Behavioral Responses of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) to Semiochemicals of Seven Ant Species

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ABSTRACT Bioassays were performed to determine the behavioral responses of *Coptotermes formosanus* Shiraki soldiers to ants or ant extracts. *C. formosanus* responded differently to semiochemicals of different ant species. A significantly greater proportion of termite soldiers responded aggressively to *Pheidole megacephala* (F.) than to other ant species. Termite soldiers were significantly less likely to respond aggressively to *Monomorium floricola* (Jerdon), *Tetramorium simillimum* (F. Smith), and *Ochetellus glaber* (Mayr) than to other ant species. Termites avoided contact with *O. glaber* in 85.3% of trials, but only avoided contact with other ant species in 18% or less of trials. Termite soldiers were significantly more likely to respond to freshly killed ants than to solvent-treated ants of the same species. Termites also responded similarly to freshly killed ants and dead termites treated with ant extracts, indicating that termite responses were mediated, at least in part, by chemical cues. The ability of *C. formosanus* to distinguish among different ant species on the basis of semiochemicals could be an adaptive response that allows them to recognize particularly threatening enemies.

KEY WORDS semiochemicals, *Coptotermes formosanus*, ants

Semiochemicals play an important role in insect societies and influence a wide variety of behaviors such as colony recognition, trail recruitment, control of reproduction and caste determination (Ali & Morgan 1990, Winston 1992). Some termites may use cuticular lipids as species or colony recognition cues (Howard et al. 1982, Haverty & Thorne 1989, Bagneres et al. 1991, Grace 1991). Semiochemicals may also be important in the recognition of potential enemies.

Ants are the greatest insect enemies of termites. Many species of ants prey on termites opportunistically, and several ant genera are specialized termite predators (Deligne et al. 1981, Holldobler & Wilson 1990). Because both ants and termites are extremely abundant in tropical ecosystems, ants could potentially play an important role in limiting the distribution of termites through predation, competition for nesting sites, and disruption of foraging activities. It is likely that termites would respond differently to different ant species because some pose a significant threat, whereas others coexist with termites in a commensalistic or possibly even mutualistic relationship (Higashi & Ito 1989).

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is the most economically important insect pest in the Hawaiian Islands (Yates & Tamashiro 1990). We have observed several species of ants in Hawaii that prey on termites and nest in termite galleries in wood. In this study, we examine the behavioral responses of Formosan subterranean termites, *C. formosanus* to seven ant species and determine if differences in termite responses to different ant species are mediated by chemical cues.

Materials and Methods

Termite and Ant Collections. Formosan subterranean termites were collected on the Manoa campus of the University of Hawaii using a trapping technique described by Tamashiro et al. (1973) in which foraging termites were collected in boxes constructed of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, lumber. A wooden stake was placed in the ground and covered by a wooden box. The box was protected by a 22.5-liter cylindrical metal can, with the bottom removed, which was placed over each box and covered with a metal sheet.

Ants frequently invade our termite collection traps and nest in termite galleries. Ant colonies of the following species were collected from termite traps at several different locations on campus and maintained in the laboratory at ambient conditions (≈25°C) in uncovered plastic boxes (30 by 16 cm): *Anoplolepis longipes* (Jerdon), *Paratrechina longicornis* (Latreille), *Pheidole* *megacephala* (F.), *Technomyrmex albipes* (F. Smith), and *Ochetellus glaber* (Mayr). Two species, *Monomorium floricola* (Jerdon) and *Tetramorium simillimum* (F. Smith), were obtained from laboratory colonies kept in an insectary at the University of Hawaii at Manoa. Ant colonies were generally composed of hundreds of workers, one or more queens, and brood. The sides of the boxes were coated with Fluon (Northern Products, Woonsocket, RI), to prevent ants from escaping. Ants were provided with a constant supply of water from a water-filled 15-dram plastic vial which contained small holes in the sides of the container and was positioned upside down in the ant box so that ants could collect water droplets when needed. Each box also contained a cap from a plastic Falcon test tube (17 by 100 mm) filled with honey. Freshly killed termites were added to boxes for protein as necessary. Termites were killed by heating them in a microwave for 20 s.

Analysis of Ant-Termite Interaction. The behavioral response of termite soldiers to ants or ant extracts was tested by presenting a soldier with a test insect in a petri dish (4.5-cm diameter). The termite soldier was first gently transferred to the petri dish with a wooden stick and allowed to acclimate for 1–2 min. Within 1–2 min, the termite would generally begin to walk around the edge of the dish. The test insect was then placed along the edge of the petri dish on the opposite side from the termite. In tests with living ants, a Fluon-coated plastic dish was used to prevent ants from escaping. In tests using freshly killed ants or extract-treated termites, the glass dish was rinsed with methanol and then water and dried after every trial. For each trial, both a new termite and a new ant were used. Tests were performed using workers of each ant species. Soldiers of *C. formosanus* showed four distinct responses which were rated as follows: Avoidance: soldier avoids contact with the test insect by backing up and going the other way or walking around the test insect without contacting it. No response: soldier shows no response if it contacts the test insect and either walks over or around it without any other positive or negative response. Aggressive: soldier opens its mandibles after making tactile contact with the test insect or soldier attacks the test insect by snapping at it or grabbing it in its mandibles.

For each trial, observations were made on the first two encounters, and the encounter eliciting the highest score was recorded. Thus, termites had to avoid contact with the test insect in both encounters for the interaction to be scored as avoidance and termites had to encounter the test insect twice without eliciting an aggressive response for the interaction to be scored as no response.

Response of Termite Soldiers to Live Ants. Experiments were performed using the workers of *P. longicornis*, *T. simillimum*, and *O. glaber* and both major and minor workers of *P. megacephala*. There were 50 replicates for each experiment.

Response of Termite Soldiers to Freshly Killed Ants. Each ant species was tested using termite soldiers collected from three or four different colonies. There were 50 replicates for each termite colony. Ants used in each experiment were obtained from the same colony.

An experiment was also performed to determine if the response of termite soldiers to freshly killed ants was a chemically-mediated response. Worker ants of *P. megacephala* and *O. glaber* were killed on dry ice and then washed in 1 ml dichloromethane for 15 min and allowed to dry for at least 5 min. The response of termite soldiers to freshly killed ants and solvent-treated ants of the same species was compared. There were 25 replicates for each experiment.

Response of Termite Soldiers to Ant Extracts. Extracts of whole *P. megacephala*, *T. simillimum*, and *O. glaber* workers were made by killing 500 ants on dry ice and then washing them in 1 ml hexane for 15 min. Extracts were applied to a termite worker that had been collected from the same trap as the termite soldiers. Extracts (2 μl) were applied to termite workers, which had been frozen and washed in 1 ml dichloromethane to remove cuticular hydrocarbons, at a concentration of approximately one ant equivalent per termite. The response of termite soldiers to ant solvent extracts was determined by performing bioassays as described above. There were 50 replicates for each experiment. Termite soldiers were collected from a single colony because results of the previous experiment indicated that termites from the four colonies tested responded similarly to these ant species.

Because five of the ant species were found occupying termite galleries in the traps from which termites used in this study were collected, an experiment was performed to determine whether the response of termite soldiers collected in the field was a learned response. Experiments were performed using a laboratory-reared colony of termites that had always been maintained in an ant-free environment. This laboratory colony was established in 1984 from a pair of alates collected in the field. The colony was maintained in the laboratory in a metal can and provided with water and Douglas-fir lumber. Experiments were performed to determine the response of termite soldiers from the laboratory colony to hexane extracts of *P. megacephala* and *O. glaber* applied to solvent-treated termite workers from the same colony. There were 25 replicates for each ant extract.

Statistical Analysis. Percentage of responses of termite soldiers to freshly killed or living ants of different species were transformed by the arcsine of the square root and subjected to analysis

Table 1. Percentage of *C. formosanus* soldiers responding to living ants (50 replicates per ant species)

Ant species	% Termite responses		
	Avoid	No response	Aggressive
O. glaber	2a	44b	54b
T. simillimum	0a	66a	34c
P. longicornis	0a	16c	84a
P. megacephala			
Minor worker	0a	14c	86a
Major worker	0a	2c	98a

Percentages in the same column followed by the same letter are not significantly different ($P > 0.05$).

of variance (ANOVA) and means were separated with Ryan-Einot-Gabriel-Welsch multiple F test (SAS Institute 1987). Termite responses to ant extracts and to freshly killed ants compared with solvent-treated controls were analyzed using a chi-square test (SAS Institute 1987).

Results

Response of Termite Soldiers to Live Ants. There was a greater proportion of aggressive responses by termites to living ants than occurred in our experiments using freshly killed ants with all four ant species. However, significantly fewer termite soldiers responded aggressively to *T. simillimum* and *O. glaber* than to the other two species and termites responded the least aggressively to *T. simillimum* ($F = 22.8$, df = 4, $P = 0.0001$) (Table 1).

Response of Termite Soldiers to Freshly Killed Ants. There were significant differences in termite responses to freshly killed ants of different species. Termites avoided contact with *O. glaber* in 85.3% of trials but only avoided contact with the other ants species in 18% or less of trials ($F = 136.1$, df = 9, $P = 0.0001$) (Table 2). Termite soldiers were significantly less likely to respond aggressively to *M. floricola*, *T. simillimum*, and *O. glaber* than the other ant species ($F = 30.2$, df = 9, $P = 0.0001$). Termite soldiers did not respond to *M. floricola* and *T. simillimum* in 84 and 87% of trials, respectively (Table 2). Termite soldiers responded aggressively to *P. megacephala* significantly more often than other species (63% of trials) ($F = 30.2$, df = 9, $P = 0.0001$).

Termite soldiers were significantly more likely to attack freshly killed *P. megacephala* ants than dichloromethane-treated ants ($\chi^2 = 8.57$, $P = 0.003$) and they were more likely to avoid freshly killed *O. glaber* ants than dichloromethane-treated ants ($\chi^2 = 11.36$, $P = 0.001$), suggesting that these behaviors are mediated by chemical cues.

Table 2. Percentage of *C. formosanus* responding to freshly killed ant species

Ant species	n	% Termite responses		
		Avoid	No response	Aggressive
O. glaber	150	85.3a	8.6d	6.0c
T. simillimum	150	0.0c	87.3a	12.6c
P. longicornis	150	1.3c	55.3b	43.3b
P. megacephala	200	0.5c	36.5c	63.0a
M. floricola	200	5.0c	84.0a	11.0c
T. albipes	200	18.0b	44.5bc	37.5b
A. longipes	200	16.0b	42.0c	42.0b

Percentages in the same column followed by the same letter are not significantly different ($P > 0.05$).

Response of Termite Soldiers to Ant Extracts. Termite soldiers responded aggressively to termite workers treated with *P. megacephala* extract in 48% of trials but responded aggressively to termites treated with extracts of *T. simillimum* in only 8% of trials ($\chi^2 = 19.84$, $P < 0.0001$). However, termite soldiers responded more aggressively to freshly killed ants than to termites treated with extracts for all three ant species. Termite soldiers avoided contact with *O. glaber* extract-treated termites in all 50 trials, whereas they avoided contact with *P. megacephala* or *T. simillimum* extract-treated termites in only 18 or 0% of trials, respectively. Soldiers responded to *O. glaber* extract from a distance of ≈1 cm, indicating that they were repelled by a volatile fraction.

Field collected and laboratory-reared termite soldiers responded in a similar manner to *O. glaber* and *P. megacephala* extracts. Laboratory-reared termite soldiers responded aggressively to termite workers treated with *P. megacephala* extract in 52% of the trials, indicating that previous exposure to *P. megacephala* is not necessary to elicit an aggressive response. Both field collected and laboratory-reared termite soldiers avoided contact with termite workers treated with *O. glaber* extract in all trials.

Discussion

Coptotermes formosanus responded differently to semiochemicals of different ant species. Termite soldiers were significantly more likely to respond to freshly killed ants than to dichloromethane-treated ants of the same species. Termites also responded similarly to freshly killed ants and extract-treated termites, indicating that termite responses were mediated, at least in part, by chemical cues. However, tactile cues may also be involved because termite soldiers responded more aggressively to freshly killed ants than the respective extracts for all three species.

Termite soldiers responded the most aggressively to *P. megacephala*. Our preliminary observations indicate that *P. megacephala* is particularly aggressive in occupying termite galleries in our termite traps that are set up at various locations on the island of Oahu. The high level of aggression exhibited by *C. formosanus* toward *P. megacephala* could be an adaptive response to a particularly threatening enemy. Although interactions among *C. formosanus* and these ant species in Hawaii are the results of recent introductions rather than long-term interactions between sympatric species, it is possible that the termites have been selected to respond to cues shared by congeneric species. *Pheidole* is a widespread genus that contains several species that are known to prey on termites (Holldobler & Wilson 1990). Termites showed no response toward freshly killed *M. floricola* and *T. simillimum* in 84 and 87.3% of trials, respectively. Neither of these two species has been observed occupying termite galleries on Oahu. However, both species could potentially prey on termites in the field.

Coptotermes formosanus avoided contact with freshly killed *O. glaber* and with *O. glaber* extract-treated termites. Our previous research has shown that termites will not penetrate *O. glaber* extract-treated sand barriers for at least 10 d (Cornelius & Grace 1994) and that extract-treated sand is toxic to termites when they are continuously exposed to it for 24 h (unpublished data). The identities of the active components are currently under investigation.

Chemical interference by ants is important in competition among ant species. Ant defensive compounds are used to repel other ant species from food sources (Andersen et al. 1991) and have been used effectively to disrupt the foraging behavior of other ant species (Shorey et al. 1992, 1993). *O. glaber* is a member of the subfamily Dolichoderinae (Shattuck 1992), which uses anal gland secretions as both an alarm pheromone for nestmates and as a repellent to other ant species (Cavill et al. 1982, Scheffrahn et al. 1984). These secretions also contain terpenoids, which are known to have insecticidal activity (Cavill & Houghton 1974, Cavill et al. 1976, Cavill et al. 1982).

In contrast, termite soldiers did not appear to avoid encounters with living *O. glaber* in one-on-one encounters. In these encounters, ants generally attempted to avoid confrontation and escape. We observed that termite soldiers responded aggressively to movement by ants, presumably caused by substrate vibration or air currents. In many cases, *O. glaber* remained motionless when approached by a termite soldier and by this strategy might have escaped detection. Because *O. glaber* did not engage in combat with termites in our experiment, it is possible that these individuals did not secrete the chemical components responsible for eliciting the repellent behavior in termites. When large groups of ants are introduced into arenas with foraging termites, we have observed that termite soldiers are repelled by *O. glaber* (unpublished data).

Experience could play a role in determining termite behavior toward different ant species, but we did not find any evidence of this in our study. Termite soldiers from a laboratory colony that had no exposure to ants for 8 yr had a response similar to that observed in field collected soldiers, which strongly suggests that the response to *Pheidole* and *Ochetellus* is not learned.

The interaction between ants and termites has influenced the evolution of both insect groups. For example, it is generally thought that the defensive secretions of termite soldiers evolved primarily as deterrents against ants (Deligne et al. 1981). The ability of *C. formosanus* to distinguish among different ant species on the basis of semiochemicals could be an adaptive response that allows them to recognize particularly threatening enemies. We are continuing research to assess the impact of introduced ant species in Hawaii on termite colonies and to determine which species pose the greatest threat to termite colonies.

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References Cited

Ali, M. F. & E. D. Morgan. 1990. Chemical communication in insect communities: a guide to insect pheromones with special emphasis on social insects. Biol. Rev. 65: 227–247.

Andersen, A. N., M. S. Blum & T. H. Jones. 1991. Venom alkaloids in *Monomorium "rothsteini"* Forel repel other ants: is this the secret to success by *Monomorium* in Australian ant communities? Oecologia (Berl.) 88: 157–160.

Bagneres, A., A. Killian, J. Clement & C. Lange. 1991. Interspecific recognition among termites of the genus *Reticulitermes*: evidence for a role for the cuticular hydrocarbons. J. Chem. Ecol. 17: 2397–2420.

Cavill, G.W.K. & E. Houghton. 1974. Volatile constituents of the Argentine ant, *Iridomyrmex humilis*. J. Insect Physiol. 20: 2049–2059.

Cavill, G.W.K., E. Houghton, F. J. McDonald & P. J. Williams. 1976. Isolation and characterization of dolichodial and related compounds from the Argentine ant *Iridomyrmex humilis*. Insect Biochem. 6: 483–490.

Cavill, G.W.K., P. L. Robertson, J. J. Brophy, D. V. Clark, R. Duke, C. J. Orton & W. D. Plant. 1982. Defensive and other secretions of the Australian cocktail ant, *Iridomyrmex nitidiceps*. Tetrahedron Lett. 38: 1931–1938.

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

Deligne, J., A. Quennedey & M. S. Blum. 1981. The enemies and defense mechanisms of termites, pp. 1–76. *In* H. R. Hermann [ed.] Social insects, vol. 2. Academic, NY.

Grace, J. K. 1991. Semiochemical mediation and manipulation of *Reticulitermes* behavior (Isoptera: Rhinotermitdae). Sociobiology 19: 147–162.

Haverty, M. I. & B. L. Thorne. 1989. Agonistic behavior correlated with hydrocarbon phenotypes in dampwood termites, *Zootermopsis* (Isoptera: Termopsidae). J. Insect Behav. 2: 523–543.

Higashi, S. & F. Ito. 1989. Defense of termitaria by termitophilous ants. Oecologia (Berl.) 80: 145–147.

Holldobler, B. & E. O. Wilson. 1990. The ants. Harvard University Press, Cambridge, MA.

Howard, R. W., C. A. McDaniel, D. R. Nelson, G. J. Blomquist, L. T. Gelbaum & L. H. Zalkow. 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* and their role as potential species and caste-recognition cues. J. Chem. Ecol. 8: 1227–1239.

SAS Institute. 1987. SAS/STAT guide for personal computers, version 6th ed. SAS Institute, Cary, NC.

Scheffrahn, R. H., L. K. Gaston, J. J. Sims & M. K. Rust. 1984. Defensive ecology of *Forelius foetidus* and its chemosystematic relationship to *F. (=Iridomyrmex) pruinosus* (Hymenoptera: Formicidae: Dolichoderinae). Environ. Entomol. 13: 1502–1506.

Shattuck, S. O. 1992. Generic revision of the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). Sociobiology 21: 1–121.

Shorey, H. H., L. K. Gaston, R. G. Gerber, P. A. Phillips & D. L. Wood. 1992. Disruption of foraging by Argentine ants, *Iridomyrmex humilis* (Mayr) (Hymenoptera: Formicidae), in citrus trees through the use of semiochemicals and related chemicals. J. Chem. Ecol. 18: 2131–2142.

Shorey, H. H., L. K. Gaston, R. G. Gerber, C. B. Sisk & D. L. Wood. 1993. Disruption of foraging by *Formica aerata* (Hymenoptera: Formicidae) through the use of semiochemicals and related chemicals. Environ. Entomol. 22: 920–924.

Tamashiro, M., J. K. Fujii & P.-Y. Lai. 1973. A simple method to observe, trap, and prepare large numbers of subterranean termites for laboratory and field experiments. Environ. Entomol. 2: 721–722.

Winston, M. L. 1992. Semiochemicals and insect sociality, pp. 315–333. *In* B. D. Roitberg & M. B. Isman [eds]. Insect chemical ecology: an evolutionary approach. Chapman & Hall, NY.

Yates, J. R. & M. Tamashiro. 1990. The Formosan subterranean termite in Hawaii. Hawaii Institute of Tropical Agriculture and Human Resources Research Extension Series 117, University of Hawaii, Honolulu.

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