Ecol Res (2015) 30: 93–100
DOI 10.1007/s11284-014-1213-7

ORIGINAL ARTICLE

Kenji Matsuura · Takeshi Matsunaga

Antifungal activity of a termite queen pheromone against egg-mimicking termite ball fungi

Received: 21 September 2014 / Accepted: 4 November 2014 / Published online: 15 November 2014

Abstract The sophisticated colony organization of eusocial insects is attributed to their elaborate chemical communication systems. Pheromones mediate most behaviors involved in colony organization including foraging, defense, brood care, and caste regulation. The number of candidate compounds available to regulate multiple systems may be biosynthetically finite and the production of several compounds instead of a single one may be more costly. Therefore, strong selection pressures encourage the use of single natural products for many purposes. Such versatility of signal substances is especially characteristic of queen pheromones in eusocial Hymenoptera. However, little is known about the multifunctionality of the recently identified termite queen pheromone. Here, we demonstrate that volatile compounds in the queen pheromone of a termite, *Reticulitermes speratus* (Kolbe), have fungistatic properties. Application of the pheromone compounds *n*-butyl-*n*-butyrate and 2-methyl-1-butanol significantly reduced the germination rates of the egg-mimicking parasitic termite ball fungus. These pheromone compounds also suppressed mycelial growth of the termite ball fungus and some entomopathogenic fungi. However, the inhibitory activity of each substance differed among fungal strains. Termites likely employ these antimicrobial volatiles to protect eggs and queens, and secondarily as communication agents informing queen fertility. This study supports the notion of evolutionary parsimony, wherein pheromones are originally used as defensive compounds and their communicative function develops secondarily, which is well-documented in social Hymenoptera.

Keywords Queen pheromone · Termite ball · Semiochemical parsimony · Insect-fungal interaction · Antimicrobial compounds

K. Matsuura (✉)
Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwakecho, Kyoto 606-8502, Japan
E-mail: kenjijpn@kais.kyoto-u.ac.jp
Tel.: +81757536136
Fax: +81757536136

T. Matsunaga
Faculty of Agriculture, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan

Introduction

Chemical communication is one of the most widespread forms of communication occurring among bacteria, fungi, plants, and animals (Wyatt 2003). Social insects have evolved sophisticated societies, characterized by efficient communication systems based on chemical signals (Wilson 1971; Vander Meer et al. 1998). The capacity to synthesize chemical compounds is biosynthetically finite and costly, potentially leading to strong evolutionary pressure to use single products parsimoniously for multiple purposes (Blum 1996; Steiger et al. 2011). Because various substances are emitted for non-communicative purposes, these chemicals provide multiple starting points for the evolution of communication. For example, cuticular anti-desiccation compounds are also used for recognition in the social Hymenoptera (Howard 1993; Greene and Gordon 2003; Steinmetz et al. 2003; Howard and Blomquist 2005; Nehring et al. 2011). Most organisms emit a multitude of defensive chemicals to protect themselves against enemies, such as antimicrobial compounds against pathogens, and repellents and venoms against predators. The secondary use of defensive compounds, which are primarily emitted for non-communicative purposes, as communication signals for sex, aggregation, alarm, or trail following occurs in a variety of insects (Blum and Brand 1972; Blum 996; Nojima et al. 2005; de Brujin et al. 2006; Geiselhardt et al. 2009).

Recently, a queen-produced volatile pheromone consisting of *n*-butyl-*n*-butyrate (nBnB) and 2-methyl-1-butanol (2M1B) was identified in the subterranean ter-

mite *Reticulitermes speratus* Kolbe (Matsuura et al. 2010; Yamamoto et al. 2012). This queen pheromone plays a variety of roles in reproductive regulation by indicating queen fertility, including inhibition of neotenic queen differentiation (Matsuura et al. 2010) and regulation of colony-level egg production (Yamamoto and Matsuura 2011). Importantly, the two volatiles in the queen pheromone are also emitted by eggs (Matsuura et al. 2010). These compounds indicate egg presence and function as an orientation pheromone guiding workers to care for eggs. The dual production of an inhibitory pheromone by reproductive females and eggs may provide a mechanism ensuring honest signaling of reproductive status, with a close relationship between fertility and inhibitory power (Matsuura 2012).

In social insect colonies, individual members cooperate to ensure colony growth, survival and reproduction; only a few individuals, the queens and their mates, produce offspring, whereas most individuals perform tasks such as foraging, nest construction, and offspring care. The dependence of the colony on a small number of reproductive individuals means that the fitness of all members of the society is jeopardized when the queen succumbs to a pathogen infection. Therefore, we would expect that the queen in a social insect colony should be subject to special protection, similar to germ lines that are subject to immune privilege (Cremer et al. 2007).

Brood survivorship is another determining factor of colony productivity. Brood protection is especially important in *Reticulitermes* termites, since eggs cannot survive in their microorganism-rich habitat without being groomed by workers (Matsuura et al. 2000). Soon after being laid by queens, eggs are carried into nursery chambers and frequently groomed by workers, whereby they are coated with saliva rich in antibiotic substances that protect them from desiccation and pathogenic infection. Brown fungal balls, called termite balls, are often found in egg piles of various termite species (Matsuura et al. 2000; Matsuura 2006) (Fig. 1). Termite balls tended by *Reticulitermes* termites are the sclerotia of an athelioid fungus (Basidiomycota, Agaricomycotina) of the genus *Fibularhizoctonia* (Matsuura et al. 2000). To date, this egg-mimicking fungus has been identified from seven *Reticulitermes* species in Japan and the United States (Matsuura 2005; Yashiro and Matsuura 2007), as well as from *Coptotermes formosanus* Shiraki (Matsuura and Yashiro 2010). By mimicking termite eggs chemically (Matsuura et al. 2009) and morphologically (Matsuura 2006), the termite ball fungus inhabits a nearly competitor-free habitat inside termite nests. This fungus relies on termites for defense against desiccation and other microorganisms, and the frequent grooming by workers keeps the survival rate near 100 %. In practice, most termite balls are inhibited from germination, and the fungus rarely consumes the eggs (Matsuura 2006). The evidence obtained to date indicates that the interaction is parasitic, in that it is beneficial for the fungus but costly for the host termites, at least in the short term. In this sense, the termite ball fungus is a sort of fungal cuckoo, taking advantage of its host's brood care through egg mimicry.

Considering the privileged status of queens and brood in defense against parasites, together with the fact that queens and eggs emit the same volatile compounds in *R. speratus* (Matsuura et al. 2010), we hypothesized that queen pheromone compounds were originally antimicrobial defense chemicals that were later exapted to function as queen fertility signals or neotenic queen differentiation inhibitors. In this study, we examined the antifungal activity of the *R. speratus* queen pheromone, which contains nBnB and 2M1B, as a first step in testing the antimicrobial-origin hypothesis of queen pheromone evolution. We investigated the compounds' influence on the growth of the egg-mimicking parasitic fungus *Fibularhizoctonia* sp., the closely related sclerotium-forming fungus *Athelia rolfsii* (Curzi) Tu & Kimbrough, and the distantly related sclerotium-forming fungus *Sclerotium tuliparum* Klebahn. We also tested the entomopathogenic fungi *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Isaria farinosa* (Holmskjold) Fries. Pathogenicity of these entomopathogenic fungi to *R. speratus* has been demonstrated by laboratory bioassays (Shimizu and Yamaji 2002). Additionally, we compared the inhibitory effect of the queen pheromone compounds on the germination of termite balls harvested from different strains.

Materials and methods

Bioassays for queen pheromone compound effects on termite ball germination

We collected termite nests from rotten pinewood, *Pinus densiflora* Siebold & Zuccarini, because both the host termite *R. speratus* and the termite ball fungi *Fibularhizoctonia* sp. are most commonly found there (Matsuura et al. 2000; Matsuura 2005). When termites were found, the nest wood was dismantled completely to locate the reproductive center cells, which harbored reproductive individuals, eggs, and larvae. Termite balls used in this experiment were obtained from the egg piles of three termite colonies (SE110810A, KA110727A, KA110627A) collected in Okayama, western Japan.

The termite balls collected from each termite colony were placed in a 2 mL test tube, and washed three times in sterilized distilled water (DW) using a vortex mixer. Fifty termite balls were randomly chosen from each colony and arranged on a moist unwoven cloth (25 × 25 mm) (REED Healthy-Cooking Paper, Lion Corp., Tokyo) placed on one side of a Petri dish (90 × 15 mm) as shown in Fig. 2a. We placed a filter paper square (10 × 10 mm) on a glass plate (20 × 25 mm) in the other side of the Petri dish. We applied 1 μL of a test chemical onto the filter paper as

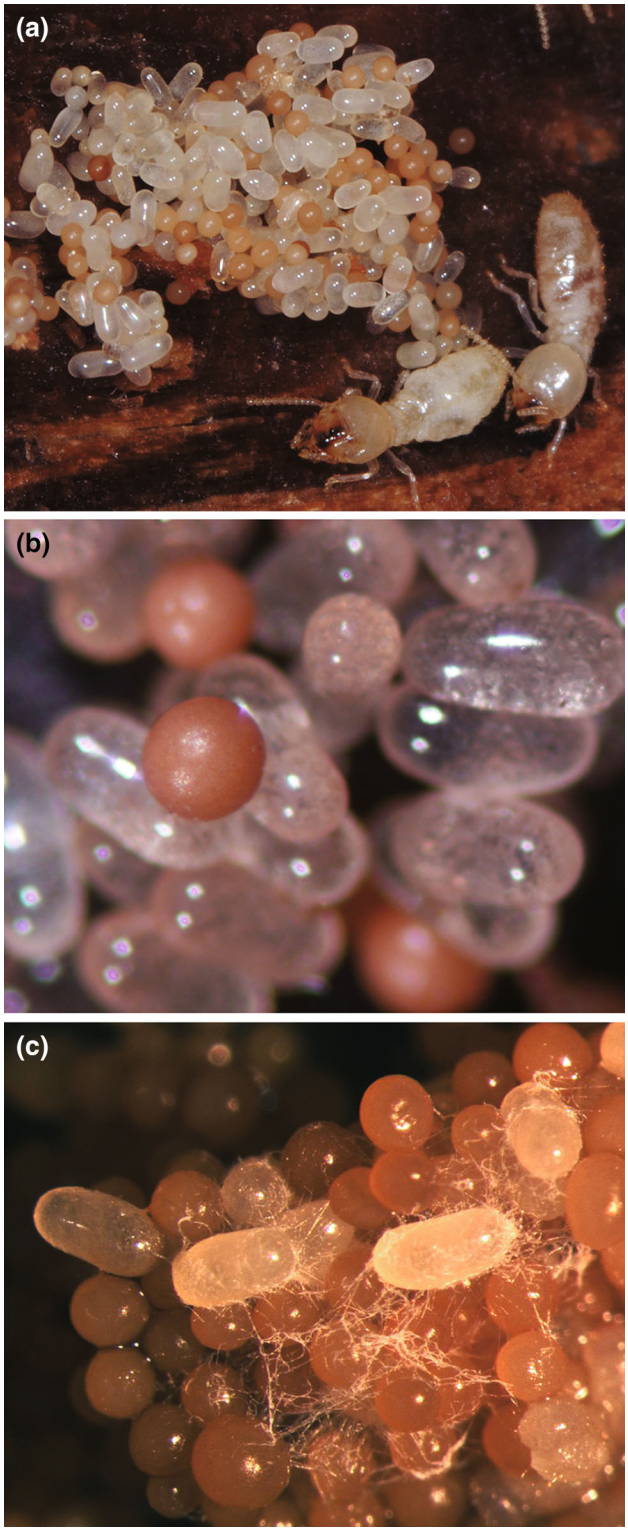

Fig. 1 Termite balls in egg piles of the termite *Reticulitermes speratus*. **a** An egg pile in a nest, **b** Close view of the egg pile. Termite balls are light brown and spherical, whereas eggs are transparent and ovoid, **c** Mycelial growth of the termite ball fungi in the egg pile, where eggs are consumed by the fungus

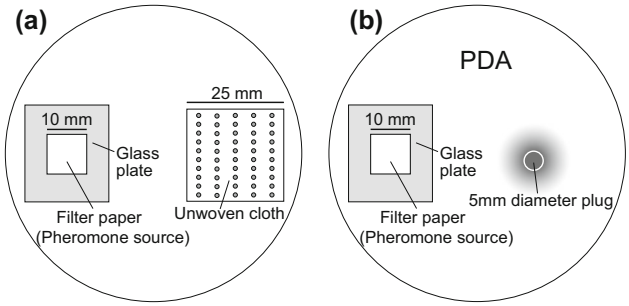

Fig. 2 Experimental set-up for the bioassay. **a** Germination test. Fifty termite balls were arranged on moist unwoven cloth. We added 1 μL of a test chemical to the filter paper, **b** Mycelial growth test. A 5 mm diameter plug of growing mycelium was placed onto potato–dextrose agar (PDA). We added 5 μL of a test chemical to the filter paper

follows: (1) nBnB; (2) 2M1B; (3) 2:1 nBnB:2M1B mixture; (4) 1:2 nBnB:2M1B mixture; (5) 1:1 nBnB:2M1B mixture; and (6) DW as a control. The Petri dishes were wrapped with two layers of Parafilm and incubated at 27 °C for 1 week. Germination rates were determined by observing the termite balls under a stereomicroscope (SZX7; Olympus Corp., Tokyo) every 24 h. Germination rates were analyzed using the Kaplan–Meier method followed by a log-rank test with Bonferroni correction. We used JMP 8.0.2 (SAS Institute, Cary, NC, USA) for this analysis.

Bioassays for queen pheromone compound effects on fungal growth

The termite ball strains (TMB strains I, II, III, and IV) used in this bioassay were isolated from the termite colonies HA100725A, TU100904A, SE110810A, and KA110727A, respectively. Colonies HA100725A, SE110810A, and KA110727A were collected in Okayama, and TU100904A was collected in Tsukuba, Japan. To isolate termite ball fungi, we extracted 5–12 termite balls from each nest. These were rinsed with sterilized DW, and then arranged on an agar plate containing 200 ppm tetracycline soon after field collection. After germination, each termite ball was inoculated on a potato–dextrose agar (PDA; BD Difco, Franklin Lakes, NJ, USA) plate and incubated at 27 °C for 3 weeks. One newly developed sclerotium was re-isolated from each plate and cultured on a new PDA plate. The strains of the sclerotium-forming fungi *A. rolfsii* (NBRC4476) and *Sclerotium tuliparum* (NBRC6168) and of the entomopathogenic fungi *B. bassiana* (NBRC5838), *I. farinosa* (NBRC8296), and *M. anisopliae* (NBRC31961), were provided by the Biological Resource Center (NBRC), National Institute of Technology and Evaluation, Tokyo, Japan. These fungal strains were cultured on PDA plates at 27 °C, as were the strains of termite ball fungi.

The assay was performed by placing a 5 mm diameter plug of growing mycelia from a 3-week-old culture onto a Petri dish with each test chemical (Fig. 2a). We placed a glass plate (20 × 25 mm) on PDA in the Petri dish (Fig. 2b). A filter paper square (10 × 10 mm) was placed at the center of the glass plate. We applied 5 μL of a test chemical onto the filter paper as follows: (1) nBnB; (2) 2M1B; (3) 2:1 nBnB:2M1B mixture; (4) 1:2 nBnB:2M1B mixture; (5) 1:1 nBnB:2M1B mixture; and (6) DW as a control. The Petri dishes were wrapped with two layers of Parafilm and incubated at 27 °C for 1 week. Five replicates were done for each treatment of each fungal strain. The radial growth of mycelia (colony diameter in mm) in all plates was measured every 24 h after inoculation. The means of two measurements of each growing colony were used for analyses. We conducted measurements for a maximum of 11 days, terminating observations when mycelial growth reached the edge of the Petri dish or glass plate. Data were analyzed by repeated measures ANOVA followed by Tukey's HSD test. We used STATISTICA 06 J (Stat Soft, Tulsa, OK, USA) for this analysis.

Results

Inhibitory effects on termite ball germination

The germination rates of the termite balls were significantly different among colonies ($p < 0.0001$, Fisher's exact probability test; Fig. 3). In the control treatment (DW), termite balls obtained from colonies SE110810A, KA110727A, and KA110627A showed germination rates of 0.88 (44/50), 0.86 (43/50), and 0.32 (16/50), respectively. Termite balls from the colony SE110810A were significantly inhibited by queen pheromone substances ($df = 5$, overall $x^2 = 96.21$, $p < 0.0001$, log-rank test) in the order 2M1B = 2TO1 = 1TO2 = 1 TO1 > nBnB > DW ($p < 0.05$, log-rank test with Bonferroni correction; Fig. 3a). There was a significant difference among termite balls from KA110727A with respect to chemical treatment (CT) ($df = 5$, overall $x^2 = 65.66$, $p < 0.0001$, log-rank test), wherein the order of inhibition was nBnB = 2TO1 > 2M1B = 1 TO1 = 1TO2 > DW (Fig. 3b). Termite balls from KA110627A had low germination rates in all treatments, and none of the queen pheromone substances showed significant inhibition ($df = 5$, overall $x^2 = 4.56$, $p = 0.47$, log-rank test; Fig. 3c).

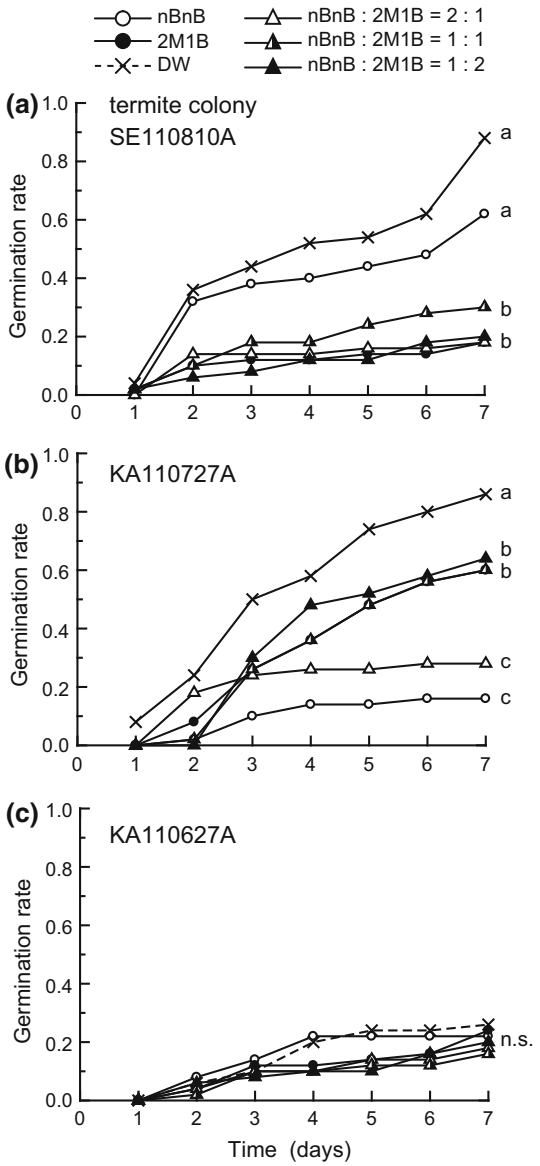

Fig. 3 Effects of queen pheromone compounds on the germination of termite balls. **a** Termite balls from colony SE110810A, **b** Termite balls from colony KA110727A, **c** Termite balls from colony KA110627A. *Different letters* indicate significant differences ($p < 0.05$, log-rank test). *nBnB* *n*-butyl-*n*-butyrate, *2M1B* 2-methyl-1-butanol

Inhibitory effects on mycelial growth

The volatile compounds of termite queen pheromone showed significant inhibitory effects on mycelial growth of the termite ball fungus (CT: $df = 5$, $F = 189.94$, $p < 0.0001$, repeated measures ANOVA; Fig. 4). The interaction effect between CT and the TMB strain was significant (Strain × CT: $df = 15$, $F = 6.86$, $p < 0.0001$, repeated measures ANOVA), where nBnB showed greater inhibition than 2M1B in strains I, II, and III, but not in strain IV. In TMB strain I, the order of inhibition was nBnB = 2TO1 = 1TO1 > 1TO2 > 2 M1B > DW ($p < 0.05$, Tukey's HSD test; Fig. 4a). In TMB strain II, the order was nBnB > 2TO1 = 1 TO1 > 1TO2 > 2M1B > DW (Fig. 4b). In TMB strain III, the order was nBnB = 2TO1 = 1TO1 = 1TO2 > 2M1B > DW (Fig. 4c). However, in TMB strain IV, the order was nBnB = 2TO1 = 1TO1 = 1TO2 = 2 M1B > DW (Fig. 4d).

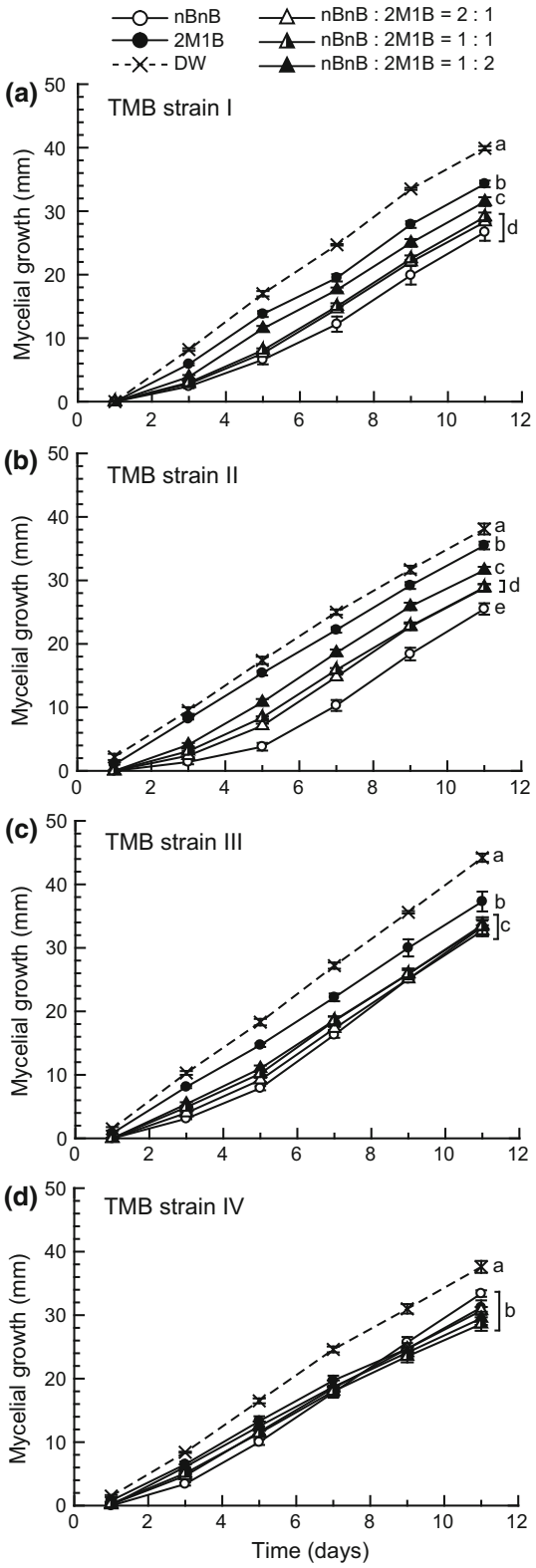

Fig. 4 Effects of queen pheromone compounds on mycelial growth of the termite ball fungus. The fungal strains I (**a**), II (**b**), III (**c**), and IV (**d**) were isolated from termite colonies HA100725A, TU100904A, SE110810A, and KA110727A, respectively. *Different letters* indicate significant differences ($p < 0.05$, Tukey's HSD test). *Error bars* denote SEM. *nBnB* *n*-butyl-*n*-butyrate, *2M1B* 2-methyl-1-butanol

Queen pheromone substances significantly inhibited the mycelial growth of the other sclerotium-forming fungi, *S. tuliparum* (CT: $df = 5$, $F = 26.668$, $p < 0.0001$; Fig. 5a) and *A. rolfsii* (CT: $df = 5$, $F = 555.75$, $p < 0.0001$, repeated measures ANOVA; Fig. 5b). The order of inhibition was nBnB = 2TO1 = 1TO1 = 1TO2 > 2M1B, DW, in both *S. tuliparum* and *A. rolfsii* ($p < 0.05$, Tukey's HSD test).

Queen pheromone significantly inhibited the entomopathogenic fungi *M. anisopliae* (CT: $df = 5$, $F = 147.06$, $p < 0.0001$; Fig. 6a) and *B. bassiana* (CT: $df = 5$, $F = 51.42$, $p < 0.0001$, repeated measures ANOVA; Fig. 6b). In *M. anisopliae*, the order of inhibition was nBnB > 2TO1 = 1TO1 > 1TO2 > 2M1B > DW (Fig. 6a). In *B. bassiana*, nBnB showed significant inhibition ($p < 0.001$), whereas 2M1B showed no significant difference from DW (Fig. 6b). In contrast, no CT significantly inhibited the entomopathogenic fungus *I. farinose* relative to DW (Fig. 6c).

Discussion

Insects and fungi have a long history of association in common habitats, where they share similar environmental conditions (Vega and Blackwell 2005). The remarkable diversity and ecological success of the social insects has been attributed to their ability to cope with the infectious microbial community inhabiting their nests and feeding sites (Wilson 1971; Hölldobler and Wilson 1990; Traniello et al. 2002). In addition to behavioral adaptations to lower disease risk (Rosengaus et al. 1999; Matsuura et al. 2002; Yanagawa et al. 2012), social insects also rely on biochemical secretions (Brough 1983; Beattie et al. 1986; Rosengaus et al. 2000; Poulsen et al. 2002; Bulmer and Crozier 2004; Rosengaus et al. 2004; de Lima Mendonça et al. 2009). In this study, the volatile compounds of a termite queen pheromone nBnB and 2M1B showed antifungal activities against the entomopathogenic termite ball fungi *M. anisopliae* and *B. bassiana*. Considering the parsimonious evolution of pheromones (Blum 1996), it is reasonable that pheromone compounds should have antimicrobial activity in various organisms (Cole et al. 1975; Matsumoto et al. 1979; Kuwahara et al. 1989; Vander Meer and Morel 1995; Ruther et al. 2001; de Brujin et al. 2006; Matsuura et al. 2007). Our results imply that the compounds nBnB and 2M1B might have originally evolved as antimicrobial defense, and that their function as termite queen pheromones might be secondary.

Germination of termite balls in egg piles imposes enormous costs on the colony because the fungus consumes surrounding eggs. The queen pheromone compounds nBnB and 2M1B are known to also be emitted by eggs (Matsuura et al. 2010). This study demonstrates that the inhibitory effects of the compounds on termite ball germination differ among colonies. Interestingly, 2M1B showed stronger inhibition than nBnB on termite

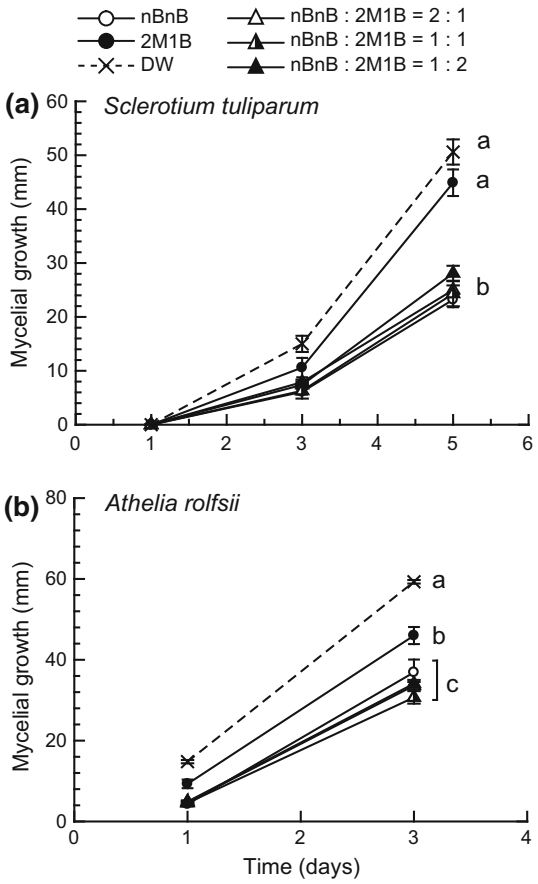

Fig. 5 Effects of queen pheromone compounds on the mycelial growth of sclerotiun-forming fungi *Sclerotium tuliparum* (**a**) and *Athelia rolfsii* (**b**). *Different letters* indicate significant differences ($p < 0.05$, Tukey's HSD test). *Error bars* denote SEM. *nBnB* *n*-butyl-*n*-butyrate, *2M1B* 2-methyl-1-butanol

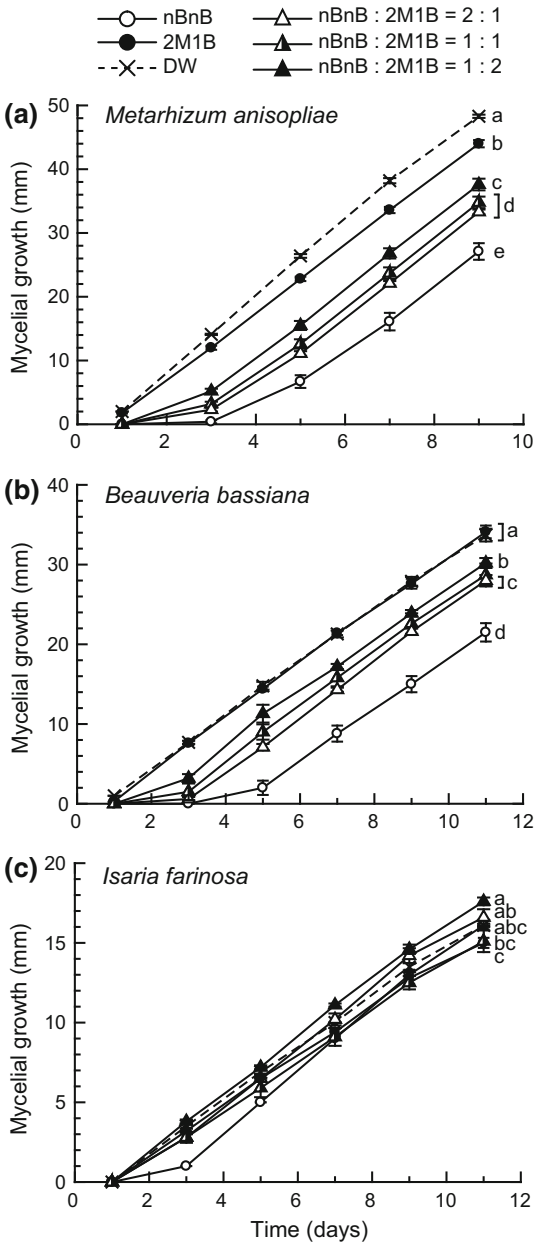

Fig. 6 Effects of queen pheromone compounds on the mycelial growth of entomopathogenic fungi *Metarhizium anisopliae* (**a**), *Beauveria bassiana* (**b**), and *Isaria farinose* (**c**). *Different letters* indicate significant differences ($p < 0.05$, Tukey's HSD test). *Error bars* denote SEM. *nBnB* *n*-butyl-*n*-butyrate, *2M1B* 2-methyl-1-butanol

balls collected from colony SE110810A (Fig. 3a), whereas nBnB showed higher suppression than 2M1B on balls from KA110727A (Fig. 3b). On average, a 2:1 ratio of nBnB and 2M1B, which matches that of queen pheromone, yielded the most stable inhibition of termite ball germination. Mycelial growth also indicated that the inhibitory effects of nBnB and 2M1B were significantly different among strains. This might explain why the termite has evolved two compounds for queen pheromones and egg volatiles.

In nature, most termite balls are inhibited from germination, and the fungus rarely consumes the eggs (Matsuura 2006). Old termite balls become shrunken and deformed. Such old, deformed, termite balls are removed from the egg piles by workers and left in a corner of the nest as garbage. The dumped termite balls germinate and grow in the nest, and newly formed termite balls are carried into egg piles. Variation in suppression level among the strains of termite ball fungus suggests that the fungus may be able to develop resistance to antifungal compounds. A single termite colony can harbor multiple strains of termite balls in its egg piles (Yashiro et al. 2011). Therefore, there might be competition among termite ball strains within a colony. If a strain were resistant to the antifungal compounds, it would be able to germinate in the egg pile and consume surrounding eggs, thereby propagating more than other strains. However, such a resistant strain would prevent reproduction by the host termite and eventually exterminate the colony. Because this fungus relies on the host for defense against desiccation and other microorganisms, severely harming the host termite would be disadvantageous for the fungus itself. This type of multi-level selection might have determined the chemical interactions between termite ball fungi and their host termites.

Acknowledgments We thank Drs C. Himuro and T. Yokoi for support and helpful discussions. We also thank T. Yashiro, W. Suehiro and Y. Yamamoto for technical assistance and discussion. This work was supported by grants from the Japan Society for the Promotion of Science (No. 21687002) to K.M. and the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry to K.M.

References

Beattie AJ, Turnbull CL, Hough T, Knox RB (1986) Antibiotic production: a possible function for the metapleural glands of ants (Hymenoptera: Formicidae). Ann Entomol Soc Am 79:448–450

Blum MS (1996) Semiochemical parsimony in the Arthropoda. Annu Rev Entomol 41:353–374

Blum MS, Brand JM (1972) Social insect pheromones: their chemistry and function. Am Zool 12:553–576

Brough EJ (1983) The antimicrobial activity of the mandibular gland secretion of a formicine ant, *Calomyrmex* sp. (Hymenoptera: Formicidae). J Invert Pathol 42:306–311

Bulmer MS, Crozier RH (2004) Duplication and diversifying selection among termite antifungal peptides. Mol Biol Evol 21:2256–2264

Cole LK, Blum MS, Roncadori RW (1975) Antifungal properties of the insect alarm pheromones, citral, 2-heptanone, and 4-methyl-3-heptanone. Mycologia 67:701–708

Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. Curr Biol 17:R693–R702

de Brujin PJ, Egas M, Janssen A, Sabelis MW (2006) Pheromone-induced priming of a defensive response in Western flower thrips. J Chem Ecol 32:1599–1603

de Lima Mendonça A, da Silva C, de Mesquita F, da Silva Campos R, Do Nascimento R, de Azevedo Ximenes E, Sant'Ana A (2009) Antimicrobial activities of components of the glandular secretions of leaf cutting ants of the genus *Atta*. Antonie Van Leeuwenhoek 95:295–303

Geiselhardt S, Schmitt T, Peschke K (2009) Chemical composition and pheromonal function of the defensive secretions in the subtribe Stizopina (Coleoptera, Tenebrionidae, Opatrini). Chemoecology 19:1–6

Greene MJ, Gordon DM (2003) Social insects: cuticular hydrocarbons inform task decisions. Nature 423:32

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

Howard RW (1993) Cuticular hydrocarbons and chemical communication. In: Stanley-Samuelson DW, Nelson DD (eds) Insect lipids: chemistry, biochemistry and biology. University of Nebraska Press, Lincoln, pp 179–226

Howard RW, Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu Rev Entomol 50:371–393

Kuwahara Y, Leal W, Suzuki T, Maeda M, Masutani T (1989) Antifungal activity of *Caloglyphus polyphyllae* sex pheromone and other mite exudates. Naturwissenschaften 76:578–579

Matsumoto K, Wada Y, Okamoto M (1979) The alarm pheromone of grain mites and its antifungal effect. Recent Adv Acarol 1:243–249

Matsuura K (2005) Distribution of termite egg-mimicking fungi (''termite balls'') in *Reticulitermes* spp. (Isoptera : Rhinotermitidae) nests in Japan and the United States. Appl Entomol Zool 40:53–61

Matsuura K (2006) Termite-egg mimicry by a sclerotium-forming fungus. Proc R Soc B 273:1203–1209

Matsuura K (2012) Multifunctional queen pheromone and maintenance of reproductive harmony in termite colonies. J Chem Ecol 38:746–754

Matsuura K, Yashiro T (2010) Parallel evolution of termite-egg mimicry by sclerotium-forming fungi in distant termite groups. Biol J Linn Soc 100:531–537

Matsuura K, Tanaka C, Nishida T (2000) Symbiosis of a termite and a sclerotium-forming fungus: Sclerotia mimic termite eggs. Ecol Res 15:405–414

Matsuura K, Fujimoto M, Goka K, Nishida T (2002) Cooperative colony foundation by termite female pairs: altruism for survivorship in incipient colonies. Anim Behav 64:167–173

Matsuura K, Tamura T, Kobayashi N, Yashiro T, Tatsumi S (2007) The antibacterial protein lysozyme identified as the termite egg recognition pheromone. PLoS One 2:e813

Matsuura K, Yashiro T, Shimizu K, Tatsumi S, Tamura T (2009) Cuckoo fungus mimics termite eggs by producing the cellulose-digesting enzyme beta-glucosidase. Curr Biol 19:30–36

Matsuura K, Himuro C, Yokoi T, Yamamoto Y, Vargo EL, Keller L (2010) Identification of a pheromone regulating caste differentiation in termites. Proc Natl Acad Sci USA 107:12963–12968

Nehring V, Evison SEF, Santorelli LA, d'Ettorre P, Hughes WOH (2011) Kin-informative recognition cues in ants. Proc R Soc B 278:1942–1948

Nojima S, Schal C, Webster FX, Santangelo RG, Roelofs WL (2005) Identification of the sex pheromone of the German cockroach, *Blattella germanica*. Science 307:1104–1106

Poulsen M, Bot AN, Nielsen MG, Boomsma JJ (2002) Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. Behav Ecol Sociobiol 52:151–157

Rosengaus R, Jordan C, Lefebvre M, Traniello J (1999) Pathogen alarm behavior in a termite: a new form of communication in social insects. Naturwissenschaften 86:544–548

Rosengaus RB, Lefebvre ML, Traniello JF (2000) Inhibition of fungal spore germination by *Nasutitermes*: evidence for a possible antiseptic role of soldier defensive secretions. J Chem Ecol 26:21–39

Rosengaus RB, Traniello JFA, Lefebvre ML, Maxmen AB (2004) Fungistatic activity of the sternal gland secretion of the dampwood termite *Zootermopsis angusticollis*. Insect Soc 51:259–264

Ruther J, Podsiadlowski L, Hilker M (2001) Quinones in cockchafers: additional function of a sex attractant as an antimicrobial agent. Chemoecology 11:225–229

Shimizu S, Yamaji M (2002) Pathogenicity of entomopathogenic fungi to the termite, *Reticulitermes speratus*. Jpn J Appl Entomol Zool 46:89–91

Steiger S, Schmitt T, Schaefer HM (2011) The origin and dynamic evolution of chemical information transfer. Proc R Soc B 278:970–979

Steinmetz I, Schmolz E, Ruther J (2003) Cuticular lipids as trail pheromone in a social wasp. Proc R Soc B 270:385–391

Traniello JF, Rosengaus RB, Savoie K (2002) The development of immunity in a social insect: evidence for the group facilitation of disease resistance. Proc Nat Acad Sci USA 99:6838–6842

Vander Meer RK, Morel L (1995) Ant queens deposit pheromones and antimicrobial agents on eggs. Naturwissenschaften 82:93–95

Vander Meer RK, Breed MD, Winston ML, Espelie KE (1998) Pheromone communication in social insects: ants, wasps, bees, and termites. Westview Press, Boulder

Vega FE, Blackwell M (2005) Insect–fungal associations: ecology and evolution. Oxford University Press, Oxford

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

Wyatt TD (2003) Pheromones and animal behaviour: communication by smell and taste. Cambridge University Press, Cambridge

Yamamoto Y, Matsuura K (2011) Queen pheromone regulates egg production in a termite. Biol Lett 7:727–729

Yamamoto Y, Kobayashi T, Matsuura K (2012) The lack of chiral specificity in a termite queen pheromone. Physiol Entomol 37:192–195

Yanagawa A, Fujiwara-Tsujii N, Akino T, Yoshimura T, Yanagawa T, Shimizu S (2012) Odor aversion and pathogen-removal efficiency in grooming behavior of the termite *Coptotermes formosanus*. PLoS One 7:e47412

Yashiro T, Matsuura K (2007) Distribution and phylogenetic analysis of termite egg-mimicking fungi ''termite balls'' in *Reticulitermes* termites. Ann Entomol Soc Am 100:532–538

Yashiro T, Matsuura K, Tanaka C (2011) Genetic diversity of termite-egg mimicking fungi ''termite balls'' within the nests of termites. Insect Soc 58:57–64