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Research article

Fungistatic activity of the sternal gland secretion of the dampwood termite *Zootermopsis angusticollis*

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Summary. In vivo and in vitro studies indicate that cuticular chemicals from the ventral region of the abdomen where the sternal gland of the dampwood termite *Zootermopsis angusticollis* is located have fungistatic properties. Germination rates of conidia of the entomopathogenic fungus *Metarhizium anisopliae* were significantly reduced from 91% (controls) to 38.5% after nymphs walked over conidia-seeded agar medium, but did not differ from controls when the sternal gland and surrounding cuticle were sealed with nail polish. In vitro studies show that germination of fungal conidia was also significantly reduced following incubation with cuticular extracts of either sternal or tergal segments suggesting that cuticular exudates in general may have antifungal properties. Extracts of sternites had greater fungistatic activity than extracts of tergites, but the difference was not statistically significant. Extracts of the sternal gland significantly reduced germination rates by up to 9%. Germination rates were significantly reduced when conidia were incubated with *n*-hexanoic acid, or its vapor. *n*-Hexanoic acid has been recovered from whole body extracts of *Zootermopsis nevadensis* and may indeed be a component of the sternal gland of *Z. angusticollis*. Here we suggest that sternal gland secretions in termites may have had the original function of controlling microbes within the nest and their prominent role in communication may have evolved secondarily.

Key words: Termites, trail pheromone, sternal gland, *n*-hexanoic acid, microbe defense, *Zootermopsis*, *Metarhizium*.

Introduction

Termites communicate information about the location of food resources through the deposition of trail pheromones (Traniello and Leuthold, 2000). Trail-laying behavior occurs in some basal groups (Stuart, 1969), although foraging never occurs outside the nest because these one-piece nest species feed on the wood they colonize (Abe, 1987). In spite of their claustral existence and apparent lack of a need to recruit nestmates to new food sources, one-piece nest termites have a well-developed sternal gland; this structure serves as the sole source of trail pheromones in the Isoptera (Grassé, 1986). Although trail-laying behavior may not occur in the context of food recruitment in basal termite species, it does appear to nevertheless play a role in communication. For example, as part of an alarm response, *Zootermopsis* nymphs drag their abdomen to lay a trail and thus recruit nestmates to an area such as a breach in the nest or a gallery in need of repair (Stuart, 1969; Grassé, 1986).

Many one-piece nest termites live among abundant and diverse microbes that colonize their decayed wood nests (see http://people.bu.edu/rrosenga/table1.htm for an extensive survey and references on the incidence of pathogen and parasite infection in termites; Rosengaus et al., 2003). Termites have in part adapted to the disease risk posed by microbes through the evolution of fungistatic compounds present in body exudates, fecal pellets and some defensive secretions (Batra and Batra, 1966; Sannasi and Sundara Rajulu, 1967; Rich, 1969; Batra et al., 1973, 1979; Olagbemiro et al., 1988; Rosengaus et al., 1998a, 2000). Does the deposition of sternal gland secretion in the nest galleries also function in the control of pathogens within the colony? Here we present the results of a series of *in vivo* and *in vitro* experiments designed to test if sternal gland secretions inhibit or delay the germination of the fungal pathogen *Metarhizium anisopliae* under controlled laboratory conditions. We show that the ventral abdominal region where the sternal gland is located in the dampwood termite *Zootermopsis angusticollis* (Hagen), a basal one-piece nest species, has antifungal properties. Furthermore, *n*-hexanoic (= *n*-caproic) acid, a cuticular component of *Zootermop-*

sis nevadensis (Hummel and Karlson, 1968; Karlson et al., 1968) and a putative sternal gland secretion in this genus, not only induces trail following in *Z. nevadensis* (Hummel and Karlson, 1968) and *Z. angusticollis* (unpublished data), but also inhibits fungal conidia germination.

Materials and methods

Termite collection and maintenance

Mature colonies of the dampwood termite *Z. angusticollis* (ranging in size from approximately 500–1000 individuals) were collected from the Redwood East Bay Regional Park in Oakland, the Pebble Beach Resort in Monterey, Palo Alto Foothill Park and Mount Tamalpais State Park, California. Termites were cultured in closed plastic containers (50 × 30 × 20 cm) with wood from the original nest logs and were maintained in an environmental chamber at 25 °C and 61.5% relative humidity (RH). The colonies were sprayed periodically with water to ensure adequate moisture.

Preparation of fungal conidia suspensions

Conidia of the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae* were harvested from cadavers of *Z. angusticollis* nymphs that had been previously exposed to conidia originally obtained from the American Type Culture Collection (batch # 93-09 media # 325; ATCC® 90448). A fungal stock suspension was prepared by scraping the conidia from several termite corpses using a flamed metallic loop and transferring them to a 0.1% Tween 80 suspension medium (Rosengaus and Traniello, 1997; Rosengaus et al., 1998b). Conidia suspensions for all experiments contained between 2.2×10^5 to 5.7×10^7 conidia/ml and their viability was determined by plating 40 µl of the conidia suspension on a thin layer of potato dextrose agar (PDA, n = 3 replicates). Germination rates for all experiments were recorded by estimating the percentage of conidia having a germ tube in each field of vision. Each field of vision contained approximately between 20 and 40 conidia, which allowed a clear distinction between germinated and un-germinated conidia. Ten to 15 consecutive fields of vision per slide were recorded over the entire length of the slide at 400X magnification after an 18-hour/25 °C/61.5% RH incubation period under a 12L:12D light cycle (Rosengaus et al., 1998a, 2000). Conidia germination in controls ranged from 90–98% in all experiments.

Measurement of inhibitory effects of fungal growth

To determine *in vivo* if conidia germination rates were reduced after termites contacted *M. anisopliae*-seeded PDA media, *Z. angusticollis* nymphs were allowed to walk individually for three hours over a microscope slide coated with a thin layer of approximately 1 ml of solidified PDA previously seeded with 40 µl of a 2.2×10^5 conidia/ml concentration of *M. anisopliae*. To ensure that termites walked over the seeded PDA slides, each agar/conidia-seeded slide was enclosed within two clean microscope slides and two cover slips secured to form a barrier around the experimental microscope slide. After removing the nymphs, the slides were incubated at 25 °C/61.5% RH under a 12L:12D light cycle for 18 hours. Germination rates of conidia were recorded as described above. The average percent germination of fungal conidia on treated slides was compared to that of control slides, which were similarly seeded with a 40 µl of a 2.2×10^5 conidia/ml concentration of *M. anisopliae* but had no termite contact. Because termite feces also reduce conidia viability (Rosengaus et al., 1998a), the germination rates of slides on which termites had defecated were eliminated from the analysis. Germination rates were recorded for 15 experimental and 15 control slides (n = 150 fields of vision; approximately 3000 conidia/treatment).

The sternal gland in *Zootermopsis* is formed by the overlap of the third and fourth sternites (Pasteels, 1972; Noirot, 1995). To determine if sternal gland secretions have antifungal properties, the abdominal segments III-VI of 15 additional nymphs were sealed with transparent nail polish. Sealing these ventral segments ensured not only that the sternal gland was completely covered but also that there would be no leakage of secretions at the boundary of the gland and the nail polish coating. This procedure had no observable effect on termite locomotion or behavior. To seal the sternal gland, nymphs placed inside Eppendorf tubes were immobilized on ice for five minutes or less and the sealant was subsequently applied. Termites were then kept on ice until the polish had dried completely (approximately 2–3 minutes). Once termites became active following the cold-immobilization procedure, each nymph was allowed to walk over a conidia-seeded PDA enclosed slide as described above. The average percent conidia germination after incubation for 18 hours was compared to that of control slides as well as slides on which untreated termites had walked. The calculation of the average germination rate of control slides seeded only with *M. anisopliae* conidia was based on 130 fields of vision, whereas the average germination rates of slides on which termites were allowed to walk (either with sealed or unsealed sternal glands) were based on 150 fields of vision, each.

Measurement of the inhibitory effect of conidia germination by cuticular and sternal gland extracts

During trail laying, the sternal gland is pressed on the surface and the secretion is directly deposited on the substrate as the abdomen is dragged (Stuart, 1969). Given the close spatial association between the sternal gland and the attached exoskeleton, we studied the inhibitory effects of both the cuticle and the exocrine gland by extracting tergites, sternites and sternal glands of *Z. angusticollis* and incubating *M. anisopliae* conidia in the presence of these extracts. To minimize contamination with fecal material, we expressed most or all fecal fluid prior to gland dissections by applying slight pressure on the abdomen of cold immobilized nymphs (Rosengaus et al., 1998a). Sternal segments (I–VIII) were removed, cleaned-off of tissue in saline and placed in a sterile 1.5 ml centrifuge tube. Tergal segments were excised and treated in the same manner. The mass of sternal and tergal segments of approximately 5–7 nymphs was 0.01 grams. In this way, we minimized quantitative differences between the sternal and tergal segments and their fungistatic influence. Cuticle was suspended in 100 µl of dimethyl sulfoxide (DMSO, Sigma) and was crushed inside an Eppendorf tube with a flamed flattened and cooled glass rod. The tubes were centrifuged for 10 minutes at 10,000 rpm at 4 °C. The supernatant containing the extract was then incubated with a conidia suspension. To test if the degree of inhibition of conidia germination was dependent on the concentration of the extract, suspensions of 1.7×10^7 conidia/ml containing either 1.5, 4.0, 8.0 or 10.0% concentrations of each extract were prepared. The extract/conidia suspension was vortexed and then 10 µl of each suspension was immediately plated on a thin layer of solidified PDA. Two slides were seeded for each cuticular extract/conidia suspension, incubated at 25 °C/61.5% RH for 18 hours and germination rates were recorded over 15 fields of vision for each slide. The average conidia germination for each cuticular extract/conidia suspension was compared to the corresponding values from cuticle-free 1.5, 4.0, 8.0 and 10.0% DMSO/conidia suspension control slides.

To separate the inhibitory effect of sternal gland secretion from that of the associated cuticular segments, the sternal glands of five additional nymphs were dissected and as much of the associated cuticle as possible was removed. The glands were then extracted in 4.0, 8.0 and 10.0% DMSO and their fungistatic activity was assayed as described above.

Measurement of the inhibitory effect of conidia germination by n-hexanoic acid

n-Hexanoic acid has been identified from whole body extracts of *Zootermopsis nevadensis* termites and appears to be an active chemical component of the trail pheromone in this species (Hummel and Karlson,

1968). To assess the inhibitory effect of *n*-hexanoic acid on conidia germination at biologically realistic concentrations, we first estimated the volume of *n*-hexanoic acid required to produce one "trail unit", defined as the amount of trail pheromone sufficient to induce trail following by at least three out of ten *Zootermopsis* nymphs along an artificial trail 10 cm in length (Karlson et al., 1968). Given that 1 mg of *n*-hexanoic acid contains 50,000 trail units, we incubated 1000 µl of a 5.7×10^5 conidia/ml solution *M. anisopliae* with 1 µl of *n*-hexanoic acid (99% purity, Sigma), creating a final conidia concentration containing the equivalent of 50 trail units. Five minutes after the addition of *n*-hexanoic acid to the conidia suspension, we vortexed the suspension and subsequently 40 µl of the conidia/*n*-hexanoic acid suspension was plated on solidified PDA on a microscope slide. The same conidia/*n*-hexanoic suspension was also allowed to stand for 10 hours at 13.5 °C before plating on new PDA slides. Average percent germination for both of these treatments and the controls were scored after the slides were incubated at 25 °C/61.5% RH for 18 hours (n = 3 slides/incubation time).

Volatile effect of n-hexanoic acid on conidia germination

To test if *n*-hexanoic acid can affect the development of conidia through its volatility, 10 solidified PDA plates (60 mm in diameter × 15 mm in height) were seeded with 300 µl of a 5.7×10^7 conidia/ml concentration of *M. anisopliae* (approximately 1.7×10^7 conidia). Blue food coloring (Durkee®, Burns Philip Food Inc., CA) was used to dye the conidia solution to ensure visually that the entire area of the plate had been evenly seeded. Food coloring had no effect on conidia germination (Rosengaus et al., 2000). Subsequently, a 0.5 mm × 0.5 mm piece of filter paper (Whatman # 5, particle retention > 2.5 µm, fine porosity) was suspended from the inner surface of the lid of the Petri dish approximately 10 mm above (but not in contact with) the PDA medium or conidia. Each piece of filter paper was then treated with 5 µl of *n*-hexanoic acid (99% purity, Sigma) and the lid was immediately placed on the Petri dish. The dishes were stacked inside a covered plastic box and maintained at 25 °C/61.5% RH under a 12L:12D light cycle. In addition to the experimental treatments, 10 control replicates were also seeded with a colored 5.7×10^7 conidia/ml concentration, but the filter paper of the controls was treated with 5 µl of a 0.1% Tween 80 solution. The surface area of the PDA plate that was free of fungal growth in both the control and experimental dishes was estimated through relative planimetry three and six days after seeding. Each control and experimental dish was inverted and photocopied, and the areas of the PDA plate with and without fungal sporulation were marked, cut out, weighed separately and their mass converted into area by obtaining the equivalence of the mass of the paper after photocopying a dish (60 mm × 15 mm) with an area of 28.3 cm^2.

Statistical analyses

Spore germination rates were not normally distributed. Therefore, differences in germination between control and experimentally treated conidia suspensions were analyzed with either a Mann Whitney U test or a Kruskal-Wallis test (SPSS, 1990).

Results

Inhibitory effects of fungal growth due to abdominal dragging behavior

Behavioral observations indicated that while walking on a conidia-seeded agar plate, termites dragged their abdomen, presumably depositing sternal gland secretions and cuticular exudates on the medium. This resulted in a significant decrease in conidia germination, from 91.5% in controls to 38.5% in the experimental slides, after nymphs were allowed to walk over the conidia-seeded PDA microscope slide (U = 1948.5, z = –10.3, p < 0.0001, Mann-Whitney U Test; Fig. 1). When the ventral segments surrounding the sternal gland were sealed with nail polish, the average germination rate was approximately 82.6%, not significantly different from that of conidia on control slides (U = 2348.5, z = –1.4, p = 0.1, Mann-Whitney U Test; Fig. 1).

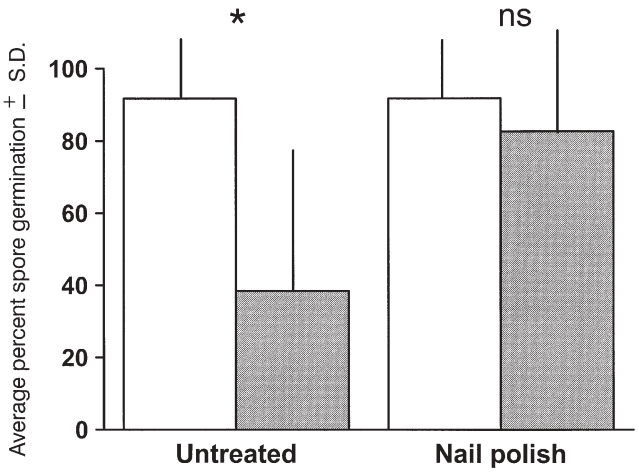

Figure 1. Average percent germination (± S.D.) of *M. anisopliae* conidia seeded on PDA slides after untreated or nail polish-treated termites were allowed to walk for three hours (filled bars) and controls (open bars). * indicates significant differences at p < 0.001 (Mann-Whitney U Test); ns indicates no statistical significance

Inhibitory effect of cuticular and sternal gland extracts on conidia germination

After the addition of DMSO at concentrations of 1.5%, 4.0%, 8.0% and 10%, the average percent germination of *M. anisopliae* conidia (± S.D.) were 97.5 ± 5.0, 96.2 ± 4.11, 93.1 ± 16.7 and 89.9 ± 9.5, respectively, whereas conidia plated in the absence of DMSO had an average percent germination of 96.1 ± 16.5. Thus, DMSO at concentrations of 8.0 and 10.0% resulted in a small difference in conidia germination, ranging from 3.0 to 6.0% relative to controls lacking DMSO. Although conidia viability in the presence of DMSO was significantly different from that of conidia that had no contact with DMSO ($\chi^2 = 26.6$, df = 4, p < 0.001, Kruskal-Wallis Test), germination rates of DMSO controls were high enough to establish that the incubation of sternal and tergal DMSO extracts further reduced germination. Because the fungistatic effects of sternites and tergites were compared to this corresponding DMSO control concentration, we are confident that our data reflects a reduction in conidia germination due to the chemicals extracted from the cuticular segments rather than the DMSO itself.

Fungistatic activity was dependent on the concentration of cuticular extract (Fig. 2). Concentrations of 1.5% and 4.0%

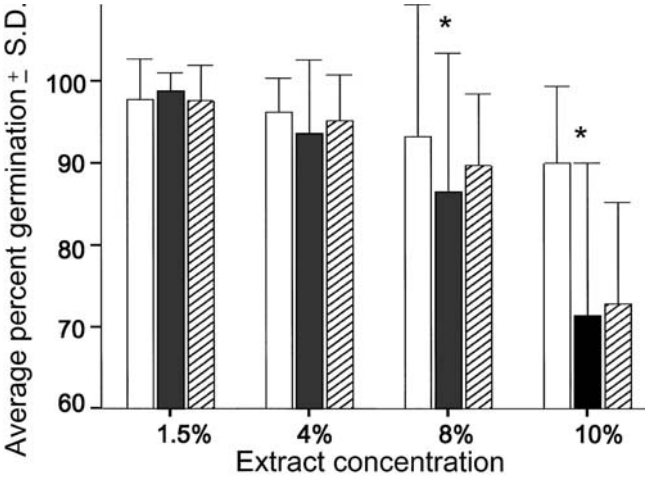

Figure 2. Average percent germination (± S.D.) of *M. anisopliae* conidia incubated with extracts of sternites and tergites at various concentrations. Controls (DMSO and conidia; open bars), sternite extract (filled bars) and tergite extract (diagonal bars). * denotes significance at $p < 0.005$ by Kruskal-Wallis Test

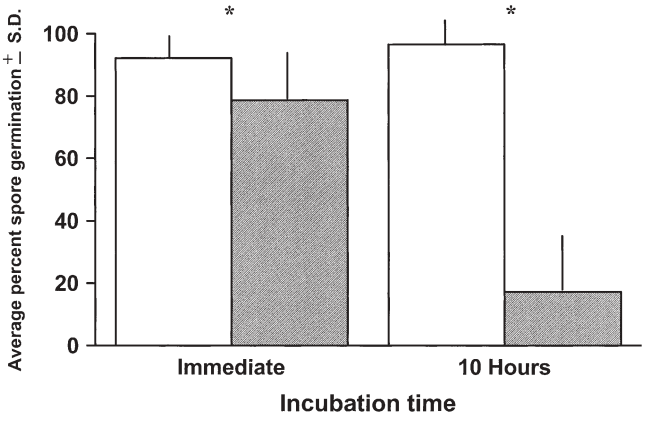

Figure 3. Average percent germination (± S.D.) of *M. anisopliae* conidia incubated with *n*-hexanoic acid for either 5 minute (immediate treatment; n = 30 fields of vision) or 10 hours prior to seeding on PDA growth media, which was subsequently incubated for 18 hours (n = 30 fields of vision; filled bars). Control (open bars; n = 30 fields of vision) had no *n*-hexanoic acid. * indicates significant differences by Mann-Whitney U Test at $p < 0.001$

had no significant antifungal properties relative to controls ($\chi^2 = 0.4$, df = 2, $p > 0.5$ and $\chi^2 = 0.5$, df = 2, $p > 0.5$, respectively, Kruskal-Wallis Test; Fig. 2). However, conidia germination was significantly reduced at concentrations of 8.0% ($\chi^2 = 11.7$, df = 2, $p < 0.005$, Kruskal-Wallis Test) and 10% ($\chi^2 = 13.1$, df = 2, $p < 0.005$, Kruskal-Wallis Test; Fig. 2). The reduction in conidia germination by approximately 4-7% in the 8.0% extract and 18% in the 10.0% extract relative to the corresponding DMSO controls suggests that cuticular chemicals are fungistatic. For the 4.0, 8.0 and 10.0% extract concentrations, sternal segment extracts had a slightly higher fungistatic effect than tergal segment extracts (Fig. 2), but these differences were not statistically significant (U = 442, 406 and 119, $p > 0.5$, respectively, Mann-Whitney U Test).

Conidia incubated with extracts of the sternal gland from which most of the attached surrounding cuticle had been removed also tended to also have lower germination rates. The average conidia viability of the 4.0% DMSO extract was significantly reduced from 91.3 ± 16.0 in controls to 82.6 ± 23.8 in experimental treatments (n = 30 fields of vision for each treatment, U = 325.5, z = –2.0, p = 0.04, Mann-Whitney U Test). Although conidia germination in the 8% DMSO sternal gland extract was reduced from 84.4 ± 19.3 in controls to 78.7 ± 20.7, this difference was not statistically significant (n = 30 fields of vision for each treatment, U = 375.5, z = –1.1, p = 0.2, Mann-Whitney U Test). No reduction in the average germination rates was observed at the 10% DMSO extract, probably due to the greater effect of DMSO on conidia germination at this high concentration than the effect of the extracted glands themselves on conidia germination.

Inhibitory effect of n-hexanoic acid on conidia germination

Germination was significantly reduced by 13.5% when *n*-hexanoic acid was added to conidia solutions and plated on PDA slides within five minutes of contact (n = 30 fields of vision, U = 256.5, z = –2.9, $p < 0.005$, Mann-Whitney U Test; Fig. 3). A greater reduction in germination was found when *n*-hexanoic acid was in contact with the conidia solution for 10 hours prior to plating on PDA. In this case, the germination rate of controls was 96.5 ± 7.8 while that of the *n*-hexanoic treated conidia was 17.2 ± 19.8 (n = 30 fields of vision for each treatment, U = 0.5, z = –6.9, $p < 0.0001$, Mann-Whitney U Test; Fig. 3).

Volatile effect of n-hexanoic acid on conidia germination

n-Hexanoic acid had a strong volatile effect on conidia germination, evident within three days after treated paper was suspended over a conidia-seeded medium. The average area (± S.D.) free of fungal growth was 28.2 ± 0.3 cm^2 while that of controls was 1.7 ± 1.5 cm^2 (n = 10 replicates/treatment, U = 0.0, z = –3.9, $p < 0.0001$, Mann-Whitney U Test). The volatile inhibitory effect persisted at six days: the average area free of fungal growth was 25.9 ± 4.1 cm^2 at this time, while that of controls was 0.6 ± 0.9 cm^2 (n = 10 replicates/treatment, U = 0.0, z = –3.8, $p < 0.0001$, Mann-Whitney U Test). The area free of fungal growth of *n*-hexanoic treated PDA plates did not differ significantly between three and six days (U = 40.0, z = –0.9, p = 0.4, Mann-Whitney U Test). In contrast, the area free of fungal growth in control replicates decreased significantly from the third (1.7 ± 1.5 cm^2) to the sixth day (0.6 ± 0.9 cm^2; U= 24.5, z = –1.9, p = 0.05, Mann-Whitney U Test).

Discussion

The results of our laboratory studies support the hypothesis that the sternal gland secretion, *n*-hexanoic acid and the gen-

eral cuticular chemistry of *Z. angusticollis* have fungistatic properties. The *in vivo* deposition of sternal gland secretions and/or cuticular chemicals on PDA/conidia-seeded agar plates and slides reduced the viability of fungi by 53%, but termites with sealed sternites walking on similarly seeded medium did not significantly reduce conidia germination. The results of *in vitro* experiments indicated that extracts made from the sternal gland (following the removal of the associated cuticle) and other sternal segments also significantly reduced conidia germination by 9% and 18%, respectively. The greatest inhibition of fungal growth (approximately 80%) was found when conidia were incubated with *n*-hexanoic acid for 10 hours prior to seeding the PDA medium with fungi. Although Hummel and Karlson (1968) extracted *n*-hexanoic acid from whole *Z. nevadensis* without specifically demonstrating its presence in the sternal gland, our research indicates that *n*-hexanoic acid is involved in trail communication (unpubl. data) as well as infection control in *Z. angusticollis.*

The inhibitory effect of extracts of sternites and tergites also indicate that cuticular substances in *Z. angusticollis*, perhaps fatty acids, lipids and/or waxes, may be also important fungistats, as has been demonstrated in other insects (Koidsumi, 1957; Sannasi and Sundara Rajulu, 1967; Smith and Grula, 1982; St Leger, 1991). The sternal gland secretions of other termite species, such as *Nasutitermes exitiosus*, may also have antimicrobial properties. Although no attempt was made to identify the source of the antimicrobial secretions in this species, abdominal extracts inhibited development of *Staphylococcus aureus* by 92% in comparison to extracts of the head or thorax (Cruse, 1998). The observed antibacterial effect of the *N. exitiosus* abdominal extracts may have been due to terpenoid compounds originating from the sternal gland (Moore, 1966; reviewed in Traniello and Leuthold 2000). Indeed, the terpenoids of the frontal glands of other *Nasutitermes* species have fungistatic properties (Rosengaus et al., 2000). In addition to terpenoids, naphthalene, fecal material, cuticular and defensive chemicals and labial gland secretions also inhibit the growth of bacteria and fungi in several species of termites (Blum et al., 1982; Olagbemiro et al., 1988; Chen et al., 1998; Cruse, 1998; Rosengaus et al., 1998a, 2000 and references therein). Recently, an inducible antibiotic protein in the salivary glands of *Pseudacanthotermes spiniger* was identified (Lamberty et al., 2001). The deposition of antibiotic proteins on the cuticle of nestmates during allogrooming could deactivate conidia, acting in consort with other infection defenses. Cuticular chemicals and glandular secretions in *Z. angusticollis* may thus complement social and individual disease control mechanisms.

Roaches, which are phylogenetically related to termites (Cleveland et al., 1934; Hennig, 1981; Nalepa, 1984; Kambhampati, 1995; Maekawa and Kitade, 1999; Lo et al., 2000), have numerous tergal and sternal exocrine glands; their number and function vary widely across families (Brossut and Sreng, 1985; Sreng, 1985, 1993). These glands appear to play an important role in courtship behavior (Brossut and Sreng, 1982; Sreng, 1985, 1993). Although the number, location and structure of sternal glands varies among termite species, the gland is the only reported source of trail pheromone in the Order Isoptera, suggesting that it evolved early and once (Pasteels and Bordereau, 1998). The ancestral function of the sternal and tergal glands in termites is hypothesized to concern mate attraction through pheromonal calling (Pasteels and Bordereau, 1998). Indeed, sex pheromones have been isolated from the sternal glands of *Kalotermes, Trinervitermes, Pseudacanthotermes* and *Reticulitermes* (reviewed in Pasteels and Bordereau, 1998). Although sex pheromones have also been reported in *Zootermopsis* (Pasteels, 1972; Pasteels and Bordereau, 1998), the involvement of sternal glands in sexual attraction in *Z. angusticollis* is unclear (Stuart, 1969; Pasteels, 1972; Pasteels and Bordereau, 1998). An alternative explanation for the origin of the sternal and tergal gland secretions of roaches and termites, both of which exploit microbially rich environments, may lie in the control of nest pathogens. Antimicrobial fatty acids and phenolic and naphthalene-based compounds have been isolated from the cuticular exudates and glandular secretions of several roach and termite species (Koidsumi, 1957; Sannasi and Sundara Rajulu, 1967; Brossut et al., 1975; Smith and Grula, 1982; Chen et al., 1998; St Leger, 1991). Here we have shown that *n*-hexanoic acid, a compound extracted from *Zootermopsis* that may be present in the sternal gland, also has fungistatic activity. Interestingly, fungal and bacterial growths are absent from the reservoir of the sternal gland of the cockroach *Nauphoeta,* suggesting not only that present day roaches may rely on their sternal gland secretions to control disease (Sreng, pers. comm.), but also that the ancestral function of the sternal gland could have been the production of antibiotics.

Termites do not appear to have evolved a gland analogous to the ant metapleural gland (Hölldobler and Wilson, 1990; Ortius-Lechner et al., 2000; Poulsen et al., 2002) for the sole purpose of synthesizing and secreting antibiotics (Rosengaus et al., 1998a, 2000; Thorne and Traniello, 2003). The evolution of chemical protection may have been constrained by the nutritional dependence of termites on gut symbionts, which are highly susceptible to antibiotics (Haverty and Howard, 1979). Alternatively, termites may use a series of lower-potency exudates and glandular secretions having higher specificity (Thorne and Traniello, 2003). The selection pressures favoring the origin of the sternal gland and the expansion of its role from microbial control to sexual attraction, defense and/or trail communication could have taken place without major evolutionary innovation.

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