



Differential undertaking response of a lower termite to congeneric and conspecific corpses

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Undertaking behaviour is an essential activity in social insects. Corpses are often recognized by a postmortem change in a chemical signature. *Reticulitermes flavipes* responded to corpses within minutes of death. This undertaking behaviour did not change with longer postmortem time (24 h); however, *R. flavipes* exhibited distinctively different behaviours toward dead termites from various origins. Corpses of the congeneric species, *Reticulitermes virginicus*, were buried onsite by workers with a large group of soldiers guarding the burial site due to the risk of interspecific competition; while dead conspecifics, regardless of colony origin, were pulled back into the holding chamber for nutrient recycling and hygienic purposes. The burial task associated with congeneric corpses was coupled with colony defence and involved ten times more termites than retrieval of conspecific corpses. Our findings suggest elicitation of undertaking behaviour depends on the origin of corpses which is associated with different types of risk.

How to deal with the dead is a problem faced by all social animals. One of the major concerns associated with the deceased individuals is the risk of pathogen transmission, and this may be particularly true for eusocial animals due to their enclosed living quarters^{1,2}. In eusocial insects, corpse management is an integral part of the behavioural repertoire for controlling disease. Termites, social bees, wasps, and ants, employ an array of behavioural adaptations (e.g., isolation of sick from healthy individuals^{3,4}, corpse management^{5–9}, and "social vaccination" of nestmates by pathogen-exposed individuals^{10,11}) to manage diseases. To prevent further direct contact with corpses, social insects practice undertaking behaviour to segregate dead individuals from colony members. These behaviours include necrophobic (avoidance), necrophoric (removal), cannibalism, and burial behaviour^{5,6,9,12}. Overlapping generations and cooperative brood care, two of the defining characteristics of eusociality, make social organisms more vulnerable to pathogens through extensive contact¹. As a result, hygienic behaviours are consistently correlated with the evolution of eusociality. Most ant species dispose of dead colony members by discarding them away from the nests, placing them into refuse piles, or carrying them to special refuse chambers^{6,13,14}. Honey bees remove nestmate corpses and drop them from the hive^{7,15}. In contrast, termites exhibit complex undertaking behaviours to deal with the dead, including avoidance⁹, cannibalism^{3,12}, removal, and burial behaviour^{4,9,16–18}.

Distinguishing the dead from the living depends on accumulation or loss of chemical cues. Postmortem accumulation of unsaturated fatty acids (e.g., oleic or linoleic acid) is common among diverse taxa. Undertaking responses in two ant species (*Pogonomyrmex badius* and *Solenopsis saevissima*) were elicited by fatty acids, particularly oleic acid, produced after death⁵. This "fatty acid death cue" has been found in other ant species, honey bees, and termites^{5–7,17–20}. Recently, the converse of a death cue was found in Argentine ants, *Linepithema humile* (formerly *Iridomyrmex humilis*), where the dissipation of vital chemical signs (dolichodial and iridomyrmecin) after death elicited the undertaking response¹⁴.

Undertaking behaviour in termites has been studied as a part of the efforts to refine pest management tactics^{3,9,21}. Su and his colleagues investigated the foraging behaviour of *Coptotermes formosanus*, a subterranean termite and a devastating structural pest. *Coptotermes formosanus* corpses resulted from physical contacts with synthetic soil insecticides, including fipronil and thiamethoxam, were walled off by healthy workers to prevent future contacts⁹. Burial behaviour was also observed in a *Reticulitermes* species, when challenged with *Metarhizium anisopliae*, a generalist fungal pathogen and a well-studied biological control agent³. Recently, there has been renewed interests in undertaking behaviour^{17,18,22,23}. In a fungus-growing Macrotermitinae species, *Pseudacanthotermes spiniger*, wingless primary reproductives buried other dealate corpses to prevent potential pathogen outbreak¹⁷. Ulyshen and Shelton reported that the presence of dead insects elicited building behaviour



in *R. virginicus*, regardless of corpse types including nestmates, predators, and others¹⁸. Neoh *et al.*²² suggested that undertaking responses vary among species, associated with their feeding behaviour and nesting ecology, and undertaking behaviours are complex with different responses depending on the nature of corpses. In *Coptotermes formosanus*, when fungus (*Metarhizium anisopliae*) induced mortality was low, cannibalism was the primary undertaking response; however at higher levels, burial was predominant²³.

Undertaking responses may also vary within species depending on the social context and experimental setup. In Florida harvester ant *Pogonomyrmex badius*, oleic acid served to release undertaking behaviour when the major activity in a colony was midden work or nest maintenance. In contrast, undertaking response was not elicited when the individuals were mainly involved in foraging or convening²⁴. In *Reticulitermes*, termites isolated fungus-infected cadavers in a dish assay³, whereas in a planar arena, termites opted to cannibalize corpses exposed to the same pathogen²⁵.

Competition between two colonies can lead to injury and mortality of both colonies through aggression. As a result the encounter rate with dead individuals from the competing species may be high²⁶. Interspecific corpses induced sand/soil deposition, and the resultant dead individuals were eventually incorporated into the tunnel building materials to block future contacts (e.g., *Coptotermes formosanus* and *Coptotermes gestroi*²⁷, *Cornitermes cumulans* and *Procornitermes araujo*²⁸). The eastern subterranean termite, *Reticulitermes flavipes*, is one of the most common termite species in the continental United States²⁹. A congeneric species, *R. virginicus*, is morphologically and ecologically similar to *R. flavipes*. In the field we observed that the two species sometimes nested adjacently and occasionally one nesting site was replaced by the other species, therefore territorial competition between the two species would lead to aggression and result in dead individuals of both species. Previous studies suggested that interspecific and intraspecific interactions differ in *R. flavipes*. *Reticulitermes flavipes* has been reported to lack intraspecific aggression and as a result colony fusion occurs³⁰, but to show agonistic behaviour toward *R. virginicus* in laboratory³¹. Given the fact that *R. flavipes* and *R. virginicus* have distinctly different chemical signatures³², and potentially represent different risks such as interspecific competition, we hypothesized that *R. flavipes* would respond differently toward congeneric and conspecific corpses. Similarly, each *R. flavipes* colony carries its unique chemical ID, which may be diet mediated³³ and/or due to genetic variations³⁴. We expected differential undertaking responses toward intraspecific corpses (nestmate and non-nestmate). To test these hypotheses, we presented *R. flavipes* with dead individuals from the same and different *R. flavipes* colonies, and corpses from a congeneric species, *R. virginicus*.

“Increased death cue”^{25,17,18} and “diminished vital sign”¹⁴ are the two reported mechanisms of corpse recognition. In both cases the changes should be dependent on time after death. To gain a better understanding of the cues eliciting undertaking process in termites, we determined the temporal profile of *R. flavipes* undertaking behaviour when exposed to nestmates from 0 to 24 h after death.

Results

Undertaking response to corpses with different postmortem time.

We examined the responses of *R. flavipes* workers to dead nestmates with various postmortem time by placing corpses at the holding chamber opening in a testing arena (Fig. 1). When a group of 10 dead nestmates were introduced, workers carried the corpses into the holding chamber irrespective of postmortem time. Total removal time did not differ significantly among treatments ($F_{6,14} = 1.166$, $P > 0.05$ for colony KY-4; $F_{6,14} = 1.416$, $P > 0.05$ for colony KY-15; 3 replications for each colony; Fig. 2a).

The undertaking response to dead nestmates started with the inspection of corpses by worker termites while one or more soldiers guarded the entrance. Typically, the first worker coming out of the

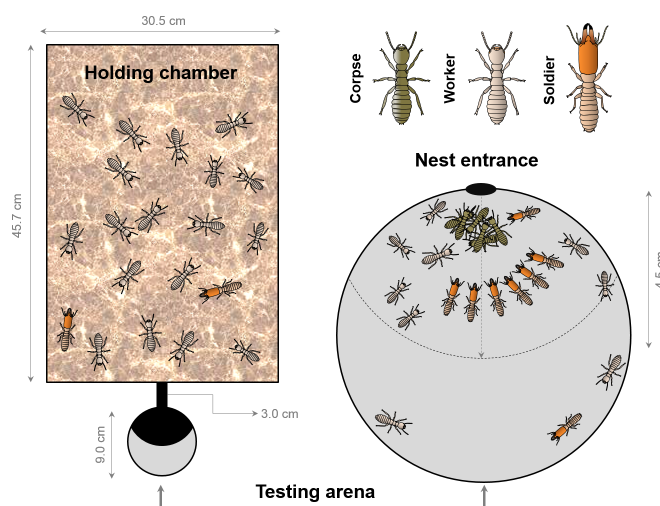


Figure 1 | Schematic drawing of experimental set-up. Colonies were maintained in holding chamber. A 9.0 cm-diameter testing arena was connected to the holding chamber with a 3.0 cm plastic tubing. The testing arena was covered with a lid to avoid disturbance by air movement. Corpses were placed in the vicinity of nest entrance, and the activities of termites in the testing arena were videotaped.

holding chamber contacted a corpse with its antennae. Immediately after antennation, the worker grasped the corpse using its mandibles (Fig. 3j–k). Workers usually pulled the corpses straight to the entrance (Fig. 3l), but the removal path could be circuitous. Soldiers sometimes contacted corpses with their antennae, but there was no agonistic response toward conspecific corpses (Fig. 3i).

Undertaking response to corpses of different origin.

The same undertaking behaviour pattern was observed toward intra- (nestmate) and inter-colony corpses 1 h postmortem. Conspecific corpses were removed from the testing arena and carried back to the holding chamber by workers, usually in less than 15 minutes (see Supplementary Video S1 online). Removal time between treatments with intra- and inter-colony corpses did not differ significantly (unpaired *t*-test: $t_8 = 1.170$, $P > 0.05$ for colony KY-173; $t_8 = 0.200$, $P > 0.05$ for colony KY-174; 5 replications per colony Fig. 2b).

In contrast to conspecific corpses, corpses from the congeneric species, *R. virginicus*, triggered alarm behaviours by both soldiers and workers, aggression behaviour by soldiers, and burial behaviour by workers (see Supplementary Video S2, S3 online). A soldier from the holding chamber inspected the corpses with antennae and then immediately initiated an attack with its mandibles (Fig. 4e–f). More soldiers were recruited from the holding chamber (Fig. 4g). Workers that contacted *R. virginicus* corpses quickly retreated into the nest, and did not come out until more soldiers gathered around the corpses. Within ten minutes after the introduction of congeneric corpses, workers began to carry out soil from the holding chamber and place it onto the corpses, and some workers were observed to coat the soil with saliva at the burial site. The burial behaviour continued while a group of soldiers surrounded the pile of corpses (Fig. 4h). Within 12 h, the group of 10 *R. virginicus* corpses was buried by *R. flavipes* workers with soil particles, forming a compact and moist mound (Fig. 4i). Alarm behaviours characterized by rapid walking of agitated termites and/or vigorous vibrations of their bodies (as previously described by Crosland *et al.*³⁵) were consistently observed during the 1 h observation period. In contrast, display of alarm behaviour was significantly less frequent in treatments with conspecific corpses (means of 36.75 ± 11.49 and 1.75 ± 1.44 for congeneric and intercolony corpses during first 15 min observation period; unpaired *t*-test: $t_6 = 3.022$, $P < 0.05$; 2 replications per colony

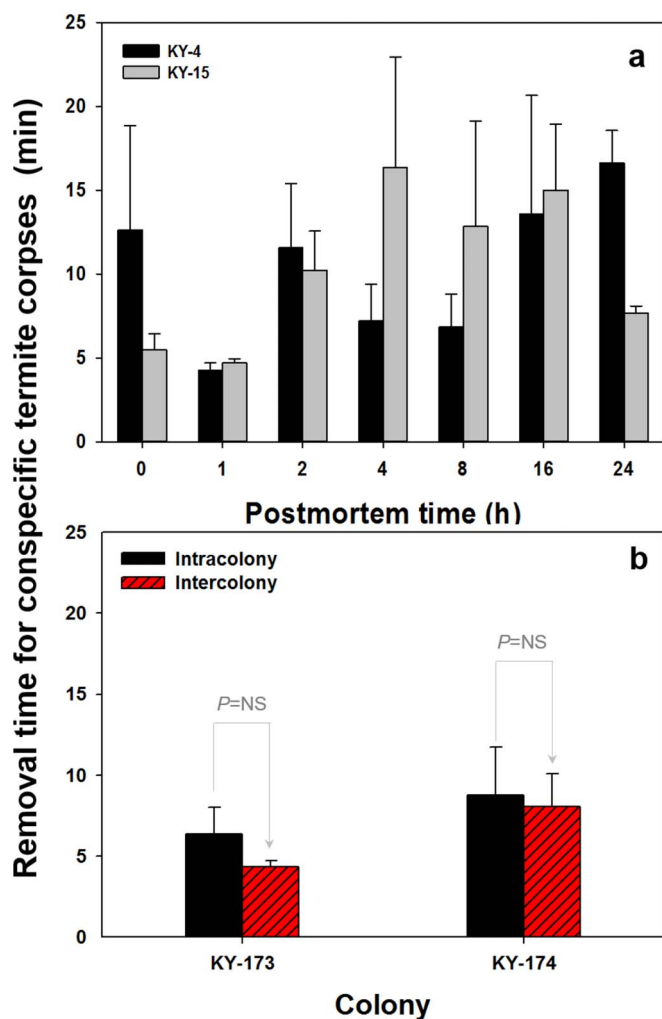


Figure 2 | Corpse removal time by *Reticulitermes flavipes*. (a) Removal time of dead nestmates with different postmortem time in two colonies, KY-4 and KY-15 (mean \pm standard error). Unpaired *t*-test ($P > 0.05$) detected no significant difference among treatments with different postmortem time in both colonies, (b) Removal time of dead nestmates or inter-colony corpses in two colonies (mean \pm standard error). NS represents no significant difference between removal times based on unpaired *t*-test, $P > 0.05$.

in KY-173 and -174). Alarm behaviour facilitated a rapid recruitment of both soldiers and workers during the first hour after the introduction of congeneric corpses (Fig. 4a–d), while termites involved in treatments of conspecific corpses were much fewer (Fig. 3a–h). Because of high variation in numbers between replicates, results are presented separately (Fig. 3a–h, 4a–d).

There was no significant effect of block or colony on the mean number of workers per 1 h observation period ($F_{1,7} = 0.15$, $P > 0.05$ and $F_{1,7} = 0.30$, $P > 0.05$). The source of the dead termites had a significant effect on number of workers observed ($F_{2,7} = 11.80$, $P < 0.01$) (Fig. 5). Recruitment of workers was greater with the *R. virginicus* corpses than with intra- or inter-colony *R. flavipes* corpses (means of 19.9, 1.4, 1.6 workers per observation, respectively; Tukey HSD Comparison Test). There was no significant effect of block or colony on the mean number of soldiers per observation period ($F_{1,7} = 0.24$, $P > 0.05$ and $F_{1,7} = 0.03$, $P > 0.05$). The source of the dead termites had a significant impact on the recruitment of soldiers ($F_{2,7} = 37.88$, $P < 0.01$). Many more soldiers were recruited to dead *R. virginicus* than dead intra- or inter-colony *R. flavipes* (means of 10.7, 0.15, 0.89 soldiers per observation, respectively;

Tukey HSD Comparison Test). There was no significant effect of block or colony on the proportion of soldiers observed ($F_{1,7} = 1.48$, $P > 0.05$ and $F_{1,7} = 1.98$, $P > 0.05$); however, the source of the dead termites had a significant effect ($F_{2,7} = 5.94$, $P < 0.05$). It is worth noting that in treatments with congeneric and inter-colony corpses, the soldier caste accounts for $\sim 40\%$ of the total number of termites in the testing arena, which is substantially more than the natural level in a field colony (4–5%)³⁶, indicating a greater recruitment of soldiers in the undertaking process.

Discussion

Chemical cues have been attributed to the undertaking behaviour in both Hymenoptera and Isoptera. The involvement of tactile cues (e.g. shape and texture) in the undertaking process in *R. flavipes* is currently under investigation, however, the possible mechanism for death recognition has already been confirmed in a congeneric species, *R. virginicus*¹⁸, suggesting the recognition of death in termites involves multi-channel signalling, including chemical and tactile cues. Recognition of a “fatty acid death cue” has been studied extensively over the past 50 years and showed phylogenetic conservation or convergence across diverse taxa. Oleic acid and linoleic acid are the two major compounds of fatty acid -based necromone to induce undertaking behaviour in ants^{5,6,19} and to lead to avoidance in a wide range of arthropods including terrestrial Isopoda, Collembola, cockroaches, and social caterpillars²⁰. In this study, the brief latency between corpse introduction and release of behaviour in both conspecific treatments and congeneric treatment suggests a chemical change has already occurred. However, this study alone does not discriminate between “increased death cue”²⁵ and “diminished vital sign”²⁴ hypotheses.

The disposal of dead colony members is a characteristic behaviour in ants and honey bees^{5,7}, and it is true in *R. flavipes*. Workers showed rapid recognition and response to nestmate corpses within 1 h after death. This is consistent with honey bees⁷ and ants^{6,14}, confirming undertaking response toward dead nestmates evolved convergently in eusocial insects. However, unlike ants^{5,14,37}, which take corpses away from the nest and deposit them into the refuse pile; and in honey bees^{7,38}, which remove corpses out of the hive; *R. flavipes* carried dead nestmates back into the holding chamber. The fate of those corpses was not examined in the current study. However, based on previous reports^{3,22} and our on-going investigation, the conspecific corpses were likely cannibalized (QS and XZ, unpublished data). Cannibalism/necrophagy has been used by many termites to recycle nutrients^{3,39}, which is, in part, due to their nutritionally poor cellulose diet⁴⁰. Cannibalism also functions as a hygienic tactic, and the cannibalism of fungus-killed nestmates was found in *R. flavipes*²⁵. Consuming corpses destroys the source of potential epidemic pathogens, and by ingesting the dead body it can also inhibit the growth of existing entomopathogens due to antimicrobial activity in the gut⁴¹.

As hypothesized, results from this study indicate that *R. flavipes* have differential responses to congeneric and conspecific corpses; however, conspecific corpses of nestmates and non-nestmates were disposed in the same manner under laboratory conditions. Introduction of dead *R. virginicus* induced an intense response from members of the *R. flavipes* colony, including rapid recruitment of soldiers and workers via alarm vibration exhibited by both castes, aggressive behaviours by soldier caste, and burial behaviour by workers. Nest soil consisting of chewed mulch and feces was used by *R. flavipes* workers to bury congeneric corpses. The propensity of termites for tunnel building plays an important role in their burial behaviour (compared with ants and bees). The use of fecal material and nest soil for burial provide antifungal components^{17,21,42}. Burial behaviour has been observed in other species. For example, in *Coptotermes formosanus*, a large number of corpses were sealed off by their nestmates^{9,23}, and in *Pseudacanthotermes spiniger* and *R. virginicus*, burial or building behaviour induced by dead nestmates

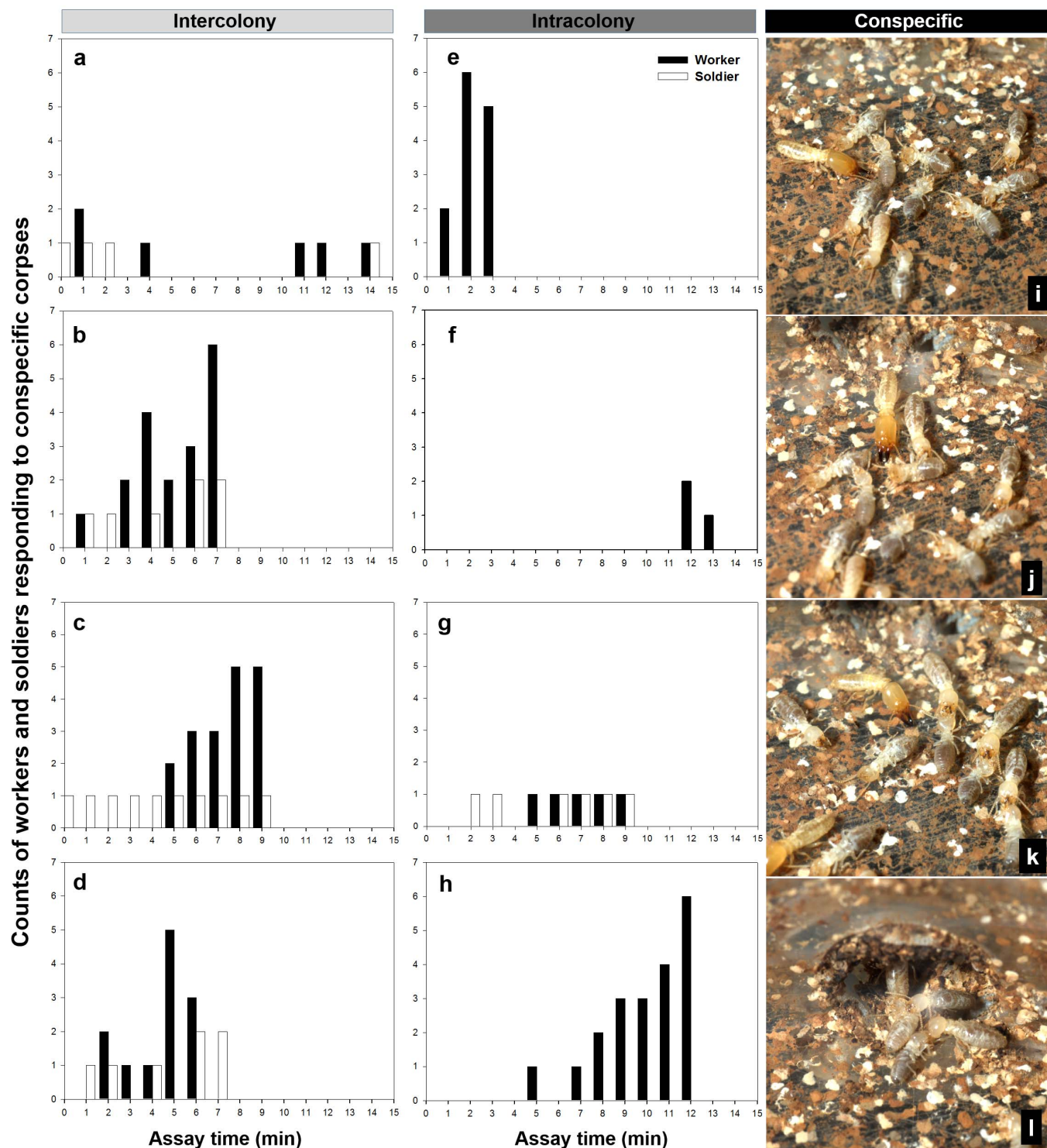


Figure 3 | Undertaking responses of *Reticulitermes flavipes* to conspecific corpses. (a)–(d) Counts of workers and soldiers which were recruited to inter-colony corpses. (a) and (b) represent two replications in colony KY-173, while (c) and (d) represent two replications in KY-174. (e)–(h) Counts of workers and soldiers which were recruited to intra-colony corpses. (e) and (f) represent two replications in colony KY-173, while (g) and (h) represent two replications in KY-174. (i) A soldier touching the corpse with antennae. (j) A worker attempting to grasp a corpse with its mandibles after antenation. (k) Three workers, each of them carrying a corpse, respectively. (l) Corpses being dragged into the holding chamber through the entrance by workers. (i)–(l) are the results of inter-colony treatments, but behaviours were the same with intra-colony treatments.

were reported^{17,18}. Unlike cannibalism, burial behaviour isolates corpses from other nest members to prevent the spread of pathogens. An increased risk of infection is possible when encountering dead foreign species that may carry a pathogen not tolerated by *R. flavipes*, given the fact that disease resistance varies among species. For

example, susceptibility to the soil fungal pathogen, *Metarhizium anisopliae*, varies among seven termite species from six families. Specifically, *Mastotermes darwiniensis*, *Hodotermopsis sjostedti* and *Nasutitermes voeltzkowi* are highly tolerant, Rhinotermitidae species, *R. flavipes* and *Proprhinotermes canalifrons*, are moderately

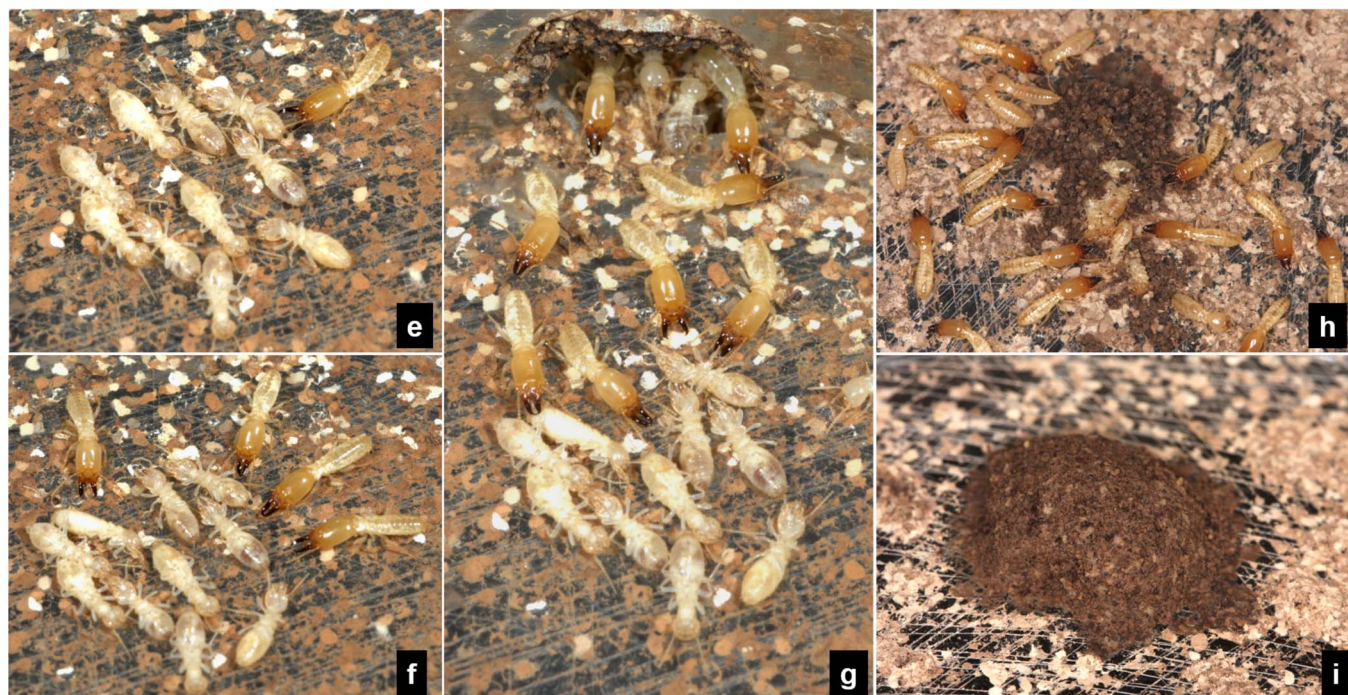
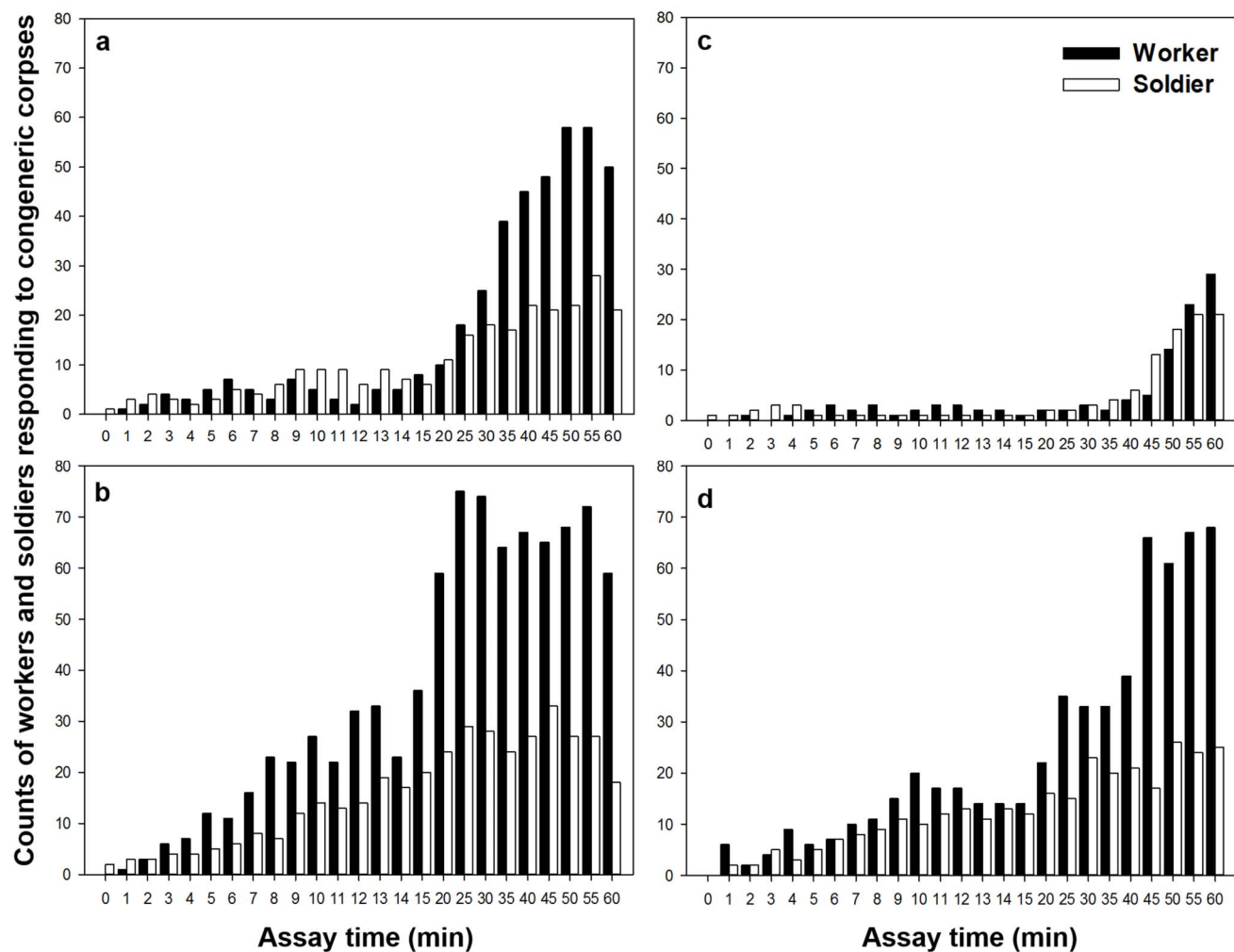


Figure 4 | Undertaking responses of *Reticulitermes flavipes* to *R. virginicus* corpses. (a)–(d) Counts of workers and soldiers which were recruited to congeneric corpses. (a) and (b) represent two replications in colony KY-173, while (c) and (d) represent two replications in KY-174. (e) A soldier touching the corpse with antennae and attacking it with open mandibles. (f) Soldiers attacking the corpses. (g) More soldiers being recruited. (h) Corpses being buried by workers while a group of soldiers guarding the burial site. (i) Ten *R. virginicus* corpses were completely buried.

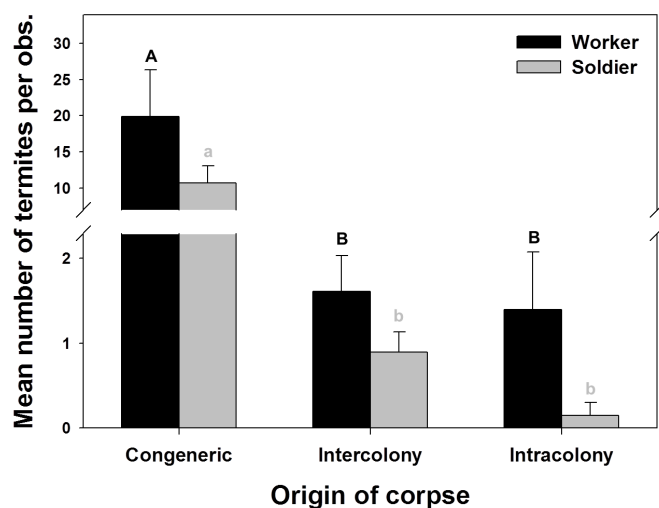


Figure 5 | Number of termites involved in treatments with corpses of different origin. Mean number of termites per observation in treatments with congeneric (*R. virginicus*), inter-colony, and intra-colony corpses. For each treatment, two colonies were used (KY-173 and KY-174) with 2 replications. Error bars represent standard error of 4 replications. Means between groups denoted with same letters were not significantly different ($P > 0.05$, Tukey HSD Comparison Test).

susceptible, whereas *Kalotermes flavicollis* and *Hodotermes mossambicus* are highly vulnerable⁴³.

In addition to a hygienic function, burial behaviour is also a colony defence mechanism. A previous report demonstrated that *Coptotermes formosanus* and *Coptotermes gestroi* sealed corpses, respectively, after interspecific aggression and avoided reopening the tunnel where two species encountered each other²⁷. In this study, *R. flavipes* isolated alien corpses with thick layers of mud, and building behaviour at the nest opening utilizing mud was frequently observed. Consequently, burying corpses of congeneric species by *R. flavipes* may facilitate colony defence against competitors. Aggressive responses by the soldier caste is typical in colony defence⁴⁴, however, when treated with dead *R. virginicus*, aggressive behaviour exhibited by soldiers is also an integral part of corpse management. The absence of worker aggression to *R. virginicus* in this study is likely the result of the presence of large number of soldiers. This is consistent with the damp-wood termite, *Hodotermopsis sjostedti*, in which the aggression level of workers was modulated by the presence of other castes. Specifically, the presence of reproductives aggravated the aggression level of workers, whereas the presence of soldier caste neutralized worker aggression⁴⁵. Therefore, aggression and guarding behaviour exhibited by soldiers may ensure that workers focus on burial tasks during the undertaking process. Inter-colony aggression was not observed, which was similar to other studies with *R. flavipes*^{30,31}.

Reticulitermes workers distinguish conspecific and congeneric individuals primarily by cuticular hydrocarbons⁴⁶. When presented with congeneric corpses, *R. flavipes* soldier exhibited strong aggression behaviour, indicating that they are capable of nestmate recognition, but might not be able to recognize the dead. Similarly, the soldier caste of fungus-growing ant, *Atta mexicana*, is not sensitive to oleic acid, the death cue, but responds to an alarm pheromone⁴⁷. Interestingly, Choe *et al.*¹⁴ found that triglycerides, which are common chemical components in insects, elicited both aggressive and undertaking behaviour in Argentine ants. If this is true in *Reticulitermes*, it is possible that additional chemicals in *R. flavipes* inhibit aggressive responses by conspecific individuals, or, elicit aggression to interspecifics. Further quantitative analysis of chemical cues is warranted to examine these hypotheses. In addition, we

predict that workers and soldiers have different sensory or central processing of these cues.

Our results showed that *R. flavipes* distinguish between their own corpses and those of a congener, eliciting corpse retrieval behaviour or burial behaviour, respectively. Depending on the origin of corpses, undertaking behaviour functions as a hygienic tactic as shown in response to nestmate corpses, and as both hygienic and colony defensive strategy as shown in response to dead congeners. The ability to switch between behavioural repertoires in *R. flavipes* is similar to the undertaking behaviours observed in an ant species *Temnothorax lichtensteini*, in which newly deceased congeneric individuals were buried while aged corpses of nestmates were removed outside the nest⁴⁸. The differential response in termites to corpses of different origins can be advantageous. Burial is extremely costly in terms of time, energy, and resources committed to this form of disposal, but may be warranted when the risk of exogenous pathogens is high, or if the workers perceive a lingering risk of interspecific aggression. In addition to recycling nutrients, corpse retrieval requires less time, energy and resources than burial. However, inoculation of the colony with exogenous pathogens or the presence of intruders may have direct costs. A differential response to corpses of different origins may serve to mitigate the risks and costs associated with corpses and their disposal.

Methods

Termites. *Reticulitermes flavipes* colonies (KY-4, -15, -173, and -174) and a congeneric *R. virginicus* colony (KY-18) were collected from The Arboretum, a 100-acre botanical garden located on the University of Kentucky campus (Lexington, KY). Colonies were obtained using termite trapping stations filled with spirally coiled cardboard during spring and summer. Colony KY-4, KY-15 and KY-18 were collected in 2010, while Colony KY-173 and KY-174 were collected in 2011.

Reticulitermes colonies were maintained in sealed plastic boxes in complete darkness (L:D = 0:24), at $27 \pm 1^\circ\text{C}$, $80 \pm 1\%$ RH, and were provisioned with pine wood and hardwood mulch. Intercolony individuals were obtained from colony OH-A8, which was a gift from Dr. Susan Jones (The Ohio State University). It was originally collected from Columbus, OH, and has been maintained in the laboratory for 9 years. The KY- and OH- colonies were collected from two different states and at least 7 years apart and therefore should have a low degree of genetic relatedness. The identity of colonies as *R. flavipes* and *R. virginicus*, respectively, were verified by a combination of soldier morphology and 16 s mitochondrial ribosomal gene sequencing⁴⁹. Termites were considered workers if they did not possess any sign of wing buds or distended abdomens, and had pronotal widths wider than mesonotal widths⁵⁰.

Experimental set up. Termites used in the following experiments had been acclimated under the laboratory conditions for at least 3 months from the time of field collection. Each laboratory colony contains at least 5000 termites. Our experimental setup includes a holding chamber, a sealed plastic box ($45.7 \times 30.6 \times 15.2$ cm, Pioneer Plastics, Inc., Dixon, KY) containing the original stock colony, and a testing arena, a covered 9 cm-diameter Petri dish (1.5 cm height, VWR Inc, Radnor, PA) where observations of undertaking were made. The bottom surface of the dish was scratched with No. 7 insect pins to facilitate movement. Behavioural experiments began at least two weeks after the Petri dish was connected, when the bottom of the testing arena was covered with nest material by workers. An acrylic tube (inner diameter: 0.7 cm; length: 3 cm) connects the testing arena to the holding chamber (Fig. 1). The entire experimental setup was held in environmental chambers under the above mentioned rearing conditions. A video camcorder (Sony DCR-SR60, Tokyo, Japan) was placed directly above the testing arena.

Preparation of corpses. Undertaking responses toward corpses with different postmortem times were tested in two colonies (KY-4 and KY-15). Seven groups of workers from the same KY *R. flavipes* colony were frozen to death at -20°C for 15 min and kept in environmental chambers at $27 \pm 1^\circ\text{C}$, $80 \pm 1\%$ RH, for 0, 1, 2, 4, 8, 16, and 24 h, respectively, in covered Petri dishes before subjecting to the behavioural bioassay. The 0 h old (freshly killed) corpses were left at room temperature for 5 min before they were placed in the arena. To discern the behavioural responses toward corpses of different origin, two colonies were tested (KY-173 and KY-174) with three types of corpses introduced: (a) intracolony corpses: dead workers collected from the same KY *R. flavipes* colony; (b) intercolony corpses: dead workers collected from a OH *R. flavipes* colony (OH-A8); and (c) congeneric corpses: dead workers collected from a KY *R. virginicus* colony (KY-18). Based on the result from the postmortem time experiment, we opted to use 1 h postmortem corpses as the age of corpses for the differential response study. Corpses were prepared by freezing as described above.

Undertaking behavioural bioassay. Behavioural assays were conducted at room temperature, and the experimental setup including a holding chamber and a testing arena was acclimated for 30 min before experiments. In each bioassay, the lid of



testing arena was gently removed, 10 corpses were then placed into the arena using a pair of feather-weight forceps (BioQuip Products Inc., Gardena, CA). Corpses were placed in the vicinity of the nest entrance, and the arena was gently covered with a clear Petri dish lid to avoid disturbance by the air movement. Video recording was initiated immediately after the introduction of corpses to the testing arena.

For different postmortem times, behavioural bioassays were replicated 3 times in both KY-4 and KY-15 colonies. For corpses with different origins, bioassays were replicated 5 times in KY-173 and KY-174 colonies. Based on the length of undertaking processes toward different types of corpses, we empirically determined a 15 and 60 min recording time, respectively, for the assays with conspecific and congeneric corpses. Undertaking processes toward conspecific corpses (body removal) are concluded within a 15 min time frame. Undertaking process toward congeneric corpses (burial behaviour), however, usually lasts for 5 h or more. Nevertheless, the entire behavioural repertoire including antennation, alarm, recruitment, aggression, burial, and guarding was clearly displayed within the first 60 min of the undertaking process. Behavioural bioassays on the same colony were separated by at least 5 h to avoid the potential influence of short-term memory. To analyze the involvement of worker and soldier castes in the undertaking process, the number of workers and soldiers appearing under the viewing area were recorded, respectively. Picture frames were paused every minute during the observation period, and termites within the viewing area (a radius of 4.5 cm from the nest entrance in the testing arena, Fig. 1) were counted. To analyze the intensity of alarm behaviour, the frequency of alarm vibration was counted within a 15 min observation period immediately after the introduction of corpses.

Data analysis. Results from the experiments with different postmortem time were analyzed by ANOVA. Removal time was recorded from the time that the first termite contacted a corpse until all 10 corpses were removed. For the experiments of corpses with different origins, removal time toward conspecific (inter- and intra-colony) corpses, and frequency of alarm vibration toward congeneric and conspecific (inter-colony) corpses, were analyzed by unpaired *t*-test at significance level of 5%. Finally, the counts of workers or soldiers observed were summed over all observation periods. The numbers of observation periods varied because the task of removing dead workers was completed in minutes (within 15 min), while burial behaviour lasted hours (beyond the 60 min recording time). The mean number of workers or soldiers per observation was calculated. These means were log transformed ($\log(n+1)$) prior to analysis of variance, which was conducted with block, colony, and treatment (intra-colony, inter-colony, or congeneric *R. virginicus*) as factors. The mean proportion of soldiers was arcsin transformed ($\arcsin(\text{square root}(p))$) prior to analysis of variance. All statistical analyses were conducted using Statistix 9.0 (Analytical Software, Tallahassee, FL).

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Author contributions

Conceived and designed the experiments: Q.S. and X.Z. Performed the experiments: Q.S. Analyzed the data: Q.S., K.F.H. and X.Z. Contributed reagents/materials/analysis tools: X.Z. Wrote the paper: Q.S., K.F.H. and X.Z. All authors read and approved the final manuscript.

Additional information

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