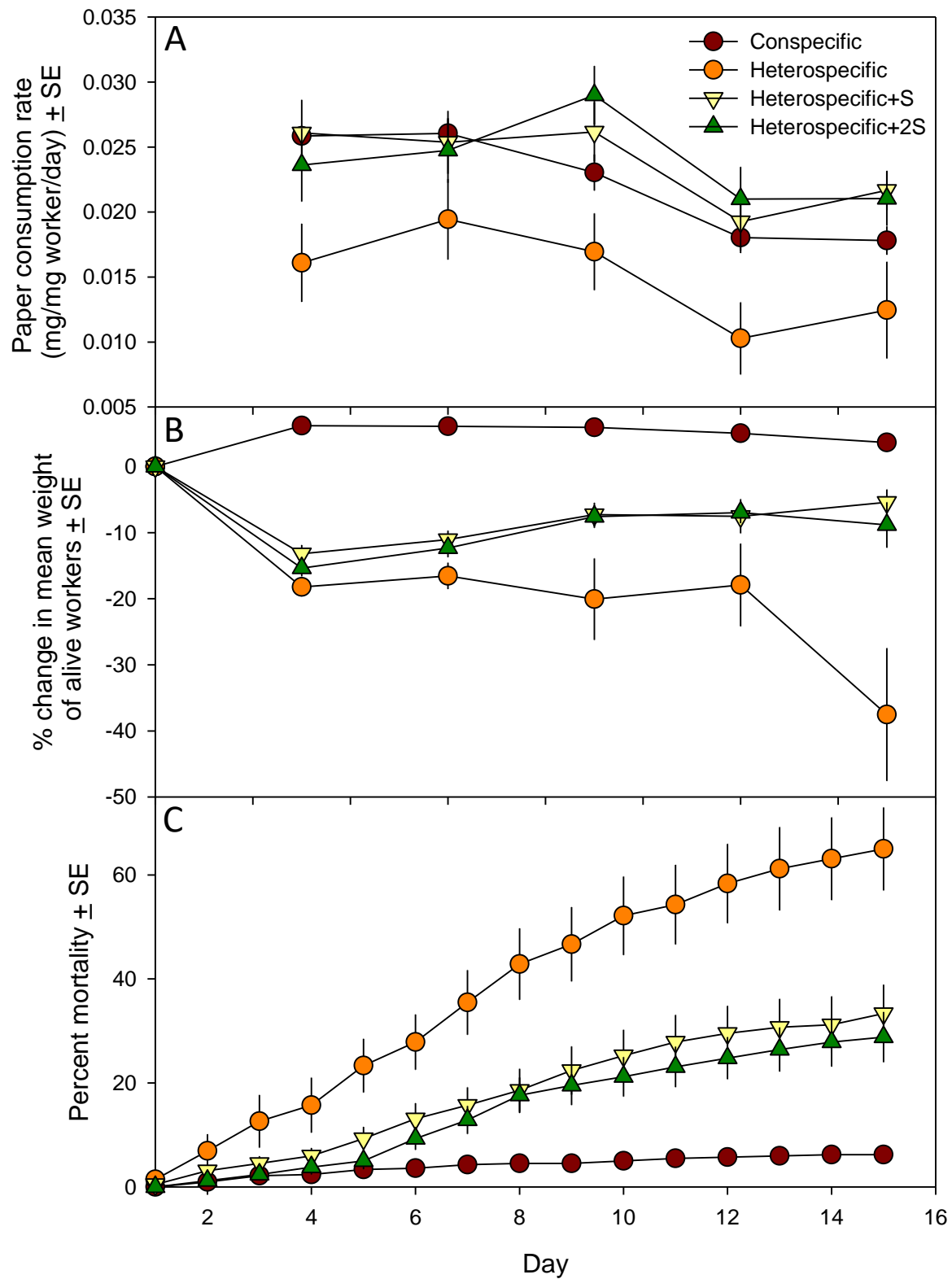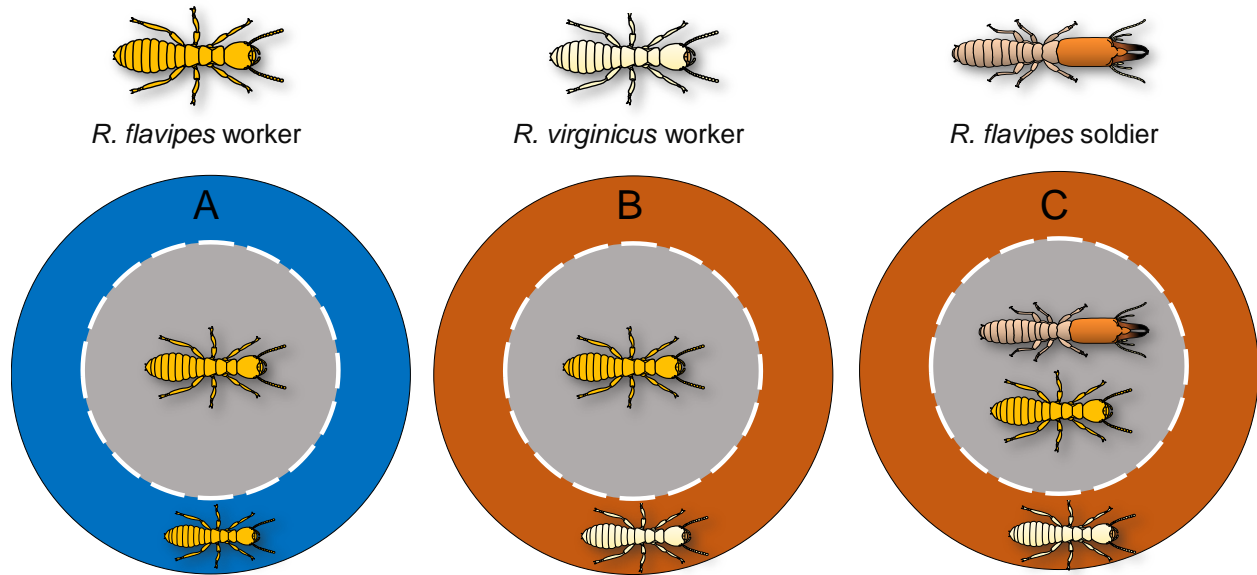
Supplementary Materials

Supplementary Figure

454

Figure S1. Schematic drawing of the experimental setup. *Reticulitermes flavipes* workers (in

yellow), with/without a soldier, were confined in the inner ring (in grey). Wall of the center dish

was cut vertically to make 1mm-wide silts. Competition risk was perceived by *R. flavipes*

workers by antennation through the slits. In control (A), nestmate workers of *R. flavipes* were

placed in the outer ring (areas in blue), while for treatments, *R. virginicus* workers or *R. flavipes*

workers from other colonies (non-nestmates), the presumed competitors, were placed in the outer

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