# Preliminary Fusion Testing Between Whole Young Colonies of *Coptotermes gestroi* (Isoptera: Rhinotermitidae)

by

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## ABSTRACT

Every year in Brazil, colonies of the exotic termite *Coptotermes gestroi* increase their production of alates, which is the caste responsible for the foundation of new colonies. Colonies of this invasive species have huge populations, and their economical impacts have increased in urban areas during recent years. To simulate the meeting of neighboring colonies in field situations, we arranged intercolony interactions in the laboratory. Additionally, we exchanged one king or queen between colonies to observe how termites react to foreign reproductives. After 15 days of interactions, the mixed colony showed survival of both queens in 80% of the replicates and death of one king in 100% of the replicates. The present study also demonstrated tolerance of *C. gestroi* colonies to foreign reproductives. The low agonism of the neuter caste (workers and soldiers) in the mixed colonies and the stability of the termite populations confirm the fusion of *C. gestroi* colonies. Probably, the few introductions of this species in Brazil resulted in populations of *C. gestroi* that are genetically very similar with high inter-colony relatedness.

Keywords**:** agonism; colony interactions; subterranean termites

## INTRODUCTION

The subterranean termite *Coptotermes gestroi* is an oriental species that was first introduced into Brazil in two port cities, Rio de Janeiro and Santos, and has caused significant economical damage in urban areas (Araujo 1958, Costa-Leonardo *et al.* 2007). Every year, colonies of this termite increase their production of alates, which is the caste responsible for foundation of new colonies (Costa-Leonardo & Barsotti 1998, Costa-Leonardo *et al.*

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2005). Additionally, in Brazil, this termite species has the potentiality of developing secondary reproductives (Lelis 1999, Costa-Leonardo *et al.* 2004, Costa-Leonardo 2007) and is characterized by the production of nymphs throughout the year (Costa-Leonardo, unpublished data). Because of this, alates appear in the colonies at different times of the year. Nevertheless, the synchronized flights occur mainly in the months of August and September (Costa-Leonardo & Barsotti 1998). After the nuptial flights, the couples of *C. gestroi* dig into the soil to establish new colonies, sometimes close to each other. Therefore, over the years and with the development of these colonies, the occurrence of encounters between neighboring colonies seems plausible. Thus, the goal of this study was to verify how entire colonies of *C. gestroi* react in interactions with other colonies of same age, and toward the exchange of one of their reproductives. This study was also undertaken to determine if strong potential for colony fusion is implicated in the ability of this species to form enormous colonies.

## MATERIALS AND METHODS

### Termites

This study used entire colonies of *C. gestroi* obtained through laboratory pairing of alates. These reproductives were collected in swarming flights which occurred in the *campus* of the São Paulo State University, located in the city of Rio Claro, SP, Brazil (22°23'45.7" S / 47°32'38.3" W; 626.5 m above sea level).

### Manipulation tests

We performed tests simulating a field situation where a population of a colony meets another neighboring colony. All colonies had a complete census of individuals before (Table 1) and after the interactions. We chose colonies established and kept in 6.0-cm-diameter Petri dishes, which had small populations for facilitating the observation and manipulation. The tests described below were conducted in 14-cm-diameter glass Petri dish arenas, lined with moistened filter paper and containing a small plastic lid (~2.0 cm-diameter) filled with wet vermiculite to maintain humidity. Bioassays were kept in a dark room at a temperature of 25 ± 2°C. Prior to these experiments, we tested topical ink markers to identify the individuals of each colony. Unfortunately, all the paint marks failed, probably

Table 1. Census of the colonies before interactions.

| Colony | King | Queen | Workers | Soldiers | Total |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 18 | 4 | 24 |
| 2 | 1 | 1 | 17 | 2 | 21 |
| 3 | 1 | 1 | 48 | 5 | 55 |
| 4 | 1 | 1 | 53 | 7 | 62 |
| 5 | 1 | 1 | 39 | 3 | 44 |
| 6 | 1 | 1 | 29 | 2 | 33 |
| 7 | 1 | 1 | 30 | 2 | 34 |
| 8 | 1 | 1 | 26 | 2 | 30 |
| 9 | 1 | 1 | 17 | 1 | 20 |
| 10 | 1 | 1 | 42 | 4 | 48 |

due to topical toxicity that caused the death and/or stimulated the killing and cannibalization of the marked termites. Thus, it was not possible to determine what occurred with each individual from the different colonies during the interaction tests.

## Interactions of paired colonies

Different two-year-old colonies of *C. gestroi* were paired for this test, totaling 10 colonies and 5 replicates (Table 2). A small amount of the nest material from each original colony was placed on opposite sides in the arena (14-cm-diameter Petri dish) for housing the individuals. The number of live reproductives in each arena was recorded daily under red light for 15 days. After this period of interactions, a new census of the remaining live termites was also conducted. After three months of interactions, the number of reproductives was checked again.

Table 2. Number of individuals of different castes, before (B) and after (A) 15 days of interactions between entire colonies of *Coptotermes gestroi.*

| Colony interaction | Kings | | Queens | | Soldiers | | Workers | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | B | A | B | A | B | A | B | A | B | A |
| 1x2 | 2 | 1 | 2 | 2 | 6 | 5 | 35 | 35 | 45 | 43 |
| 3x4 | 2 | 1 | 2 | 2 | 12 | 12 | 101 | 96 | 117 | 111 |
| 5x6 | 2 | 1 | 2 | 2 | 5 | 4 | 68 | 54 | 77 | 61 |
| 7x8 | 2 | 1 | 2 | 2 | -4 | 3 | 56 | 44 | 64 | 50 |
| 9x10 | 2 | 1 | 2 | 1 | 5 | 5 | 59 | 54 | 68 | 61 |

## Reproductive permutations

This bioassay involved 12 two-year-old colonies. All colonies were placed individually in Petri dish arenas (14-cm-diameter) containing a small portion of the nest material from the original colony in the center. Three pairs of colonies had their queens permuted. Then, each colony selected for pairing manipulation received a foreign queen from the other colony, and the same procedure was carried out with kings of six other colonies. The termite behavior related to the new queen or king was observed during 15 days after the reproductive exchanges.

## Data analysis

A Student's t-test was performed to compare the number of individuals of each caste between each pair of colonies (Zar 1996). The same test was also conducted to compare the total number of kings, queens, soldiers and workers before and after the period of interaction. When data failed to fulfill the parametric test prerequisites, even after their transformation by the square root, the t-test was replaced by the nonparametric Kruskal-Wallis test. The level of significance adopted was 5% in all data analyses.

# RESULTS

All entire colonies used in the bioassays presented similar population sizes according to the statistical analysis ($t>3.84$, $p>0.05$). As a result of the interaction experiments, we observed the migration of the individuals from one colony and occupation of only one side of the dish for both colonies. Some reproductives were killed in the interactions between *C. gestroi* colonies, and the first one attacked and killed was one of the kings. After 15 days, the whole mixed population was nearby the reminiscent reproductives and behaving as a merged colony.

## Interactions of paired colonies

The result of the interactions between colonies showed no significant mortality of individuals belonging to the neuter caste (workers and soldiers) (Table 2; workers: $t=0.480$, d.f.=8, $p=0{,}644$; soldiers: $t=0.280$, d.f.=8, $p=0.787$). After 15 days of interactions, the mixed colony showed survival of both queens in 80% of the replicates. Conversely, one king died in 100% of the replicates (Table 2; $H=25.00$, d.f.=1, $p=0.003$). The reproductive deaths occurred in the first 24h of the interaction period. After this period, individuals of all castes

Table 3. Number of individuals of each caste before (B) and after (A) 15 days of the reproductive permutations between entire colonies of *Coptotermes gestroi*.

| | Kings | | Queens | | Soldiers | | Workers | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | B | A | B | A | B | A | B | A | B | A |
| Permutation of Kings | 1 | 1 | 1 | 1 | 4 | 2 | 28 | 30 | 41 | 40 |
| | 1 | 1 | 1 | 1 | 4 | 4 | 42 | 45 | 58 | 61 |
| | 1 | 1 | 1 | 1 | 2 | 3 | 30 | 30 | 37 | 36 |
| | 1 | 1 | 1 | 1 | 2 | 4 | 39 | 42 | 57 | 57 |
| | 1 | 1 | 1 | 1 | 3 | 2 | 34 | 36 | 47 | 42 |
| | 1 | 1 | 1 | 0 | 4 | 4 | 38 | 37 | 58 | 49 |
| Permutation of Queens | 1 | 1 | 1 | 1 | 4 | 4 | 34 | 35 | 40 | 41 |
| | 1 | 1 | 1 | 1 | 2 | 2 | 30 | 29 | 34 | 33 |
| | 1 | 1 | 1 | 1 | 4 | 3 | 22 | 21 | 28 | 26 |
| | 1 | 1 | 1 | 1 | 2 | 2 | 13 | 12 | 17 | 16 |
| | 1 | 1 | 1 | 1 | 3 | 3 | 30 | 30 | 35 | 35 |
| | 1 | 1 | 1 | 1 | 2 | 1 | 23 | 24 | 27 | 27 |

moved inside the whole Petri dish and acted as a single colony. In addition, the population size of the fused colony remained statistically similar after the 15-day period compared to the population size at the beginning of the interaction (t=0.615, d.f.=8, p=0.556). Three months after the interaction, the same reproductives reported in Table 2 were still alive.

### Reproductive permutations

The results show that the permutations of the reproductives caused no significant mortality of the individuals of any caste. Thus, the colonies maintained stable population sizes after the 15-day-period (Table 3; permutation of queens: t=0.104, d.f.=10; p=0.919; permutation of kings: t=0.389; d.f.=10; p=0.705), and we may assume that the foreign reproductives were successfully accepted by the host colonies. Additionally, the foreign queens laid eggs in the new colonies, and the workers took care of them, as occurs in normal colonies. This fact was observed in all bioassays. When foreign reproductives, queens and kings were introduced in the host colonies, they were always carefully inspected by the antennas of the other termites without causing agonistic responses.

## DISCUSSION

According to Thorne & Haverty (1991), the use of entire colonies in experiments of interaction is more realistic, because the social structure of the termite colony remains intact. All the results of the interactions between entire colonies of *Coptotermes gestroi* showed colony fusion. These data are similar to

those described by Leniaud *et al.* (2008) for the invasive *Reticulitermes urbis* in France. The explanation is that *R. urbis* behaves as a single "genetic entity". Thorne *et al.* (2003) observed that agonistic interactions between colonies of different sizes often resulted in attacks directed toward the individuals of the smaller colony, mainly toward their reproductives. Pickens (1934 *apud* Thorne & Haverty 1991) reported that incipient colonies of *Reticulitermes hesperus* were readily destroyed by workers from mature colonies, and that the introduction of primary reproductives into foreign colonies also resulted in their immediate destruction.

The present study shows that some aggression against reproductives may be observed even in situations where the size of the colonies is statistically similar. The bioassays also demonstrated the tolerance of the original colony to foreign reproductives. Since parthenogenesis does not occur in *C. gestroi* (Costa-Leonardo & Barsotti 1998), the colony ensures their reproductive success by avoiding attacks on foreign kings or queens in the absence of the original royal pair. This tolerance is less pronounced when two entire colonies interact and the royal couples from both colonies are present. In this case, significant mortality of the reproductives may occur. In most of the interactions of paired colonies, both queens and only one of the kings survived. It is difficult to explain the systematic agonistic behavior addressed to the king during intercolony interactions. The recognition mechanisms are connected to the cuticular hydrocarbon composition and reflect the genetic relationship among colonies (Dronnet *et al.* 2006). Thus, low aggression among individuals of *C. gestroi* colonies may be interpreted as a genetic consequence, given the fact that this species was introduced in Brazil on only a few occasions.

For Grace (1996) and Cornelius & Osbrink (2003), the high degree of kinship and a low degree of agonism between two conspecific colonies may contribute to possible fusion. This process may be a strategy in termites, ensuring an accelerated initial growth of the population and avoiding unnecessary energy expenditure in battles with other colonies of the same species (Matsuura & Nishida 2001).

The low agonism of individuals in bioassays and the population stability of colonies confirm the fusion of *C. gestroi* colonies in this experiment. However, future studies are needed to understand the factors that determine the direction of attacks against the reproductives, a fact also observed in *Neotermes tectonae* by

Kalshoven (1959 *apud* Thorne & Haverty 1991). Additionally, colony fusion may represent an adaptive way of outbreeding without the risk of predation that occurs in the swarming of the alates (Matsuura & Nishida 2001).

Merged colonies have rapid initial population growth (Thorne *et al.* 2002) and may become "super-colonies" that are considered groups of nests exhibiting no intraspecific aggression (Leniaud *et al.* 2008). The fusion of *C. gestroi* colonies may increase the competition with native species of subterranean termites and represents a significant challenge to the control of this termite pest in urban centers of Brazil.

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