# Impact of Low Temperature Conditioning on Intercolonial Agonism in *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Reduction of agonism between non-nestmates (both groups and individuals) of *Coptotermes formosanus* Shiraki was demonstrated for a duration of 4-24 hrs after low temperature (3±1°C for 1 hr) conditioning. Doubling the length of the conditioning period did not extend the duration of the effect to 72 hrs. Investigations of acute agonism between conditioned and unconditioned termite soldiers suggested that one or more recognition cues are temporarily suppressed in the chilled termites, reducing aggression from unconditioned non-nestmates. This mechanism would be consistent with possible temporary suppression of a glandular secretion in the conditioned termite.

## INTRODUCTION

Intraspecific agonistic behavior has been observed in many subterranean termites, but the mechanism by which non-nestmates of some species recognize each other is unknown (Thorne & Haverty 1991; Su & Haverty 1991; Shelton & Grace 1996). In particular, the kin recognition system of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, has not been completely described (Su & Haverty 1991; Shelton & Grace 1996). Physiological or behavioral manipulations of termites can provide useful information on social insect communication (Andrews 1911; Dropkin 1946; Springhetti & Sapigni 1984, 1990), and a reduction in aggression has been observed in termite species conditioned by exposure to low temperature (Dropkin 1946; Howick & Creffield 1980; Springhetti & Sapigni 1984, 1990). The following study focuses on a particular manipulation of intercolonial agonistic behavior after low temperature conditioning of *C. formosanus*.

Dropkin (1946) examined the transference of host-specific gut symbionts between various species of kalotermitids after exposing the termites to low temperatures. Dropkin (1946) was able to maintain

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mixed colonies consisting of two *Kalotermes* spp., as well as *Kalotermes* spp. mixed with a *Neotermes* sp. or a *Zootermopsis* sp. (Termopsidae) without visible agonism after exposing the termites to low temperatures. This study demonstrated that movement and survival of gut symbionts between different species of kalotermitids is possible in the absence of agonistic behavior. Dropkin (1946) concluded that this ease of transmission of gut fauna suggested yet another purpose for aggression in Isoptera: to prevent trophallactic contact between termites of separate species, thereby inhibiting the movement of gut fauna from one species to the next.

Springhetti and Sapigni (1984, 1990) demonstrated not only interspecific but interfamily trophallaxis following a reduction in interspecific aggression by exposure of *Reticulitermes lucifugus* (Rossi) (Rhinotermitidae) and *Kalotermes flavicollis* (Fabricius) (Kalotermitidae) to low temperatures. The authors concluded that the cues releasing trophallactic behavior must be similar in both species for this to occur (Springhetti & Sapigni 1990).

In work with *Coptotermes acinaciformis* (Froggatt) in Australia, Howick and Creffield (1980) observed a reduction in aggressive behavior between non-nestmates following a low temperature manipulation. An incubation regime of 3°C for a period of two hours enabled the formation of intercolonial mixed groups of *C. acinaciformis*. Although the chilled termites were capable of remaining in non-nestmate groupings for short periods of time, only 3 of 16 groups survived for 2 weeks, and only 2 of these 3 groups survived for 24 days. Non-nestmate groupings were also found to consume significantly less wood than groups composed solely of nestmates. The surviving groups were composed of all possible two-colony combinations from 3 original colonies, ruling out the possibility that survival of these few groups resulted from a lack of agonism between specific parent colonies.

The present study represents an examination of low temperature conditioning in *C. formosanus* and the impact of low temperature conditioning on response to non-nestmates.

We hypothesized two possible mechanisms for a reduction in agonistic behavior from low temperature conditioning that could be observable in bioassays mixing groups of chilled and unchilled termites. The first possibility is that a glandular secretion used in recognition may cease as a result of chilling, or some other "colony odor" may be eliminated or suppressed. Agonism, then, would be predicted to occur only when low temperature conditioned termites recognized and attacked unchilled (ambient) non-nestmates. A second possibility is that low temperature conditioned termites are unable to recognize other termites as non-

nestmates due to an effect on their sensory receptors (e.g., chemoreceptors) or other aspects of the neurological system involved in kin recognition. Under this hypothesis, agonism would only occur when low temperature conditioned termites are recognized and attacked by unchilled non-nestmates.

In the first part of our study, we used termite mortality as an indicator of chronic agonism between paired groups of termites. However, chronic agonism studies alone cannot provide the information necessary to discriminate between the two possible mechanisms hypothesized above. Rather, an indication of which termite, low temperature or ambient conditioned, is first to recognize the other is necessary to differentiate between these possibilities. Therefore, we also used acute agonism, or observed battle, as the means of determining agonism between paired opponents.

## MATERIALS AND METHODS

### Part I: Chronic Agonism Studies

**Termite collection and bioassay conditions.** Previous investigations performed on the University of Hawaii at Manoa campus, and elsewhere on Oahu, documented the location of discrete colonies of Formosan subterranean termites (Lai 1977; Su *et al.* 1984; Shelton & Grace 1997). For several years, foraging territories of these *C. formosanus* colonies have been regularly delineated using mark-release-recapture techniques based upon a modified Lincoln index method (Begon 1979). We designated these discrete colonies by the letters A, B, C, D, E, F, G, H, and I (Shelton & Grace 1997).

Experiments in this study were designed to test the effects of low temperature on intercolonial agonism, using previously established agonism patterns for Oahu termite colonies (Shelton & Grace 1997), as well as the patterns reported by Su and Haverty (1991), as a guide. *C. formosanus* foragers were collected on the Manoa campus from aggregation traps as described by Tamashiro *et al.* (1973) immediately prior to their use in behavioral assays. Foragers were counted and placed into labeled petri dishes (9.0 cm x 2.0 cm i.d., Pyrex USA), separated by colony, prior to temperature conditioning. After conditioning, the groups were paired in labeled petri dishes that contained a single filter paper disk (Whatman #2, 90 mm diameter, Whatman International Ltd., Springfield Mill, England) moistened with 1.0 ml distilled water. Dishes with termites were incubated in the dark at 28±1°C for 4 hr.

**Agonism 4 hrs after conditioning (trial 1).** Termites from the mutually agonistic D and G colonies were selected for examining the

effects of low temperature conditioning on agonism four hours after the end of the conditioning period. Experimental units consisted of groups of 10 termites from each colony, 9 workers plus 1 soldier, to approximate foraging caste proportions for *C. formosanus* (Haverty 1977). The termites were conditioned at 3±1°C or at 24°C (ambient temperature) for 1 hr and then paired for 4 hr as described above. Positive agonistic treatments, where both non-nestmate groups were ambient temperature conditioned (i.e., unchilled), were included.

Termite mortality, inclusive of any moribund and ataxic individuals, was assessed following the 4 hr time period. Proportional mortality data were transformed by the arcsine of the square root and subjected to ANOVA (SAS Institute 1987). Means significantly different at the 0.05 level were separated by the Ryan-Einot-Gabriel-Welsch Multiple F Test (SAS Institute 1987).

**Agonism 4 hr after conditioning (trial 2).** A second examination of agonism 4 hr after conditioning was performed using termites stained with the fat-soluble dye Sudan Red 7B to enable discrimination of surviving termites. In this study, pairings of low temperature conditioned termites (LTC) with ambient temperature conditioned (ATC) nestmates were included to examine which termites (LTC or ATC) survived these encounters.

Termites were collected from aggregation traps at colonies I and C, which represented an agonistic combination. Termites from each colony were split into 2 groups, and incubated at 28°C in petri dishes lined with either 2 stained or unstained filter papers (Whatman #2), each moistened with 1 ml distilled water. The filter papers were stained with 1% (wt/wt) Sudan Red 7B dye in acetone, as described by Su and Scheffrahn (1988).

After 7 days incubation, termites were removed and counted into groups of 10 nestmates (9 workers and 1 soldier). These groups were randomly allocated to either 1 hr ambient ( 24°C) or low temperature (3±1°C) conditioning, then paired (stained groups vs. unstained groups) and incubated as described for trial 1.

Termite mortality was assessed following the 4 hr time period. Combined proportional mortality data were transformed by the arcsine of the square root and subjected to ANOVA (SAS Institute 1987). Means significantly different at the 0.05 level were separated by the Ryan-Einot-Gabriel-Welsch Multiple F Test (SAS Institute 1987). Stained and unstained median percentage mortalities for each treatment were subjected to a Mann-Whitney procedure ( =0.05) (Minitab Inc. 1994), with a null hypothesis of equivalent mortalities for each, and an alternative hypothesis of unequal mortality.

**Agonism 24 hr after conditioning.** Based upon the results of the 4 hr experiments, a second bioassay examined agonism 24 hr after low temperature conditioning using agonistic colonies B and G. Treatments were included in which only one of the two termite groups were low temperature conditioned to examine how temperature conditioning affects agonism with unchilled nestmates and non-nestmates. Since different colonies might react differently to low-temperature conditioning, reciprocals of each one-sided treatment were included. Termites were collected from the B and G colonies, and temperature-conditioned as previously described for the 4 hr experiments. Total termite mortality in each replicate was assessed after post-conditioning incubation at 28°C for 24 hrs. Proportional mortality data were analyzed as previously described.

**Agonism 72 hr after conditioning (trial 1).** In this experiment, the time period for assessing mortality due to agonistic interactions was extended to 72 hr after low temperature conditioning. A different bioassay was designed to accommodate the extended time period of this experiment.

Three 15 dram plastic vials, (3.5 cm wide and 6.0 cm tall, Fisher Scientific, Pittsburgh, PA), were connected in series with short pieces of glass tubing (3.5 cm long, 0.5 cm i.d.) through holes drilled near the base of each vial (Fig. 1). A 4.0 cm long strip of corrugated cardboard (0.4 cm wide) moistened with 0.2 ml distilled water was inserted into each glass tube to facilitate termites moving between vials. Twenty grams of washed and oven-dried silica sand (Fisher Scientific, Pittsburgh, PA) moistened with 2.5 ml of distilled water was added to each of the three vials. A 1.5 cm length of hardwood tongue depressor (2 cm

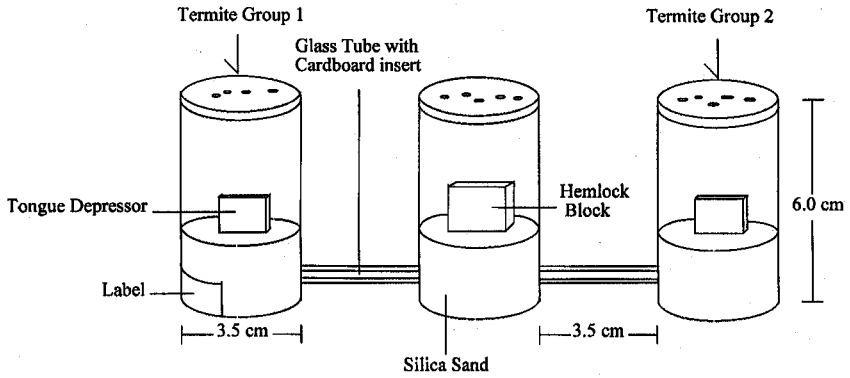


Fig. 1. Bioassay for assessing *Coptotermes formosanus* agonism 72 hr after low temperature conditioning.

wide; Fisher Scientific, Pittsburgh, PA) was added to each of the two vials on either end of the setup as initial food sources. A single western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) block (2 x 2 x 2 cm) was added to the center vial. Each vial was capped with a plastic lid containing small holes made with an insect pin.

In this experiment, one group of 100 worker termites in each replicate was marked with Sudan Red 7B dye in order to establish the colony identity of the survivors. Soldiers were not used since this dye is not passed through trophallaxis to any extent, and does not stain soldiers reliably (Su *et al.* 1983). Termites from colonies B and G were collected from the field and. placed in an incubator at 28°C in petri dishes lined with dyed or undyed moist filter paper, as described for the second 4 hr trial.

Following the 7-day incubation period, groups of stained and unstained termite workers were separated into units of 100 workers, and each unit was subjected to either ambient or low-temperature conditioning for 1 hr. Each group of termites was added to an individual vial at opposite ends of the experimental unit (Fig. 1). Each replicate contained both stained and unstained workers, to allow discrimination between groups. The experimental units were held in an unlighted incubator at 28°C for a period of 72 hr, after which the numbers of live stained and unstained workers, not including moribund or injured individuals, in each vial were counted.

Proportional mortality data were analyzed as previously described. For treatments in which 1 group was low temperature conditioned and 1 was not, mortality data for unstained and stained termites were compared using the Mann-Whitney test (Minitab Inc., 1994), with the null hypothesis of equivalent proportional mortality.

**Agonism 72 hr after conditioning (trial 2).** To ensure that results did not reflect the effects of a unique interaction between those two specific colonies, the 72 hr post-conditioning experiment was repeated with termite workers from colonies C and D. Procedures were the same as those previously described with a few exceptions: vials were capped with foam plugs (S/P Dispo Plugs, Baxter Scientific Products, IL), low temperature conditioned controls were not included, a treatment was included to test whether a longer 2 hr low temperature conditioning period would prolong suppression of intercolony agonism, and 3 rather than 5 replicates were used. Proportional mortality data were analyzed as previously described.

**Part II: Acute Agonism Study**

**Termite collection.** Foraging *C. formosanus* termites were collected from Douglas-fir (*Pseudotsuga menziezii* [Mirbel] Franco) aggregation traps (28.5 x 8.5 x 7.5 cm) according to the methods of Tamashiro *et al.* (1973) from colonies C and I, 1 day before initiation of the experiment. Termites were extracted from the traps and placed into plastic boxes (21 x 34 x 9 cm) with plastic lids in an unlit incubator at 28±1°C for the duration of the experiment.

**Termite conditioning and bioassays.** Soldiers were used in these bioassays since their large mouthparts made possible more accurate observations of their behavior than was the case with workers. There were 30 replicates of each treatment, with 10 bioassays per treatment performed each day over a period of 3 days.

Each day, 30 *C. formosanus* soldiers were chosen at random from the boxes containing each colony's foragers. The soldiers were placed into individual uncovered petri dishes (1.5 cm x 5 cm i.d.; Pyrex U.S.A.). Termites were then conditioned either by chilling at 3±1°C (low temperature conditioned, LTC) or exposure to ambient laboratory temperature ( 24°C; ambient conditioned, ATC) for 1 hr. After conditioning, termites were placed in individual uncovered petri dishes (1.5 cm x 5 cm i.d.) containing a single piece of Whatman #2 filter paper wet with 0.4 ml of distilled water. Termites were then placed in an unlit incubator at 28±1°C for 4 hr.

Soldiers from each of the 2 temperature regimes were paired in a clean petri dish (Pyrex 1.5 cm x 5 cm i.d.) arenas, and observed for 3 minutes. Since responses only occurred when the soldiers were in close proximity, 1 individual (identified by conditioning treatment) was followed during observations to maintain the treatment identity of the soldiers. The number of bites delivered by each termite toward the other was recorded. The first termite soldier to bite the other was recorded as the first to attack. Preliminary studies suggested that no biting occurred between nestmate soldier pairs. Controls consisted of pairings of ATC non-nestmates.

**Data analysis.** Some replicates were not included in data analysis due to either handling mortality of an individual or lack of termite encounter in the trial. First attack data were summarized, and replicates where no biting occurred were listed in the "no response" category. Numbers of bites between colony I and colony C soldiers in each treatment were compared using a Mann-Whitney test (Minitab Inc. 1994). Colony I and colony C responses were log (x+1) transformed and separately subjected to ANOVA, with means significantly different at the 0.05 level separated by the Tukey-Kramer Test (Minitab Inc. 1994).

## RESULTS

### Part I: Chronic Agonism Studies

**Agonism 4 hr after conditioning (trial 1).** The mean mortality in the positive agonism treatment was significantly higher than in either the nestmate controls or the low-temperature conditioned non-nestmate treatment for colonies G and D ($P$>F=0.0015; F=8.27; DF=3) (Table 1).

**Agonism 4 hr after conditioning (trial 2).** Total mean mortalities 4 hr after low temperature conditioning of colonies I and C are summarized in Table 2. The mean mortality of the positive agonistic treatment (I (ATC) and C (ATC)) was significantly greater than both of the non-nestmate pairings where one group was LTC and the other ATC ($P$>F=0.0001; F=13.71; DF=5). These 3 treatments had significantly greater mortality than the 2 nestmate-only controls. Mortality in the treatment where both non-nestmate groups were LTC overlapped with that of both the nestmate controls and the LTC/ATC non-nestmate treatments ($P$>F=0.0001; F=13.71; DF=5) (Table 2).

Analyses of the mortalities of the conditioned and ambient termites in each treatment resulted in only 1 significant difference between each pair (Table 2). In the positive agonistic treatment, there was a significant difference ($P$=0.0122) between mortality in colony I (ATC) (30% median) and colony C (ATC) (100% median). Mann-Whitney tests could not be performed with the 2 nestmate ambient controls since in each case either the stained or the unstained mortality sets contained only zeroes (Table 2).

**Agonism 24 hr after conditioning.** The mean mortality 24 hr after low temperature conditioning for colonies B and G is summarized in Table 3. The only significant difference occurred when LTC colony B termites were paired with ATC colony G termites ($P$>F =0.004; F=3.22; DF=9). Mean mortalities in the reciprocal cross of ATC colony B termites and LTC colony G termites, the colony B nestmate ATC control, and the positive agonistic treatment overlapped other treatments (Table 3).

**Agonism 72 hr after conditioning (trial 1).** Results of the 72 hr post-conditioning trial with colonies B and G were analyzed for overall termite mortality (Table 4) and separately for stained and unstained termites in the non-nestmate treatments where 1 termite group was low temperature conditioned. Mortality in same-colony control pairings (both ATC and LTC) was significantly lower than that in all other treatments ($P$>F= 0.0001; F=42.78; DF=7). However, mortality from pairing colony B (LTC) with colony G (LTC) did not significantly differ from that in the positive agonistic treatment of B (ATC) and G (ATC) ($P$>F= 0.0001; F=42.78; DF=7). Pairing ambient colony B termites with colony G (LTC) termites,

Table 1. Mean percentage mortality (± standard deviation) of paired *C. formosanus* colonies (G vs D) in agonism bioassays 4 hr after low temperature conditioning (trial 1).

| Paired Colonies[a] | Mean % Mortality ± SD[b] |
|---|---|
| G (LTC) and D (LTC) | 4.00±4.18b |
| G (LTC) and G (LTC) | 0.00±0.000b |
| D (LTC) and D (LTC) | 2.00±2.74b |
| G (ATC) and D (ATC) | 39.00±34.89a |

[a]Letters D and G refer to colony labels; LTC = Low Temperature Conditioned (3°C for 1 hour), ATC = Ambient Conditioned (24°C for 1 hour); 5 replicates per treatment.
[b]Means followed by the same letter do not differ significantly at the 0.05 level (ANOVA of transformed proportions, Ryan-Einot-Gabriel-Welsch Multiple F Test).

Table 2. Mean total percentage mortality (± standard deviation) of paired *C. formosanus* colonies (I vs C) in agonism bioassays 4 hr after low temperature conditioning (trial 2).

| Paired Colonies[a] | | Overall Mean % | Median Mortality by Group[c] | | |
|---|---|---|---|---|---|
| Stained | Unstained | Mortality ± SD[b] | Stained | Unstained | P value |
| I (LTC) | C (ATC) | 23.00 ± 16.05b | 10.0 | 20.0 | 0.9168 |
| C (LTC) | I (ATC) | 33.00 ± 20.80b | 70.0 | 0.0 | 0.0758 |
| I (ATC) | C (ATC) | 63.00 ± 8.37a | 30.0 | 100.0 | 0.0122 |
| I (ATC) | I (ATC) | 1.00 ± 2.24c | 0.0 | 0.0 | -[d] |
| C (ATC) | C (ATC) | 0.00 ± 0.00c | 0.0 | 0.0 | -[d] |
| I (LTC) | C (LTC) | 14.00 ± 17.82bc | 0.0 | 10.0 | 0.2506 |

[a]Letters I and C refer to colony labels; LTC = Low Temperature Conditioned (3°C for 1 hour); ATC = Ambient Conditioned (24°C for 1 hour); stained groups were dyed with 1% Sudan Red 7B for 7 days; 5 replicates per treatment.
[b]Means followed by the same letter do not differ significantly at the 0.05 level (ANOVA of transformed proportions, Ryan-Einot-Gabriel-Welsch Multiple F Test).
[c]P-values determined by Mann-Whitney tests of $H_0$: Termite mortalities are equal vs. $H_1$: Termite mortalities are not equal.
[d]Mann-Whitney tests could not be performed due to some data sets containing all zeroes.

but not the reciprocal cross, resulted in mortality significantly greater than when the two ambient colonies were paired. When the mortality occurring in each group of stained and unstained termites was analyzed, no significant differences were found between the chilled and unchilled groups. Mean percentage mortality for the control treatments ranged from 15.2 to 31.0% (Table 4), perhaps indicative of low vigor of these colonies.

**Agonism 72 hr after conditioning (trial 2).** In the 72 hr experiment with colonies C and D, mortality in treatments where at least 1 group of non-nestmates was chilled did not differ significantly from that occurring when unchilled termites from the two colonies were mixed (Table 4). A 2 hr chilling period did not result in mortality significantly

Table 3. Mean percentage mortality (± standard deviation) of *C. formosanus* colony pairs (B vs G) in agonism bioassays 24 hr after low temperature conditioning.

| Paired Colonies[a] | | Mean % Mortality ± SD[b] |
|---|---|---|
| B (LTC) | G (ATC) | 35.00 ± 23.18a |
| B (ATC) | G (LTC) | 25.00 ± 35.36ab |
| B (LTC) | B (LTC) | 2.00 ± 2.74b |
| G (LTC) | G (LTC) | 0.00 ± 0.00b |
| B (ATC) | G (ATC) | 18.00 ± 24.14ab |
| B (LTC) | G (LTC) | 2.00 ± 2.74b |
| B (ATC) | B (ATC) | 3.00 ± 2.74ab |
| G (ATC) | G (ATC) | 2.00 ± 2.74b |
| B (LTC) | B (ATC) | 0.00 ± 0.00b |
| G (LTC) | G (ATC) | 0.00 ± 0.00b |

[a]Letters B and G refer to colony labels; LTC = Low Temperature Conditioned (3°C for 1 hour), ATC = Ambient Conditioned (24°C for 1 hour); 5 replicates per treatment.
[b]Means followed by the same letter do not differ significantly at the 0.05 level (ANOVA of transformed proportions, Ryan-Einot-Gabriel-Welsch Multiple F Test).

Table 4. Mean percentage mortality (± standard deviation) of paired *C. formosanus* colonies (100 workers/colony; B vs G, C vs D) in agonism bioassays 72 hr after low temperature conditioning (trials 1 and 2).

| Paired Colonies[a] | | |
|---|---|---|
| Stained | Unstained | Mean % Mortality ± SD[b] |
| B (LTC) | G (ATC) | 89.20 ± 14.65ab |
| B (ATC) | G (LTC) | 98.00 ± 1.58a |
| G (LTC) | B (LTC) | 90.60 ± 6.03ab |
| G (ATC) | B (ATC) | 75.20 ± 16.65b |
| B (LTC) | B (LTC) | 15.20 ± 1.92c |
| B (ATC) | B (ATC) | 24.80 ± 15.50c |
| G (LTC) | G (LTC) | 15.00 ± 2.83c |
| G (ATC) | G (ATC) | 31.00 ± 14.09c |
| C (LTC) | D (LTC) | 80.67 ± 18.47a |
| D (ATC) | C (ATC) | 47.17 ± 36.25abc |
| C (LTC) | D (ATC) | 72.50 ± 14.26a |
| D (LTC) | C (ATC) | 66.67 ± 27.91ab |
| C (ATC) | C (ATC) | 8.17 ± 3.79bc |
| D (ATC) | D (ATC) | 3.83 ± 1.26c |
| D (LTC2) | C (LTC2) | 52.17 ± 32.15ab |

[a]Letters B, C, D, and G refer to colony labels; LTC = Low Temperature Conditioned (3°C for 1 hour), ATC = Ambient Conditioned (24°C for 1 hour), LTC2 = Low Temperature Conditioned (3°C for 2 hours); stained groups dyed with 1% Sudan Red 7B for 7 days; 5 replicates per treatment in experiment 1 (B vs. G), and 3 replicates per treatment in experiment 2 (C vs. D).
[b]Horizontal line separates experiments (B vs. G, C vs. D). Means within each experiment followed by the same letter do not differ significantly at the 0.05 level (ANOVA of transformed proportions, Ryan-Einot-Gabriel-Welsch Multiple F Test).

different from treatments where termites were chilled for 1 hr. As in the first 72 hr trial, separate analysis of mortality in the stained and unstained groups did not reveal any statistically significant pattern of mortality in the chilled and unchilled groups.

**Part II: Acute Agonism Study**

Bioassays in which no biting occurred counted for nearly half of the replicates in the ambient non-nestmate treatment and the pairing of colony I (ATC) and colony C (LTC) soldiers (Table 5). The median number of bites exhibited by colony I and colony C soldiers were significantly different in the ambient non-nestmate treatment ($P$=0.0120; Table 6) as well as in the pairing of colony I (LTC) and colony C (ATC) soldiers ($P$<0.0001; Table 6). For colony I soldier mean bite numbers, the ANOVA results indicated an overlap of data between the ambient non-nestmate treatment and both of the other treatments (Table 7). However, the mean number of bites delivered by colony I soldiers in both pairings where 1 soldier is LTC and the other ATC, were significantly different from each other ($P$>F=0.004) (Table 7), with ATC colony I soldiers delivering more mean bites than LTC colony I soldiers. For colony C soldiers, no significant differences in the number of bites delivered were found among any treatments ($P$>F=0.550) (Table 7).

## DISCUSSION

Chilling reduced agonism between non-nestmate groups of *C. formosanus* which had been low temperature conditioned for 1 hr, for a period of 4-24 hr post-conditioning. This confirms the lack of aggression observed by Dropkin (1946) in chilled kalotermitids and by Howick and Creffield (1980) in *C. acinaciformis*. At 24 hr after low temperature conditioning, a depression of agonism between chilled groups of non-nestmates was evident, although this was not statistically significant due to variability in the control assays. One hr of

Table 5. Percentage of first attacks by each conditioned *C. formosanus* soldier in acute agonism bioassays to investigate effects of low temperature conditioning on kin recognition.

| Colony and Conditioning Status[a] | | | % Attacking First | | % Not |
|---|---|---|---|---|---|
| Termite #1 | Termite #2 | N[b] | Termite #1 | Termite #2 | Responding |
| I (LTC) | C (ATC) | 29 | 72.4 | 6.9 | 20.7 |
| I (ATC) | C (ATC) | 27 | 37.0 | 18.5 | 44.5 |
| I (ATC) | C (LTC) | 28 | 28.6 | 24.1 | 47.3 |

[a]Letters I and C refer to colony labels; LTC = Low Temperature Conditioned (3°C for 1 hour), ATC = Ambient Conditioned (24°C for 1 hour).
[b]Replicates with handling mortality or no termite encounters were not included.

Table 6. Median number of responses (bites) exhibited by each *C. formosanus* soldier in each treatment in acute bioassays investigating low temperature conditioning effects on kin recognition.

| Colony and Conditioning Status[a] | | | Median No. of Responses | | |
|---|---|---|---|---|---|
| Termite #1 | Termite #2 | N[b] | Termite #1 | Termite #2 | P value[c] |
| I (LTC) | C (ATC) | 29 | 2.0 | 0.0 | 0.0000 |
| I (ATC) | C (ATC) | 27 | 1.0 | 0.0 | 0.0120 |
| I (ATC) | C (LTC) | 28 | 0.0 | 0.0 | 0.6248 |

[a]Letters I and C refer to colony labels; LTC = Low Temperature Conditioned (3°C for 1 hour), ATC = Ambient Conditioned (24°C for 1 hour).
[b]Replicates with handling mortality or no termite encounters were not included.
[c]Results of Mann-Whitney tests ($H_0$: termite responses are equal, $H_1$: termite responses are not equal).

Table 7. Mean number of responses (± Standard Deviation) of each *C. formosanus* soldier by treatment in acute agonism bioassays investigating low temperature conditioning effects on kin recognition.

| Colony and Conditioning Status[a] | | | Mean No. of Responses (±SD)[b] | |
|---|---|---|---|---|
| Termite #1 | Termite #2 | N[b] | Termite #1 | Termite #2 |
| I (LTC) | C (ATC) | 29 | 2.21±2.11a | 0.28±0.84a |
| I (ATC) | C (ATC) | 27 | 1.37±1.96ab | 0.48±1.37a |
| I (ATC) | C (LTC) | 28 | 0.64±1.06b | 0.46±0.88a |

[a]Letters I and C refer to colony labels; LTC = Low Temperature Conditioned (3°C for 1 hour), ATC = Ambient Conditioned (24°C for 1 hour).
[b]Replicates with handling mortality or no termite encounters were not included in analysis.
[b]Means followed by the same letter in each column are not significantly different at the 0.05 level (Tukey-Kramer Test).

chilling did not suppress agonism 72 hrs after the conditioning period. Doubling the length of the chilling period to 2 hrs did not extend the duration of the effect.

The aggression between non-nestmates when only 1 group had been low temperature conditioned may provide a clue as to whether LTC termites have been rendered unrecognizable by the conditioning treatment, or are themselves unable to recognize other termites as non-nestmates. These two possibilities were examined in the second study of chronic agonism 4 hr after conditioning. However, the only significant difference between the mortality of stained and unstained termites was found in the positive agonistic treatment, which suggests a basic disparity in the degree of agonistic behavior exhibited by the 2 colonies in the study. Colony I suffered significantly less mortality in this treatment than colony C. Colony I also suffered less mortality overall in non-nestmate pairings than colony C, although these differences were

not statistically significant. Interestingly, the LTC-only non-nestmate pairings had some variability in mortality (Table 2). This may indicate that the low temperature effect does not suppress all of the cues responsible for colony identification.

Acute agonism results give further evidence for a disparity in the degree of agonistic behavior exhibited by colonies C and I (Tables 5, 6, 7). In the positive agonistic treatment, the number of bites exhibited by colony I soldiers was significantly greater than that of colony C soldiers (Table 6). Even with this confounding factor, a trend may be seen in both the first attack percentages and the ANOVA results for colony I soldier responses in all treatments (Tables 5 and 7). Comparing within colony performance, the trend suggests that when termite soldiers are low temperature conditioned, they bite more often than when they are ambient conditioned (Table 7). Also, comparing within colonies, it appears that low temperature conditioned soldiers initiate combat more often than their ambient opponents (Table 5). Unfortunately, the overall disparity in agonistic behavior between the 2 colonies overshadows this experiment (Table 7).

These data indicate that the observed effect of low temperature conditioning on non-nestmate recognition in *C. formosanus* soldiers is most likely due to inability of ambient conditioned soldiers to distinguish low temperature conditioned non-nestmates. This supports the our first hypothesis that chilled termites are rendered unrecognizable by the low temperature treatment; although this may result from erasure or suppression of a "colony odor," suppression of a glandular secretion, or perhaps the temporary elimination of some other physical or behavioral factor affecting non-nestmate recognition in *C. formosanus*. The erasure of a "colony odor" seems least likely since the effect of the temperature conditioning is temporary (Table 5). Erasure of a "colony odor" should last until the conditioned termites are exposed to the odor again, i.e., through contact with non-conditioned nestmates, which did not occur in the 72 hr studies. Thus, it may be more reasonable to view this conditioning as a physiological stress on the termite, temporarily eliminating or hiding one or more cues that are either recreated, such as a secretion, or uncovered by the termite over time.

The variability in response among the soldiers, particularly in the pairing of colony I (ATC) and colony C (LTC) termites, suggests confusion among the soldiers. This further suggests that perhaps only some recognition cues are suppressed, while others may still be active in low temperature conditioned termites.

Overall, our results support the "multiple stimulus hypothesis" described by Su and Haverty (1991), which proposes that kin recogni-

tion in *C. formosanus* is likely to result from a combination of many factors. These factors may include polar cuticular chemicals, glandular secretions, environmentally-based cues such as volatile digestive compounds, and intercolonial behavioral differences. Our study lends support for the presence of 1 or more cues that can be temporarily disabled through exposure to low temperatures.

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