Absence of Overt Agonistic Behavior in a Northern Population of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

by

J. Kenneth Grace[1]

ABSTRACT

Groups of termites from two each of four *Reticulitermes flavipes* colonies in the Metropolitan Toronto region were paired in a 7-day bioassay intended to simulate a field situation where two colonies nesting in discrete locations encounter each other while foraging upon the same resource. Colony members were marked with a dye, and both mortality of each colony group and the distribution of colony members within the bioassay apparatus was evaluated. Although one colony appeared to generally suffer greater mortality than the other three, there was no significant differential mortality between colonies in each pairing, nor differences in the distribution of individuals within the bioassay apparatus that would indicate agonistic interactions among these four colonies. These results provide evidence for a lack of agonistic response among at least this northern population of *R. flavipes*, although studies are needed with colonies from other parts of North America in order to demonstrate whether an absence of aggression is characteristic of North American *R. flavipes* in general, or only of particular geographic populations.

INTRODUCTION

In social insects, agonistic behavior refers to the social interactions of aggression and responses to aggression that occur when insects of different species, different colonies, or even different individuals within a single colony, encounter each other. In North America, there has been little study of such interactions in termites, with the notable exceptions of *Zootermopsis* spp. (Haverty & Thorne 1989, Thorne & Haverty 1991a), *Heterotermes* spp. (Binder 1988, Jones 1993), and *Coptotermes formosanus* Shiraki (Su & Scheffrahn 1988, Su & Haverty 1991, Shelton 1996, Shelton & Grace 1996a & 1996b). Even in these species, the mechanisms underlying agonistic responses have not yet

[1]Department of Entomology, University of Hawaii, 3050 Maile Way, Honolulu, HI 96822-2271, USA.

been elucidated, and the responses observed in bioassays are frequently highly variable.

Reticulitermes Holmgren is the most broadly distributed genus of subterranean termites in North America. Yet, research on their agonistic behavior has lagged behind research on the genera mentioned above. Thorne & Haverty (1991a) commented that intraspecific mixtures of *Reticulitermes* spp. were generally docile, but this was apparently based upon unpublished observations. In an extensive review of the topic, these authors described Pickens' (1934) record of attack on incipient colonies by established *R. hesperus* Banks colonies, and observed that "little is known about patterns of agonism in *Reticulitermes*" (Thorne & Haverty 1991b).

The present study was initiated to attempt to document patterns of agonism among *Reticulitermes flavipes* (Kollar) colonies near the northern limits of the species' distribution, in Ontario, Canada. *Reticulitermes flavipes* appears to have been introduced to the province of Ontario from the United States at least twice, first to the southernmost Point Pelee in the 1920s (Kirby 1965) and later to Toronto in the mid-1930s (Urquhart 1953). In comparison to *R. flavipes* colonies further south, where the species is endemic, northern termite colonies are characterized by reduced occurrence of alate flights (Esenther 1969), large foraging areas and populations (Esenther 1980, Grace *et al.* 1989, Grace 1990), and some unique foraging behaviors such as building shelter tubing on trees (Cooper & Grace 1987). Colonies may also be characterized by a high degree of relatedness, although the possibility of repeated termite introductions to Ontario cannot be excluded, and the species as a whole may have a very limited amount of genetic variation (Reilly 1987).

European *Reticulitermes* spp. have demonstrated both interspecific and intraspecific aggressive behavior (Clément 1978), as well as geographic and seasonal variation in the degree of intraspecific aggression of individual colonies (Clément 1986). Thus, although unpublished observations may indicate a lack of aggression among North American *Reticulitermes* spp. (Thorne & Haverty 1991a), the few published studies with this genus (Clément 1978 & 1986, Pickens' 1934) suggest that agonistic interactions are not at all uncommon. It was hoped that an investigation of *R. flavipes* colonies in Toronto might lend further support to either one viewpoint or the other, as well as possibly leading to additional study of mechanisms of colony recognition, and information on termite behavior of use in control efforts.

MATERIALS AND METHODS

Foraging termites were collected from four locations in Metropolitan Toronto, using the collection traps (cardboard rolls in short lengths of plastic pipe) described by Grace (1989). Two of these sites, Birchmont and Warden, were located in the City of Scarborough; the third, D'Arcy, was located in downtown Toronto; and the fourth, Sumach, was located in the Regent Park area of Toronto. After collection, the termites were kept for approximately 3 months in plastic boxes in an unlighted incubator (27±0.5°C, 90±5% RH). The bioassays were performed under these same conditions in a similar unlighted incubator.

The bioassay was intended to test what Shelton (1996) recently termed "chronic" agonism, or agonistic interactions resulting in elevated termite mortality. The bioassay apparatus was somewhat similar to the design of Binder (1988), and intended to simulate a field situation in which two termite colonies nesting in discrete locations encounter each other while foraging upon the same resource. This apparatus consisted of three small polystyrene vials (60 by 35 mm dia.) connected in series by short lengths of Tygon tubing (Fig. 1). Each vial contained 15g of silica sand, dampened with 3ml distilled water. A folded filter paper was placed in each of the two end (or "nest") vials as a source of food, and a ca. 2g pine block was placed in the center ("foraging") vial.

Termites from each of the four colonies were counted into groups of 40 (39 workers, or pseudergates, plus 1 soldier). To discriminate

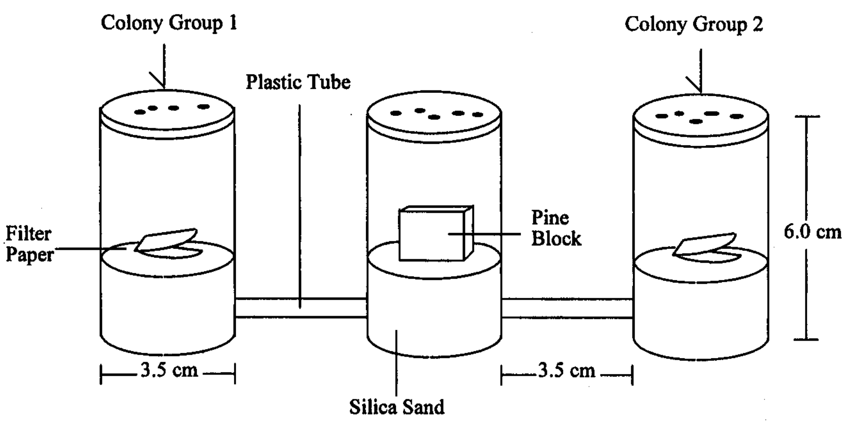

Fig. 1. Bioassay for studying agonistic interactions of two groups of termites from different colonies. Termites (one group dyed with Sudan Red 7B) were placed in vials at each end of the apparatus and their distribution and mortality were evaluated after seven days.

among colony members in the bioassays, half of the groups from each colony were marked by feeding for five days on Whatman No. 1 filter paper impregnated with a 2% (weight/weight) concentration of the dye Fat (=Sudan) Red 7B (Grace & Abdallay 1989). Each pairing of two different colonies was replicated six times, three times with the members of one colony dyed and three with the members of the other colony dyed, in order to block for any effects of the dye-marker on termite behavior. All reciprocal pairings (6) of the four colonies were performed.

In each bioassay, 40 dyed individuals from one colony were placed in one of the terminal nest vials, and 40 undyed individuals from a different colony were placed in the nest vial at the opposite end of the bioassay apparatus (Fig. 1). After a 7-day incubation period, the apparatus was carefully dismantled and the numbers of dyed and undyed individuals in each of the three vials recorded (connecting tubes were considered part of the respective end vial). Termite colony mortalities were compared within each pairing by a paired comparisons *t* test (SAS Institute 1987); and total termite mortality among the treatments was subjected to analysis of variance of proportional mortality transformed by the arcsine of the square root, with means significantly different at the 0.05 level separated by Duncan's multiple range test (SAS Institute 1987).

RESULTS AND DISCUSSION

Results of the 7-day bioassay offer little evidence of agonistic behavior among these four *R. flavipes* colonies. Although mortality differed among the colony pairings, in no case did the mortality of one colony in the pairing exceed the mortality of the other colony at the 0.05 level of significance (Table 1). There was a trend towards greater mortality of the Warden colony when paired with each of the others. However, the fairly equal distribution of members of both colonies throughout the bioassay apparatus strongly suggests that this elevated mortality is the result of an intrinsic condition of the termites from the Warden colony, rather than a reflection of attack upon them by the other colonies.

Although Clément (1978 & 1986) observed that agonism declined over time in *Reticulitermes* colonies kept in the laboratory, as did Shelton & Grace (1996) with laboratory-reared *C. formosanus*, none of the colonies used in these assays had been kept in the laboratory longer than 3 months, and all were collected in the fall, a season when Clément (1986) suggested that European *Reticulitermes* colonies were "closed," or antagonistic towards one another. Thus, the absence of

Table 1. Mean percentage termite mortality and the final average number of termites present in each compartment of a 3-vial bioassay apparatus in which 2 colonies were paired for 7 days. The 3 vials were connected serially with a nest vial on either end in which groups (39 workers + 1 soldier) from colony 1 and colony 2 were placed, and a center foraging vial containing wood.

		Mean Number of Termites Present (Colony 1 : Colony 2)			Mean Percentage Mortality (±SD)		
Colony 1	Colony 2	Nest Vial 1	Center Vial	Nest Vial 2	Colony 1*	Colony 2*	Total**
D'Arcy	Warden	10:7	17:23	7:7	6.67±5.40	12.50±9.22	9.58±4.45a
D'Arcy	Sumach	10:11	17:17	11:7	6.25±6.85	9.58±4.85	7.92±2.46a
D'Arcy	Birchmont	10:8	13:13	10:10	19.17±11.69	21.67±6.26	20.42±5.90b
Birchmont	Sumach	6:5	18:21	7:5	22.08±12.39	16.67±12.52	19.38±5.05b
Birchmont	Warden	7:5	11:5	14:15	18.33±9.17	36.67±14.63	27.50±6.57c
Warden	Sumach	6:5	4:7	13:22	41.25±23.39	14.17±14.11	27.71±5.89c

*Difference in mortality between the paired colonies was in no case significant at the 0.05 level, although the Birchmont-Warden pairing was significant at the 0.10 level (p=0.08, paired comparisons *t* test).
**Total bioassay mortalities followed by the same letter do not differ significantly at the 0.05 level (ANOVA, Duncan's multiple range test).

overt evidence of agonism in this study cannot be attributed to long-term laboratory maintenance nor to the time of collection.

The population sizes of these four colonies, although different, are certainly well in the range of "mature" *R. flavipes* colonies. Within the year prior to this study, the Birchmont colony was estimated by mark-release-recapture methods to have a foraging population of 3 million

(Grace *et al.* 1989), the D'Arcy colony was estimated at 2 million (Grace *et al.* 1989), and the Warden colony at 0.7 million (Grace 1990). The Sumach colony was not characterized, but the occurrence of termite activity and damages in two adjacent buildings over a period of several years suggested that a relatively large colony was present. This indicates that the reduced agonism noted in *C. formosanus* colonies in southern Florida by Su & Scheffrahn (1988) and attributed by those authors to the recent introduction of young (small) termite colonies to a new geographic area would be an unlikely explanation of the present results. On the other hand, reduced agonism among northern *R. flavipes* colonies certainly could be at least partially attributable to a high degree of relatedness resulting from few new introductions and frequent creation of new colonies by "budding" from existing colonies.

Low agonism among *R. flavipes* colonies in Toronto has implications for termite control, since it raises the possibility that a large number of termites from a single colony might be captured at one locale in traps such as those described by Grace (1989), treated with a dust or a topical toxicant, and then released at multiple locations to spread the toxicant into entirely different colonies. This could simplify the collection and treatment phases of the trap-treat-release methods of subterranean termite control suggested by Grace & Abdallay (1990), Myles & Grace (1991) and Myles *et al.* (1994).

I look forward to publication by other researchers of results of agonistic assays with *R. flavipes* colonies from other geographic regions. More comprehensive studies are certainly needed in order to demonstrate whether an absence of aggression is truly characteristic of North American *Reticulitermes* in general, or only of particular geographic populations.

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REFERENCES

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

Clément, J.-L. 1978. L'agression interspécifique et intraspécifique des espèces françaises du genre *Reticulitermes* (Isoptera). C.R. Acad. Sci. Paris 286: 351-354.

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

Cooper, P.A. & J.K. Grace. 1987. Association of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), with living trees in Canada. J. Entomol. Sci. 22: 353-354.

Esenther, G.R. 1969. Termites in Wisconsin. Ann. Entomol. Soc. Am. 62: 1274-1284.

Esenther, G.R. 1980. Estimating the size of subterranean termite colonies by a release-recapture technique. Internat. Res. Group on Wood Preserv. Doc. IRG/WP/112. 4 pp.

Grace, J.K. 1989. A modified trap technique for monitoring *Reticulitermes* subterranean termites (Isoptera: Rhinotermitidae). Pan-Pac. Entomol. 65: 381-384.

Grace, J.K. 1990. Mark-recapture studies with *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 16: 297-303.

Grace, J.K. & A. Abdallay. 1989. Evaluation of the dye marker Sudan Red 7B with *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 15: 71-77.

Grace, J.K. & A. Abdallay. 1990. Termiticidal activity of boron dusts (Isoptera, Rhinotermitidae). J. Appl. Entomol. 109: 283-288.

Grace, J.K., A. Abdallay & K.R. Farr. 1989. Eastern subterranean termite (Isoptera: Rhinotermitidae) foraging territories and populations in Toronto. Can. Entomol. 121: 551-556.

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

Jones, S.C. 1993. Field observations of intercolony aggression and territory changes in *Heterotermes aureus* (Isoptera: Rhinotermitidae). J. Ins. Behav. 6: 225-236.

Kirby, C.S. 1965. The distribution of termites in Ontario after 25 years. Can. Entomol. 97: 310-314.

Myles, T.G., A. Abdallay & J. Sisson. 1994. 21st century termite control. Pest Control Technol. 22(March): 64-72, 108.

Myles, T.G. & J.K. Grace. 1991. Behavioral ecology of the eastern subterranean termite in Ontario as a basis for control. Proc.Technol. Transfer Conf., Vol. II, The Multi-Media Approach: Integrated Environmental Protection. Ontario Ministry of the Environment, Toronto. pp. 547-554.

Pickens, A.L. 1934. The biology and economic significance of the western subterranean termite *Reticulitermes hesperus*. In: Termites and Termite Control (C.A. Kofoid, ed.). Univ. Calif. Press, Berkeley, Calif. pp. 157-183.

Reilly, L.M. 1987. Measurements of inbreeding and average relatedness in a termite population. Am. Naturalist 130: 339-349.

Shelton, T.G. 1996. Factors affecting colony recognition in *Coptotermes* *formosanus* Shiraki (Isopter: Rhinotermitidae). M.S. thesis, Univ. Hawaii at Manoa, Honolulu.

Shelton, T.G & J.K. Grace. 1996a. Evidence of an environmental influence on intercolonial agonism in *Coptotermes formosanus* Shiraki. Environ. Entomol., in review.

Shelton, T.G & J.K. Grace. 1996b. Impact of low temperature conditioning on intercolonial agonism in *Coptotermes formosanus* Shiraki. J. Ins. Behav., in review.

Su, N.-Y. & R.H. Scheffrahn. 1988. Intra- and interspecific competition of the Formosan and the Eastern subterranean termite: evidence from field observations (Isoptera: Rhinotermitidae). Sociobiology 14: 157-164.

Thorne, B.L. & M.I. Haverty. 1991a. A review of intracolony, intraspecific, and interspecific agonism in termites. Sociobiology 19: 115-145.

Thorne, B.L. & M.I. Haverty. 1991b. An assessment of the potential uses of agonistic behaviors in termite control. In: Proc. Symposium on Current Research on Wood-destroying Organisms and Future Prospects for Protecting Wood in Use. USDA Gen. Tech. Rep. PSW-128. pgs 24-27.

Urquhart, F.A. 1953. The introduction of the termite into Ontario. Can. Entomol. 85: 292-293.