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Evidence of cue synergism in termite corpse response behavior

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Abstract Subterranean termites of the genus *Reticulitermes* are known to build walls and tubes and move considerable amounts of soil into wood but the causes of this behavior remain largely unexplored. In laboratory assays, we tested the hypothesis that *Reticulitermes virginicus* (Banks) would carry more sand into wooden blocks containing corpses compared to corpse-free controls. We further predicted that the corpses of predatory ants would elicit a stronger response than those of a benign beetle species or nestmates. As hypothesized, significantly more sand was carried into blocks containing corpses and this material was typically used to build partitions separating the dead from the rest of the colony. Contrary to expectations, however, this behavior did not vary among corpse types. We then tested the hypothesis that oleic acid, an unsaturated fatty acid released during arthropod decay and used by ants and other arthropod taxa in corpse recognition, would induce a similar building response in *R. virginicus*. To additionally determine the role of foreign objects in giving rise to this behavior, the experiment was carried out with and without imitation corpses (i.e., small glass beads). As predicted, oleic acid induced building (a tenfold increase) but only when applied to beads, suggesting strong synergism between tactile and chemical cues. Oleic acid also significantly reduced the amount of wood consumed by *R. virginicus* and may possess useful repellent properties.

Keywords Chemical ecology · Burial · Defense · Necromone · Necrophoresis

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Introduction

Healthy group living requires good hygiene in social or otherwise gregarious arthropod species and corpses typically elicit strong responses from these organisms (Wilson 1971; Cremer et al. 2007; Yao et al. 2009). Ants, for instance, are known to dump their dead outside the nest, pack them away in deserted nest chambers, consume them, or bury them on sight (Hölldobler and Wilson 1990; Hart and Ratnieks 2002). Bees and wasps discard their deceased by dragging them from their nests (Wilson 1971; Visscher 1983). Virtually all termites eat their dead (perhaps due to their low-protein diets) (Wilson 1971; Myles 2002; Chouvenc et al. 2008) but will also remove or bury them (Pearce 1987; Zoberi 1995; Jones et al. 1996; Crosland and Traniello 1997; Myles 2002; Su 2005; Li et al. 2010; Chouvenc et al. 2011). Because corpses represent sources of contagion, these behaviors serve primarily to reduce disease risk.

Subterranean termites of the genus *Reticulitermes* feed on dead wood and share this habitat with a wide variety of other species. Although the risks of disease and predation in such an environment are high (Rosengaus et al. 2011, and references therein), it remains incompletely understood how these small and soft-bodied insects defend themselves against such threats. In addition to their meticulous grooming habits and the production of antimicrobial secretions (Rosengaus et al. 2011; Hamilton et al. 2011), their propensity for building, though modest compared to the giant mounds constructed by some tropical termites (Wood 1988), may play an important role. Members of the genus are known to fill cracks and build walls and tubes with soil and frass but what causes them to move soil into wood remains unclear. Early researchers working on other termite genera suggested that air movement (Howse 1966) or other alarm stimuli (Stuart 1967) stimulate building and soil may be carried into wood for that purpose. The practical problem of where to discard excavated soil represents another possibly explanation. Li and Su (2008), for example, speculated that the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) fills voids in wood created by their feeding activities with soil displaced by tunneling. The first objective in the current study was to formally test whether the presence of corpses induces *Reticulitermes* to bring soil into wood. We hypothesized that *Reticulitermes virginicus* (Banks), a species native to the southeastern USA, would carry more sand into blocks containing corpses than in corpse-free controls. We further predicted that the corpses of a predator, the red imported fire ant (*Solenopsis invicta* Buren), would elicit a stronger building response than those of bark beetles or nestmates.

The mechanism of corpse recognition in termites is poorly known despite a long history of work on other taxa. This line of research began when Wilson et al. (1958) found that the corpse removal response in two genera of ants was elicited by oleic acid, a fatty acid commonly released by dead arthropods. Later work revealed that another fatty acid associated with arthropod corpses, linoleic acid, has a similar effect (Yao et al. 2009, and references therein). It now appears that a wide range of other arthropods are repelled by these compounds as well, including bees, terrestrial isopods, cockroaches, Collembola, and gregarious caterpillars (Yao et al. 2009, and references therein). Chouvenc et al. (2011) recently found that corpses of the African termite *Pseudacanthotermes spiniger* (Sjöstedt) release a complex mixture of fatty acids (including oleic acid), indol, and phenol that act in combination to trigger burial behavior. The second main objective of the current study was to determine whether *R. virginicus*, like so many other arthropod taxa, uses oleic acid in corpse recognition as well as the role of foreign bodies in eliciting a response.

Methods

Experiment 1: response to corpses

This experiment followed a randomized block design with four treatments, three experimental blocks, and five replicates (i.e., 60 experimental units in total). The treatments were (1) ant corpses (major *S. invicta* workers collected from a single colony), (2) bark beetle corpses (*Ips* spp. collected using Lindgren funnel traps from a single forest), (3) termite nestmate corpses (collected from the same colonies used in the study), and (4) a corpse-free control. All insect corpses were obtained by placing field-collected specimens in the freezer ≥24 h before the experiment began. Individual ants, beetles, and termites weighed approximately 4, 3, and 2 mg, respectively. Each experimental block represented a different field-collected *R. virginicus* (Banks) colony and occupied a separate incubator shelf (see below). Termite identification was based on soldier morphology using Hostettler et al. (1995).

Each experimental unit consisted of two 3.8×3.8×2.0-cm pieces of spruce (*Picea* A. Dietr. sp.) wood held together with a nail driven through their centers (Fig. 1a–c). The nail passed through two washers (combined thickness ~2 mm) placed between the two pieces of wood to create a narrow space for termite passage. The upper surface of each lower piece of wood contained four 0.8-cm diameter holes drilled to a depth of 1 cm. Three ant, beetle, or nestmate corpses were added to each of the holes for the different corpse treatments whereas nothing was added to the holes for the control treatment (i.e., all four holes in each piece of wood received the same treatment). Each completed assembly was placed in a plastic jar containing 150 g of sand and 27 ml of distilled water (Fig. 1c). Two hundred *R. virginicus* workers (pseudergates of third instar or greater) were then added. The screw top jar lids were

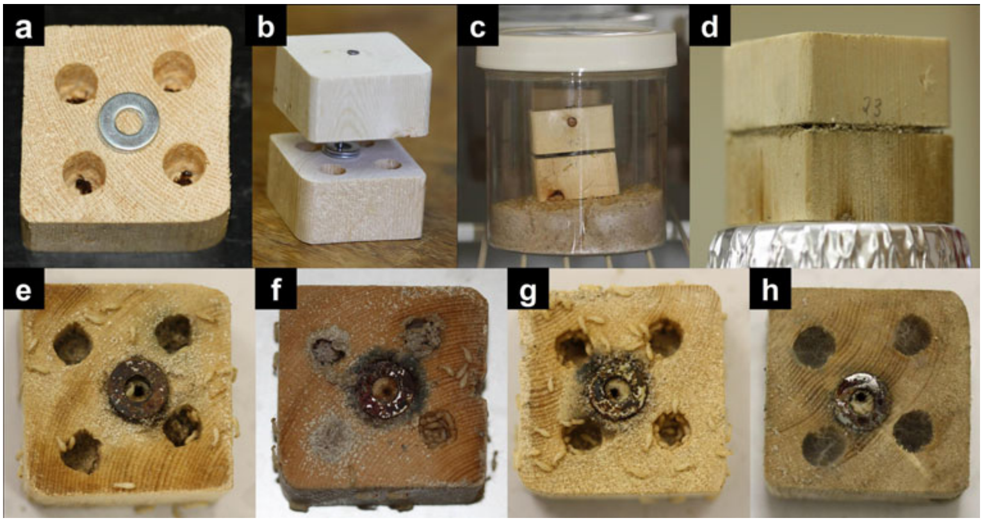

Fig. 1 Lower block showing holes and ant corpses (**a**). A nail was used to connect the two blocks with washers as spacers (**b**). Completed experimental unit (**c**). Sand used to partially fill space between blocks (**d**). Layers of sand perfectly covering corpses inside holes (**e**). Top of hole capped with sand (**f**). Control block with little sand (**g**). Fungal overgrowth (**h**)

loosened slightly to permit gas exchange. Each experimental block (i.e., termite colony) was placed on a separate shelf in an incubator (25°C and complete darkness) with the 20 jars comprising each arranged randomly.

After 17 days, the wooden blocks were removed from the jars. Survivorship was determined by counting the number of living termites from each jar. All sand stuck to the outer surfaces of the wood was brushed away whereas that found in the space between the two wooden blocks or in the holes in the lower blocks was carefully collected. Corpses were carefully removed from the sand using forceps and discarded. After drying for 24 h at 102°C and removing the washers and nails, the wood pieces were weighed to determine wood consumption (wood weights were measured before the study began following the same protocol). The sand samples were also oven dried and then burned with a butane torch to remove as much organic matter as possible before being weighed to the nearest milligram.

Analyses of variance were carried out using SAS on a dataset consisting of 47 of the original 60 experimental units (the others were excluded due to high termite mortality rates) to compare the amount of wood consumed, sand weight and survivorship among treatments. Colony (i.e., block) was included in the model as a random effect but removed when insignificant. The data were log(x+1) or square root transformed to achieve normality.

Experiment 2: response to oleic acid and imitation corpses

This experiment also followed a randomized block design with four treatments, two experimental blocks (representing different incubator shelves), and five replicates (i.e., 40 experimental units in total). The main factor of interest was whether or not oleic acid was present. This was carried out with and without the addition of imitation corpses (i.e., small black glass beads) to test whether the presence of foreign bodies alone may elicit the building response in *R. virginicus*. Thus, the four treatments were: (1) oleic acid with beads, (2) oleic acid without beads, (3) no oleic acid with beads, and (4) no oleic acid or beads. The exact same experimental units and experimental set up was used in this experiment as described above but in this case only a single colony was used. Three black glass beads (each ~10 mg in weight) were added to each hole for the bead treatments. For the oleic acid treatment, 0.05 ml of >97% oleic acid (Fisher Scientific) was added to each hole (directly onto the beads, when present) using an Eppendorf pipette. Control blocks received an equivalent amount of distilled water. The experiment was ended after 17 days and data were collected and analyzed (due to high rates of mortality in two of the jars, 38 of the original 40 experimental units were included in the final analysis) in exactly the same manner as described above for experiment 1.

Results

Experiment 1: response to corpses

It was clear at the end of the experiment that much more building had taken place in wood containing corpses (Fig. 1d–f) than in corpse-free controls (Fig. 1g). Although sand was sometimes used to fill the space between the two pieces of wood (Fig. 1d), it was more often used to construct caps of sand just above the corpses (Fig. 1e) or at the tops of the holes (Fig. 1f). Sand weight differed significantly among treatments ($F_{3,43}$=12.3, P<0.01), being higher when corpses were present than when absent (Fig. 2). There were no differences in sand weight among corpse types, however (Fig. 2). Termite survivorship ($F_{3,43}$=0.9, P=0.4) and wood consumption ($F_{3,6}$=0.2, P=0.9) did not differ among treatments. Only for wood consumption was there a significant block (i.e., colony/incubator shelf) effect.

Experiment 2: response to oleic acid and imitation corpses

Approximately ten times more sand, a significant difference ($F_{3,34}$=29.38, P<0.0001), was carried into blocks containing beads treated with oleic acid compared to those containing oleic acid alone, beads treated with distilled water or control blocks lacking both oleic acid and beads (Fig. 3). There were no differences in sand weight among these other treatments (Fig. 3). Beads treated with oleic acid elicited the same building response observed in the first experiment, resulting in the construction of caps and walls to separate them from the rest of the colony. Termite survivorship did not differ among treatments ($F_{3,34}$=0.6, P=0.6) but wood consumption was significantly lower from blocks treated with oleic acid ($F_{3,34}$=5.5, P=<0.01, Fig. 3). The block effect (i.e., incubator shelf) was insignificant for all response variables.

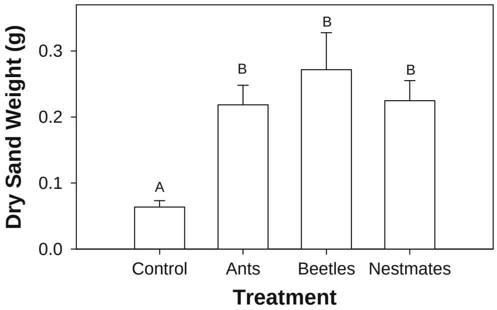

Fig. 2 Significantly more sand was collected from wooden blocks containing corpses of ants, beetles, or nestmates than corpse-free controls based on Tukey's Studentized Range Test. There were no differences among corpse types. Untransformed data (mean ± SE) are presented here

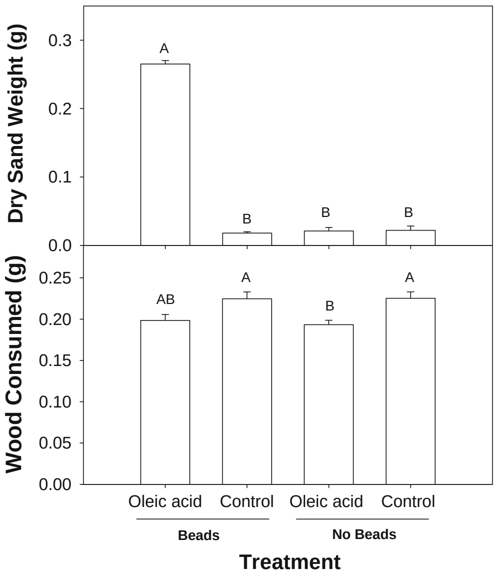

Fig. 3 Oleic acid induced a stronger building response in *R. virginicus* but only when applied to imitation corpses (beads) (*top*) and also reduced the amount of wood consumed (*bottom*) based on Tukey's Studentized Range Test. Untransformed data (mean ± SE) are presented here

Discussion

As hypothesized, the results from this study indicate that insect corpses induce building in *R. virginicus*. This response is probably aimed at mitigating the threat from fungus or other dangers associated with decay (Chouvenc and Su 2010). It should be noted that *Reticulitermes*, like other termites, are known to eat their dead (Wilson 1971; Myles 2002; Chouvenc et al. 2008) and this behavior may also have a protective function. Because termites are soft-bodied and decay rapidly, however, the extent to which nestmate corpses were consumed in this study cannot be determined. Whether a termite corpse gets buried or consumed may depend, at least in part, on whether or not it has been colonized by pathogens. Termites can distinguish between dangerous and benign fungal species (Myles 2002; Mburu et al. 2009; Yanagawa et al. 2011) and react accordingly. Kramm et al. (1982), for instance, observed that *Reticulitermes* never cannibalized dead nestmates infected with *M. anisopliae*, opting to bury them instead (but see Chouvenc et al. 2008).

To our knowledge, this is the first study to formally test a potential cause for building in *Reticulitermes*. Other cues likely to induce this response include exposure (e.g., building tunnels across exposed surfaces) and the presence of predators. Although not supported by the current study (i.e., the corpses of predatory ants did not result in more building than those of a benign beetle species or nestmates), the possibility that the presence of ants and other predators results in higher rates of building in termites deserves more attention. Ants commonly nest in dead wood (Higgins and Lindgren 2006) and subterranean termites are virtually defenseless against them in one-on-one encounters (Buczkowski and Bennett 2008). Even so, ant nests and termite colonies frequently occur in close proximity to one another (e.g., Shelton et al. 1999) which would seem to necessitate protective fortification-building of some kind. Previous work has shown that ant extracts affect feeding and tunneling behavior in *C. formosanus*, suggesting that termites are capable of detecting ant-specific odors (Cornelius and Grace 1994). Because the extracts were taken from dead ants, however, these results should be interpreted with caution.

The findings from the second experiment suggest that oleic acid, a widespread compound released during the decomposition of termites and other arthropods, could be an important chemical cue, or "necromone" used by termites to recognize the dead. This seems particularly likely considering that oleic and linoleic acids serve as necromones for cockroaches, close relatives of termites (Rollo et al. 1994), as well as many other arthropod taxa (Yao et al. 2009). These findings are not entirely consistent with those recently reported by Chouvenc et al. (2011), however. In their study, pieces of filter paper treated with oleic acid alone did not trigger burial activity in *Pseudacanthotermes spiniger*, requiring instead a blend of multiple corpse compounds. Cue synergism may provide an explanation. The results from our study suggest that corpse recognition and response requires both tactile and chemical stimuli in *R. virginicus*. Only the combination of oleic acid and imitation corpses elicited corpse response behavior; neither cue elicited a response when presented alone. This is sensible considering how energetically impractical it would be for termites to react to every foreign object or trace of oleic acid encountered when foraging. Oleic acid may prove to be just one of several chemical mechanisms by which termites recognize corpses, however. Recent research on ants, for instance, suggests that the disappearance of two chemical signals from the cuticles of living ants elicits corpse recognition and removal behaviors (Choe et al. 2009).

It is noteworthy that oleic acid significantly reduced wood consumption, even though this was only the case in our second experiment. These results suggest the compound, and perhaps others associated with termite decay, may possess important repellent properties. Oleic acid has the additional benefit of reducing leachability and has already been incorporated into at least one compound, ammonium borate oleate, for that purpose (Lyon et al. 2007).

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References

Buczkowski G, Bennett G (2008) Behavioral interactions between *Aphaenogaster rudis* (Hymenoptera: Formicidae) and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae): the importance of physical barriers. J Insect Behav 21:296–305

Choe D-H, Millar JG, Rust MK (2009) Chemical signals associated with life inhibit necrophoresis in Argentine ants. PNAS 106:8251–8255

Chouvenc T, Su N-Y (2010) Apparent synergy among defense mechanisms in subterranean termites (Rhinotermitidae) against epizootic events: Limits and potential for biological control. J Econ Entomol 103:1327–1337

Chouvenc T, Su N-Y, Elliott ML (2008) Interaction between the subterranean termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) and the entomopathogenic fungus *Metarhizium anisopliae* in foraging areas. J Econ Entomol 101:885–893

Chouvenc T, Robert A, Sémon E, Bordereau C (2011) Burial behavior by dealates of the termite *Pseudacanthotermes spiniger* (Termitidae, Macrotermitinae) induced by chemical signals from termite corpses. Insect Soc. doi:10.1007/s00040-011-0197-3

Cornelius ML, Grace JK (1994) Semiochemicals extracted from a dolichoderine ant affects the feeding and tunneling behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae). J Econ Entomol 87:705–708

Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. Curr Biol 17:693–702

Crosland MWJ, Traniello JFA (1997) Behavioral plasticity in division of labor in the lower termite *Reticulitermes fukienensis*. Naturwissenschaften 84:208–211

Hamilton C, Lay F, Bulmer MS (2011) Subterranean termite prophylactic secretions and external antifungal defenses. J Insect Physiol 57:1259–1266

Hart AG, Ratnieks FLW (2002) Waste management in the leaf-cutting ant *Atta colombica*. Behav Ecol 13:224–231

Higgins RJ, Lindgren BS (2006) The fine scale physical attributes of coarse woody debris and effects of surrounding stand structure on its utilization by ants (Hymenoptera: Formicidae) in British Columbia, Canada. In: Grove SJ, Hanula JL (eds) Insect biodiversity and dead wood: Proceedings of a symposium for the 22nd International Congress of Entomology. Gen. Tech. Rep. SRS-93. U.S. Department of Agriculture Forest Service, Southern Research Station, pp 67–74

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge

Hostettler NC, Hall DW, Scheffrahn RH (1995) Intracolony morphometric variation and labral shape in Florida *Reticulitermes* (Isoptera: Rhinotermitidae) soldiers: significance for identification. Fla Entomol 78:119–129

Howse PE (1966) Air movement and termite behavior. Nature 210:967–968

Jones WE, Grace JK, Tamashiro M (1996) Virulence of seven isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Environ Entomol 25:481–487

Kramm KR, West DF, Rockenbach PG (1982) Termite pathogens: transfer of the entomopathogen *Metarhizium anisopliae* between *Reticulitermes* sp. termites. J Invertebr Pathol 40:1–6

Li H-F, Su N-Y (2008) Sand displacement during tunnel excavation by the Formosan subterranean termite (Isoptera: Rhinotermitidae). Ann Entomol Soc Am 101:456–462

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

Lyon F, Pizzi A, Imamura Y, Thevenon M-F, Kartal SN, Gril J (2007) Leachability and termite resistance of wood treated with a new preservative: ammonium borate oleate. Holz Roh Werkst 65:359–366

Mburu DM, Ochola L, Maniania NK, Njagi PGN, Gitonga LM, Ndung'u MW, Wanjoya AK, Hassanali A (2009) Relationship between virulence and repellency of entomopathogenic isolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the termite *Macrotermes michaelseni*. J Insect Physiol 55:774–780

Myles TG (2002) Alarm, aggregation, and defense by *Reticulitermes flavipes* in response to a naturally occurring isolate of *Metarhizium anisopliae*. Sociobiology 40:243–255

Pearce MJ (1987) Seals, tombs, mummies, and tunneling in the drywood termite *Cryptotermes* (Isoptera: Kalotermitidae). Sociobiology 13:217–226

Rollo CD, Czyzewska E, Borden JH (1994) Fatty acid necromones for cockroaches. Naturwissenschaften 81:409–410

Rosengaus RB, Traniello JFA, Bulmer MS (2011) Ecology, behavior and evolution of disease resistance in termites. In: Bignell DE, Roisen Y, Lo N (eds) Biology of termites: a modern synthesis. Springer, New York, pp 165–191

Shelton TG, Vogt JT, Appel AG, Oi FM (1999) Observations of *Reticulitermes* spp. in *Solenopsis invicta* mounds (Isoptera: Rhinotermitidae, Hymenoptera: Formicidae). Sociobiology 33:265–275

Stuart AM (1967) Alarm, defense, and construction behavior relationships in termites (Isoptera). Science 156:1123–1125

Su N-Y (2005) Response of the Formosan subterranean termites (Isoptera: Rhinotermitidae) to baits or nonrepellent termiticides in extended foraging arenas. J Econ Entomol 98:2143–2152

Visscher PK (1983) The honey bee way of death: necrophoric behavior in *Apis mellifera* colonies. Anim Behav 31:1070–1076

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge

Wilson EO, Durlach NI, Roth LM (1958) Chemical releasers of necrophoric behavior in ants. Psyche 65:108–114

Wood TG (1988) Termites and the soil environment. Biol Fertil Soils 6:228–236

Yanagawa A, Fujiwara-Tsujii N, Akino T, Yoshimura T, Yanagawa T, Shimizu S (2011) Behavioral changes in the termite, *Coptotermes formosanus* (Isoptera), inoculated with six fungal isolates. J Invertebr Pathol 107:100–106

Yao M, Rosenfeld J, Attridge S, Sidhu S, Aksenov V, Rollo CD (2009) The ancient chemistry of avoiding risks of predation and disease. Evol Biol 36:267–281

Zoberi MH (1995) *Metarhizium anisopliae*, a fungal pathogen of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Mycologia 87:354–359