*Journal of Insect Behavior, Vol. 17, No. 4, July 2004 (© 2004)*

*Short Communication*

# Lack of Agonism in an Insular Caribbean Termite, *Nasutitermes acajutlae*

**Claire A. Fuller,[1,4] Punidan D. Jeyasingh,[1,2] and Leslie W. Harris[3]**

*Accepted March 17, 2004; revised May 19, 2004*

**KEY WORDS:** *Nasutitermes*; termite; alarm; agonism; nest-mate recognition; Caribbean.

## INTRODUCTION

The theory of kin selection predicts that social animals should be able to discriminate between kin and nonkin as well as among various levels of relatedness (Hamilton, 1964). Among eusocial insects, kin recognition is important in maintaining colony integrity (Fletcher and Michener, 1987). In addition, it can allow individuals to discriminate levels of relatedness among nestmates. The ability to recognize kin has been found in a variety of social insects, including bees, ants, wasps, and termites (e.g., Fletcher and Michener, 1987; Clément and Bagnéres, 1998).

Laboratory assays of agonism in small arenas are frequently used as an indicator of kin-recognition among social insects (Vander Meer *et al.*, 1998) including many species of higher (Family Termitidae) and lower (all other termite families) termites (reviewed by Thorne and Haverty, 1991; Shelton and Grace, 1996; Clément and Bagnéres, 1998). Most termites examined show pronounced aggression, including chasing, insertion of mandibles, and biting by soldiers and workers (Thorne, 1982; Binder, 1988; Su and Haverty, 1991; Thorne and Haverty, 1991; Shelton and Grace, 1996; Clément and Bagnéres, 1998). Agonism can result in immediate death or removal of appendages such as antennae and legs (Clément, 1986). Levels of agonism can vary

[1]Department of Biology, Murray State University, 334 Blackburn Hall, Murray, Kentucky 42071.
[2]Current address: Department of Zoology, University of Oklahoma, Norman, Oklahoma.
[3]Department of Biology, University of Texas, El Paso, Texas.
[4]To whom corrrespondence should be addressed. e-mail: claire.fuller@murraystate.edu.



0892-7553/04/0700-0523/0 

among species (Thorne and Haverty, 1991), among colonies (e.g., Levings and Adams, 1984; Jmhasly and Leuthold, 1999a), and by season (Clément, 1986). However, only a few populations of lower termites have shown a complete lack of agonism toward nonnestmates (e.g., *Coptotermes formosanus*: Su and Haverty, 1991; *Reticulitermes flavipes*: Grace, 1996; Bulmer and Traniello, 2002). The higher termites tested thus far have all shown some ability to discriminate nestmates from nonnestmates; most members of the genus *Nasutitermes* show high levels of aggression (Thorne and Haverty, 1991; LePonce *et al.*, 1996).

In this study, we examined both behavior and survival to test the hypothesis that a Caribbean higher termite, *N. acajutlae* recognizes nestmates. Studies on the mechanism of nestmate recognition suggest that both environmental (e.g., Matsuura, 2001) and genetic (Adams, 1991; Haverty *et al.*, 1999; Husseneder and Grace, 2001) factors play a role. Thus, we predicted that, if recognition occurs in *N. acajutlae*, it would increase with geographic distance from focal nests because differences in both environmental factors and genetic structure should increase with distance.

## MATERIALS AND METHODS

This study took place on the island of St. John, US Virgin Islands. St. John is a small (49.2 km$^2$), steep (max. elevation of 370 m) island, of which 2/3 is in the Virgin Islands National Park. *Nasutitermes acajutlae* nests occur in all areas of the island, and in a variety of habitats including mangrove, wetlands, sparse vegetation, and moist and dry forests (habitats described by Gibney *et al.*, 2000).

Termites used in this study were collected from nests in four geographic areas of St. John: the east end, the north shore, the south side, and the interior of the island (Fig. 1). Trials were conducted in July of 2001 and 2002. In 2001, agonistic behavior and survival rates for a focal nest were compared by pairing termites from the focal nest with termites belonging to four nests: (1) their own nest (Self = control), (2) with termites from the same geographic area, but that were from a neighboring nest, thus were assumed to interact in the field (NN = presumably familiar and known to be nearest neighbors in all but two cases), (3) with termites from the same geographic area but separated by at least two nests, thus we assumed that they did not interact in the field (WA = unfamiliar, but presumable similar in terms of diet and genetics), and (4) with termites from a different geographic area (DA = unfamiliar and presumably different with respect to diet and genetics).

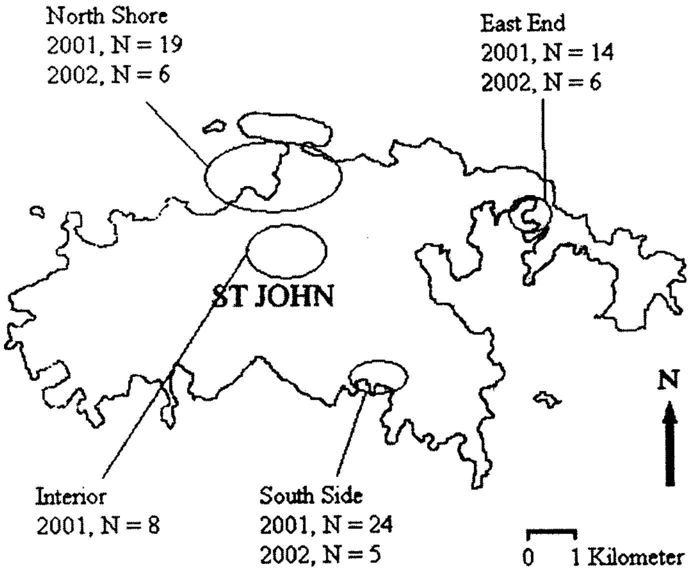


**Fig. 1.** Map of St. John, US Virgin Islands. Collection areas and the number of colonies used in behavioral trials from each area in 2001 and 2002 are indicated.

We conducted 54 trials in 2001 with 18 focal colonies and an additional 47 colonies in NN, WA, and DA treatments. In 2002, we tested the possibility that our original arenas were too small to induce agonism (see Polizzi and Forschler, 1998), thus used a similar design to that in 2001 but the trials were conducted in larger arenas (see below). We used a subset of the original colonies: we excluded the WA treatment, used nine focal colonies and an additional nine colonies in the NN (all known to be nearest neighbors) and DA treatments. All encounters in both years were between unique combinations of colonies and no individual termite was used more than once. Distances between nests were measured using a tape measure or GIS coordinates. Range of distance between colonies is shown in Table I.

Termites were collected between 14:00 and 16:00 hours by opening 20 cm of foraging galleries 1–2 m below nest cartons and brushing emerging termites into plastic containers. Termites were sorted by colony immediately before behavioral trials; trials were conducted from 19:00 to 22:00 hours. At sorting, five workers and five soldiers from a colony were placed together in a 6-cm (2001) or 10-cm (2002) Petri dish. We chose a 1:1 caste ratio because both workers and soldiers participate in defense and actual ratios vary widely (personal observation, see also Thorne, 1982). Once all termites from a focal

**Table I.** The Range of Distances Between Nests, Mean Alarm Behavior (Jittering), and Survival Exhibited by Workers and Soldiers of *N. acajutlae*

| | Self (control) | Neighboring nest | Within area | Different area |
|---|---|---|---|---|
| 2001 ($N = 18$, each treatment) | | | | |
| Range of distances | 0 | 5.5–87 | 40.7–782 | 1302–3885 |
| Worker jittering[a] | 3.67 + 0.76 | 3.22 + 1.03 | 4.17 + 0.96 | 4.44 + 1.02 |
| Soldier jittering | 10.00 + 1.71 | 11.17 + 1.50 | 12.61 + 1.73 | 10.72 + 1.62 |
| Worker survival[b] | 9.83 + 0.09 | 9.72 + 0.13 | 9.56 + 0.20 | 9.94 + 0.06 |
| Soldier survival | 9.67 + 0.17 | 9.83 + 0.09 | 9.89 + 0.08 | 9.83 + 0.09 |
| 2002 ($N = 9$, each treatment) | | | | |
| Range of distances | 0 | 9.2–67 | N/a | 741–2936 |
| Worker jittering | 2.56 + 0.69 | 3 + 1.76 | N/a | 2.78 + 1.40 |
| Soldier jittering | 4.89 + 1.22 | 7.56 + 2.65 | N/a | 4.56 + 0.93 |
| Worker survival | 9.78 + 0.15 | 9.89 + 0.11 | N/a | 9.78 + 0.15 |
| Soldier survival | 9.78 + 0.15 | 9.89 + 0.11 | N/a | 9.44 + 0.24 |

[a]Jittering is the mean (+SE) number exhibited by all workers or all soldiers per trial.
[b]Survival represents the mean (+SE) number of workers or soldiers alive out of 10, after a 10–12 h observation period.

nest and its comparison nests were sorted into dishes, trials for that focal group were conducted.

To start trials, one author put the contents of two Petri dishes into a third dish (6 cm in 2001; 10 cm in 2002) that was lined by moistened absorbent paper. A naïve observer recorded agonistic behaviors of workers and soldiers every 15 s for 5 min. After the observation period, Petri dishes were placed in a lidded plastic container and left 10–12 h. At the end of this period, the number of active and apparently healthy, moribund, and dead termites was recorded. Dead and moribund termites were pooled for statistical analysis.

### Statistical Analysis

The only alarm or agonistic behavior observed in any trials was jittering (*sensu* Traniello and Beshers, 1985). The total number of times animals were observed jittering in 5 min was summed for each comparison (Self, NN, WA, DA) for each focal nest to obtain total jittering per trial. Means and standard errors for each treatment were calculated using total jitters per trial. Total jittering among Self, NN, WA, and DA were compared using a repeated measures ANOVA. This analysis allowed us to compare all trials in which a focal nest was involved, thus taking nest differences into account. We used paired $t$-tests between the focal nest and other nests as a post hoc test if the ANOVA resulted in significant differences. Separate analyses were performed on workers and soldiers. We also summed all jittering performed by workers and by soldiers across all treatments from each colony. We compared the total number of behaviors performed by workers and soldiers by paired $t$-test, pairing within nests. Analyses for survival were as above for jittering except that we used the total number of healthy animals after 10–12 h.

## RESULTS

### Jittering

There were no significant differences among treatments in the number of times workers or soldiers jittered among treatments in 2001 (workers: $F = 0.386$; df $= 3, 51$; $P = 0.8$; soldiers: $F = 0.644$, df $= 3, 51$, $P = 0.6$) or 2002 (workers: $F = 0.023$, df $= 2, 16$, $P = 0.98$; soldiers: $F = 0.841$, df $= 2, 16$, $P = 0.45$) (Table I). However, the soldiers jittered significantly more often than workers in both years (2001: paired $t$-test = 7.82, df $= 17$, $P < 0.001$; 2002: paired $t$-test = 2.296, df = 8, $P = 0.05$; Fig. 2A).

### Survival

In 2001, survival ranged between 70 and 100% and there were no differences across treatment groups for either workers or soldiers (workers: $F = 1.46$, df $= 3, 51$, $P = 0.23$; soldiers: $F = 0.739$, df $= 3, 51$, $P = 0.5$; Table I). There was too little variation among worker treatments in 2002 to run a statistical analysis (Table I). Although there were significant differences in overall soldier survival in 2002 ($F = 4.522$, $P = 0.028$), soldiers in the DA treatment did not differ significantly from the Self treatment (paired $t$-test = 2.0, $P = 0.081$) (Table I). There were no differences between worker and soldier survival in either year (2001: paired $t$-test = 0.489, df = 17, $P = 0.63$; 2002: paired $t$-test = 0.667, df = 8, $P = 0.5$; Fig. 2B).

## DISCUSSION

We found no evidence of agonism among colonies of *N. acajutlae* from St. John. We never observed chasing or biting by either caste or spraying of defensive compounds by soldiers. The only alarm behavior we observed was jittering. Although soldiers jittered more often than workers in both years of our study, there were no differences in the number of jitters observed among control and treatment categories. Thus, *N. acajutlae* increased alarm behavior in response to being placed in Petri dishes rather than to the presence of nonnestmates. We only found significant differences in survival in one caste in 1 year of the study: the overall ANOVA for soldiers in 2002 was significant. However, the difference does not seem to be related to agonism:

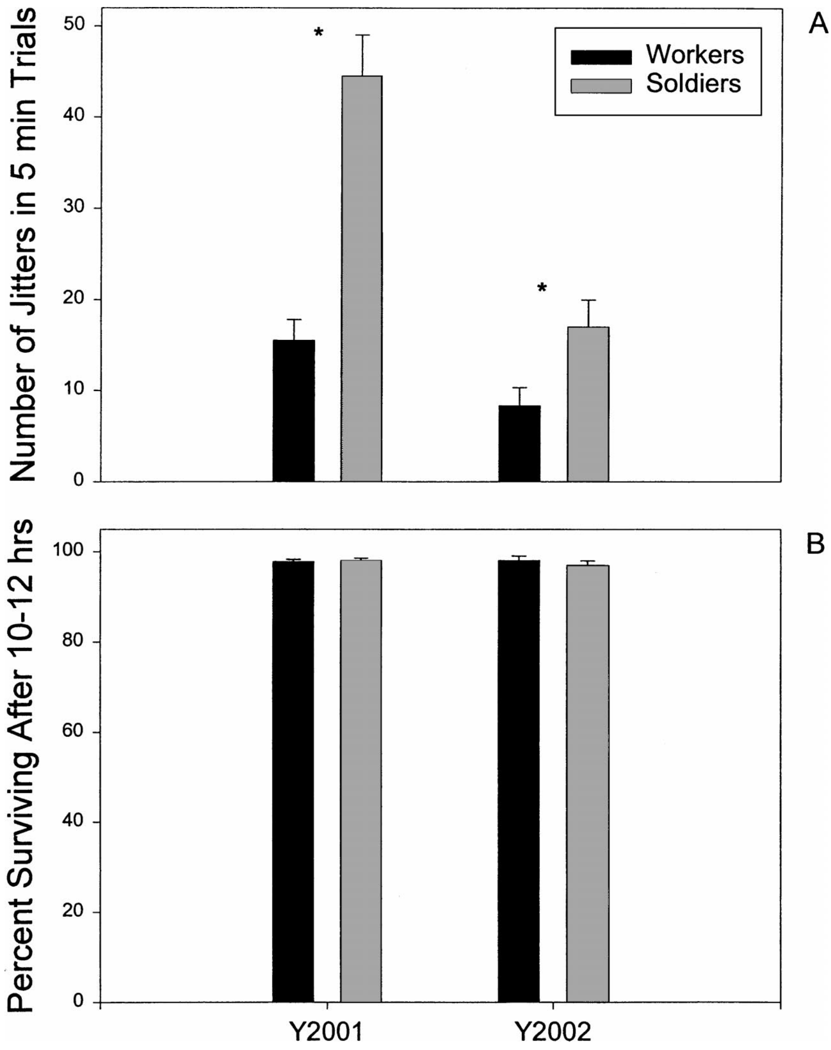


**Fig. 2.** Total jittering (A) and percent survival (B) for all workers and all soldiers, summed across treatments each year. Soldiers jittered significantly more often than workers in both years. There were no significant differences in survival between workers and soldiers in either year. $N = 18$ focal nests in 2001; $N = 9$ focal nests in 2002. $* = P < 0.05$.

survival of DA soldiers was not significantly lower than controls and we never observed any wounded termites. Finally, it is possible that termites from the two different colonies in each Petri dish separated themselves spatially, thus avoiding encounters (*sensu* Bulmer and Traniello, 2002). While we did not measure spacing, we did observe that all termites were found together in a clump after 10–12 h, with no apparent grouping.

All other higher termites examined thus far have been found to discriminate between nestmates and nonnestmates. Most species, especially nasutes, exhibit high levels of aggression, often resulting in death and dismemberment (Thorne and Havery, 1991; LePonce *et al.*, 1996; Jmhasly and Leuthold, 1999b; Kaib *et al.*, 2002). *Macrotermes bellicosus* did not exhibit aggression but did show differences in examination behaviors between nestmates and nonnestmates (Jmhasly and Leuthold, 1999b), thus demonstrating an ability to discriminate. Many of these studies used assays very similar to ours. Thus, the observed behavioral responses of *N. acajutlae* to conspecifics on St. John seem to be highly unusual among higher termites. It is currently unclear whether all *N. acajutlae* show low aggression, or whether this phenomenon is specific to St. John and/or other small islands.

Lower termites also discriminate between nestmates and nonnestmates in most studies (Thorne and Haverty, 1991; Shelton and Grace, 1996; Getty *et al.*, 2000). However, exceptions include reduced aggression in *Reticulitermes* during the non-swarming season (Clément, 1986) or when merging of colonies could benefit both colonies (Matsuura and Nishida, 2001). Neither of these mechanisms seems likely in our study. Late summer is the swarming season for *N. acajutlae* on St. John and 85 and 89% of trials in 2001 and 2002, respectively, involved colonies that had nymphs or alates at the time of testing (determined by sampling the nest carton with a 2.5-cm diameter soil corer). Colony merging may be a rare event in *N. acajutlae* because they build foraging trails to new food sources, rather than move the entire colony to a new (perhaps occupied) site. Colonies probably move when a nest is destroyed or severely damaged (Thorne and Haverty, 2000), but this seems to be a rare occurrence (CAF, personal observation of $> 200$ marked colonies in 4 years).

Lack of recognition could be due to a lack of cues. If cues are primarily environmental (e.g., food source: Shelton and Grace, 1997; or intestinal bacteria: Matsuura, 2001), colonies with similar environments should give off similar cues. However, our test colonies came from four geographic areas separated by $> 700$ m. Moreover, each area consisted of different habitats and, generally, different tree species within habitats. Therefore, it seems likely that colony-specific odors should differ enough for recognition to be possible. If cues are primarily genetically based, the ability to discriminate should be correlated with the diversity of genetically determined cues (e.g., Su and Haverty, 1991; Grace, 1996; Haverty *et al.*, 1999). Lack of recognition in some introduced social insects has been attributed to genetic bottlenecks during introduction (e.g., Su and Haverty, 1991; Tsutsai *et al.*, 2000). Harris (2002) showed that colonies can be differentiated genetically, but that between-colony variability in *N. acajutlae* on St. John only accounted for 8% of the total population variability, while the other 92% was from within colonies. Thus, there may not be enough genetic variability among populations on St. John for discrimination to occur. Although *N. acajutlae* is probably native to our study sites, it may have undergone successive genetic bottlenecks during its colonization of the Caribbean islands (Thorne *et al.*, 1994); tropical storms and hurricanes could also produce bottlenecks if most colonies subsequently die and recolonization is by survivors. On the other hand, storms could actually facilitate gene flow and increase genetic homogeneity (e.g., Calsbeek and Smith, 2003).

Alternatively, *N. acajutlae* may discriminate between nestmates and nonnestmates without exhibiting aggression or alarm. Lack of aggression could benefit colonies under certain circumstances. Kaib *et al.*, (2002) showed a neighbor-stranger effect in which neighbors exhibited less aggression toward each other than nonneighboring colonies. However, because neighboring colonies in our study were no more or less aggressive toward each other nonneighboring colonies, low aggression in *N. acajutlae* does not seem to fit the "dear-enemy" hypothesis (Temeles, 1994). Agonistic behaviors may not result in significant net gain when resources are abundant (Holway *et al.*, 1998). The volume of available dead wood on St. John was approximately 10-fold greater (Jeyasingh and Fuller, in press) than that reported by Eggleton *et al.*, (1996) from southern Cameroon. Although hurricanes and tropical storms may damage nests, they may also provide a constant source of food on St. John, thus *N. acajutlae* may benefit by avoiding conflict and investing more energy in foraging.

## ACKNOWLEDGMENTS

We thank B. L. Thorne and two anonymous reviewers for critically reviewing the manuscript. Special thanks goes to S. El-Amine, H. Ivins, K. Phillips, and A. Smith, as well as A. M. and N. M. Sikkel for their hard work. T. Turner and K. Brown provided logistical support. We thank the Virgin Islands Environmental Resource Station (VIERS) for use of facilities and glassware. Funding was provided by the Biology Departments at UTEP, and MSU, NSF (Grant #9973712), and Earthwatch/Durfee Foundation grants to CAF. This research was conducted under US Park Service Permit# VIIS-2001-SCI-001 and VIIS-2002-SCI-009.

## REFERENCES

Adams, E. S. (1991). Nestmate recognition based on heritable odors in the termite *Microtermes arboreus*. *Proc. Natl. Acad. Sci.* 88: U.S.A. 2031–2034.

Binder, B. F. (1988). Intercolonial aggression in the subterranean termite *Heterotermes aureus* (Isoptera: Rhinotermitidae). *Psyche* **95:** 123–137.

Bulmer, M. S., and Traniello, J. F. T. (2002). Lack of aggression and spatial association of colony members in *Reticulitermes flavipes*. *J. Insect Behav*. **15:** 121–126.

Calsbeek, R., and Smith, T. B. (2003). Ocean currents mediate evolution in island lizards. *Nature* **426:** 552–555.

Clément, J. L. (1986). Open and closed societies in *Reticulitermes* termites (Isoptera, Rhinotermitidae): Geographic and seasonal variations. *Sociobiology* **11:** 311–323.

Clément, J. L., and Bagnéres, A. G. (1998). Nestmate recognition in termites. In Vander Meer, R. K., Breed, M. D., Espelie, K. E., and Winston, M. L. (eds.), *Pheromone Communication in Social Insects*, Westview Press, Boulder, CO, pp. 126–155.

Eggleton, P., Bignell, D. E., Sands, W. A., Mawdsley, N. A., Lawton, J. H., Wood, T. G., and Bignell, N. C. (1996). The diversity, abundance and biomass of termites under differing levels of disturbance in the Mbalmayo Forest Reserve, southern Camaroon. *Philos. Trans. R. Soc. Lond*. *B* **351:** 51–68.

Fletcher, D. J. C., and Michener, C. D. (eds.) (1987). Kin Recognition in Animals, Wiley, New York.

Getty, G. M., Haverty, M. I., and Lewis, V. R. (2000). Agonistic behavior between recently collected and laboratory cultured Reticulitermes spp. (Isoptera: Rhinotermitidae) from northern California. *Pan-Pacific Entomol.* **76:** 243–250.

Gibney, E., Thomas, T., O'Reilly, R., and Devine, B. (2000). *The U.S. Virgin Islands Vegetation Classification System*, Conservation Data Center Publication, Virgin Islands, 24 pp.

Grace, J. K. (1996). Absence of overt agonistic behavior in a northern population of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiology* **28:** 103–110.

Hamilton, W. D. (1964). The genetical evolution of social behavior. I. *J. Theor. Bio.* **7:** 1–16.

Harris, L. W. (2002). Should I stay or should I go? Dispersal in the termite *Nasutitermes acajutlae*, Unpublished MS Thesis, University of Texas, El Paso, TX.

Haverty, M. I., Copren, K. A., Getty, G. M., and Lewis, V. R. (1999). Agonistic behavior and cuticular hydrocarbon phenotypes of colonies of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California. *Ann. Entomol. Soc. Am*. **92:** 269–277.

Holway, D. A., Suarez, A. V., and Case, T. J. (1998). Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science* **282:** 949–952.

Husseneder, C., and Grace, J. K. (2001). Evaluation of DNA fingerprinting, aggression tests, and morphometry as tools for colony delineation of the Formosan subterranean termite. *J. Insect Behav*. **14:** 173–186.

Jeyasingh, P. D., and Fuller, C. A. (in press). Habitat-specific life-history variation in the Caribbean termite *Nasutitermes acajutlae* (Isoptera: Termitidae). *Ecol. Entomol*.

Jmhasly, P., and Leuthold, R. H. (1999a). The system of underground passages in *Macrotermes subhyalinus* and comparison of laboratory bioassays to field evidence of intraspecific encounters in *M. subhyalinus* and *M. bellicosus* (Isoptera: Termitidae). *Insectes Sociaux* **46:** 332–334.

Jmhasly, P., and Leuthold, R. H. (1999b). Intraspecific colony recognition in the termites *Macrotermes subhyalinus* and *M. bellicosus* (Isoptera: Termitidae). *Insectes Sociaux* **46:** 164–170.

Kaib, M., Franke, S., Francke, W., and Brandl, R. (2002). Cuticular hydrocarbons in a termite: Phenotypes and a neighbour-stranger effect. *Physiol. Entomol.* **27:** 189–198.

LePonce, M., Roisin, Y., and Pasteels, J. M. (1996). Intraspecific interactions in a community of arboreal nesting termites (Isoptera: Termitidae). *J. Insect Behav.* **9:** 799–817.

Levings, S. C., and Adams, E. S. (1984). Intra- and interspecific territoriality in *Nasutitermes* (Isoptera: Termitidae) in a Panamanian mangrove forest. *J. Anim. Ecol.* **53:** 705–714.

Matsuura, K. (2001). Nestmate recognition mediated by intestinal bacteria in a termite, *Reticulitermes speratus. Oikos* **92:** 20–26.

Matsuura, K., and Nishida, T. (2001). Colony fusion in a termite: What makes the society "open"? *Insectes sociaux* **48:** 378–383.

Polizzi, J. M., and Forschler, B. T. (1998). Intra- and interspecific agonism in *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) and effects of arena and group size in laboratory assays. *Insectes Sociaux* **45:** 43–49.

Shelton, T. G., and Grace, J. K. (1996). Review of agonistic behaviors in the Isoptera. *Sociobiology* **28:** 155–176.

Shelton, T. G., and Grace, J. K. (1997). Suggestion of an environmental influence on intercolony agonism of Formosan subterranean termites (Isoptera: Rhinotermitidae). *Environ. Entomol.* **26:** 632–637.

Su, N. Y., and Haverty, M. I. (1991). Agonistic behavior among colonies of the Formosan subterranean termite, *Coptotermes formosanus* from Florida and Hawaii: Lack of correlation with cuticular hydrocarbon composition. *J. Insect Behav*. **4:** 115–128.

Temeles, E. J. (1994). The role of neighbors in territorial systems: When are they "dear enemies"? *Anim. Behav.* **47:** 339–350.

Thorne, B. L. (1982). Termite–termite interactions: Workers as an agonistic caste. *Psyche* **89:** 133–150.

Thorne, B. L., and Haverty, M. I. (1991). A review of intracolony, intraspecific, and interspecific agonism in termites. *Sociobiology* **19:** 115–145.

Thorne, B. L., and Haverty, M. I. (2000). Nest growth and survivorship in three species of neotropical *Nasutitermes* (Isoptera: Termitidae). *Environ. Entomol.* **29:** 256–264.

Thorne, B. L., Haverty, M. I., and Collins, M. S. (1994). Taxonomy and biogeography of *Nasutitermes acajutlae* and *N. nigriceps* (Isoptera: Termitidae) in the Caribbean and Central America. *Ann Entomol Soc Am.* **87:** 762–770.

Traniello, J. F. A., and Beshers, S. N. (1985). Species-specific alarm/recruitment responses in a neotropical termite. *Naturwissenschaften* **72:** 491–492.

Tsutsai, N. D., Suarez, A. V., Holway, D. A., and Case, T. J. (2000). Reduced genetic variation and the success of an invasive species. *Proc. Nat. Acad. Sci. U.S.A.* **97:** 5948–5953.

Vander Meer, R. K., Breed, M. D., Espelie, K. E., and Winston, M. L. (1998). *Pheromone Communication in Social Insects*, Westview Press, Boulder, CO, 352 pp.