

Immunomarking Reveals Food Flow and Feeding Relationships in the Eastern Subterranean Termite, *Reticulitermes flavipes* (Kollar)

GRZEGORZ BUCZKOWSKI,¹ CHANGLU WANG, AND GARY BENNETT

Department of Entomology, Purdue University, West Lafayette, IN 47907

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ABSTRACT Trophallaxis and feeding relationships in the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), were examined using a novel marking technique, rabbit IgG protein coupled with an enzyme linked immunosorbent assay (ELISA) to detect the marker. Transfer experiments in small dishes evaluated the trophallactic transfer of the marker from donor workers fed IgG-treated paper to recipient workers or larvae. Worker donors rapidly acquired the marker, and 100% of donors tested positive within 24 h. Trophallactic transfer from donors to recipients was relatively inefficient, and $51 \pm 2\%$ of recipient workers and $31 \pm 2\%$ of recipient larvae tested positive at 72 h. Based on the mean optical density counts, $\approx 27\%$ of marker ingested by the donors was passed on to the recipient workers in the first 24 h, 14% to recipient larvae, and 26% to recipient soldiers. The ability of soldiers to feed independently of workers was examined in dish assays. Soldiers showed no significant uptake of the marker when isolated from the workers, and uptake increased significantly when workers were present. The distribution of the marker was further studied in larger colony fragments composed of workers, soldiers, nymphs, and larvae. Marker acquisition by the different castes/developmental stages was highly variable, with workers and nymphs acquiring the marker at a faster rate than soldiers and larvae. The results of this study contribute to our understanding of the foraging ecology and social behavior in *R. flavipes*. In addition, they may help design improved control programs for subterranean termites based on baits.

KEY WORDS immunomarking, trophallaxis, termite, *Reticulitermes flavipes*, marking techniques

In social insects, such as termites and many Hymenoptera, trophallaxis is an important and highly efficient method for delivering food to the various castes and developmental stages that do not or cannot feed directly (Wilson 1971). Besides this obvious primary function, trophallaxis also plays a crucial role in the exchange of cuticular hydrocarbons among nestmates (Dahbi et al. 1999, Boulay et al. 2000), nutrient cycling (Machida et al. 2001), exchange of information about available food sources (Farina 1996), and transfer of gut symbionts (McMahan 1969), pheromones (Seeley 1995), and caste determination hormones (Moore 1969). Unlike eusocial Hymenoptera, which possess specialized structures for storing and distributing liquid food among nestmates (Eisner 1957), termites lack specialized organs for transporting and sharing food with other colony members. While trophallaxis by regurgitation of liquid food in ants is rapid and readily observed under laboratory conditions (Markin 1970), termites generally share a suspension of wood particles (Su and La Fage 1987) and require several hours or days to effectively share food with nestmates (Rosengaus et al. 1986). Termites use both stomodeal and proctodeal trophallaxis when sharing food with

nestmates. The larvae, soldiers, nymphs, and neotenic receive nutrition from foraging workers (Buchli 1958, McMahan, 1969).

In Hymenoptera, the patterns of social food transfer by trophallaxis and food allocation from foraging to nonforaging individuals have been thoroughly examined. For example, in ants, the rate of exchange of food varies with factors such as season (Khamala and Buschinger 1971), temperature (Howard and Tschinkel 1981), and starvation (Markin 1970; Meudec and Lenoir 1982). Furthermore, the differential patterns of food allocation based on food type are well documented in numerous species of ants (Wilson and Eisner 1957; Brian and Abbott 1977; Sorensen and Vinson 1981), whereby carbohydrates are mainly used by foragers, lipids by workers and some larvae, and proteins by the growing larvae and egg laying queens. In contrast, Isoptera possess radically different life history characteristics and consequently different patterns of food distribution. Termites in the genus *Reticulitermes* usually occupy a single piece of wood, which serves as their food source and harborage. As a result, all individuals in the colony receive a single type of food (cellulose). In contrast to immature stages in ants, which are immobile, immature termites (third-stage larvae and older) are capable of indepen-

¹ Corresponding author, e-mail: gbuczkow@purdue.edu.

dent feeding because of their hemimetabolous development. These factors affect the foraging ecology of termites and contribute to the unique patterns to food distribution in termite colonies.

Numerous aspects of subterranean termite foraging ecology, including foraging range, diet selection, and caste differences in feeding and trophallaxis remain poorly understood. This lack of knowledge may result from the cryptic nesting habits of termites, lack of open trail foraging in many species, and the lack of suitable markers to study foraging patterns under field conditions. Recently, however, progress has been made exploring foraging in several termite groups. Molecular techniques have shed light on the foraging range in the genus *Reticulitermes* (DeHeer and Vargo 2004). Several studies have investigated feeding and trophallaxis in subterranean (Rosengaus et al. 1986, Su and La Fage 1987, Suárez and Thorne 2000), drywood (Afzal 1984, Cabrera and Rust 1999), and dampwood (Suárez and Thorne 2000) termites. While some of these studies were purely observational, others used various radioisotope markers or the nonradioactive rubidium (Rb) incorporated into the food. Radioisotope markers have also been used to study other aspects of termite biology such as population estimation and colony structure in the field (Spragg and Paton 1980). In addition, recent studies on the horizontal transfer of insecticides in termite colonies have contributed to our understanding of the trophallactic exchange of food in termites (Sheets et al. 2000).

Recently, protein-based marking techniques for insects have been developed (reviewed in Hagler and Jackson 2001) whereby immunoglobulin G (IgG) proteins are applied to the target insect and later detected by the highly sensitive double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (Hagler 1997a, b). The protein marking technique is less costly, more sensitive, and safer than using radioactive isotopes, which had been used in previous studies in termites (Afzal 1984, Rosengaus et al. 1986, Suárez and Thorne 2000), ants (Markin 1970, Sorensen et al. 1980), and honey bees (Nixon and Ribbands 1952). Protein markers are also persistent under field conditions. They resist degradation by heat and water (Hagler 1997a) and are therefore an ideal marker for studying natural populations. Immunomarking has been used to study the flow of food in natural populations of honey bees (DeGrandi-Hoffman and Hagler 2000) and ants (Buczowski and Bennett 2006). Rabbit IgG protein was obtained by foraging individuals from sucrose feeding stations spiked with the marker and was readily transferred to other members of the colony.

The objective of this study was to investigate in laboratory assays the flow of food and feeding relationships in the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) using protein marking and sandwich ELISA. In the first phase of the study, we used small dish assays to investigate the trophallactic transfer of the marker from donor workers to other workers, larvae, and soldiers. In the second phase, we

used larger colony fragments and examined the distribution of the marker to various castes and developmental stages.

Materials and Methods

Colonies. *R. flavipes* workers and soldiers were collected on the campus of Purdue University, West Lafayette, IN, by trapping within cardboard rolls inserted into the ground and/or cavities of live, termite-infested trees. The termites were brought into the laboratory and allowed to migrate into plastic containers with cellulose powder, moistened pine wood, and laboratory paper towels provided as food and harborage. Species identity was verified by external morphology of soldiers (Nutting 1990). Colonies were maintained at 25–27°C, >80% RH, and in constant darkness. Water was added to rearing containers as needed. Colonies were held in the laboratory for 4–10 mo before use in assays to produce eggs, larvae, nymphs, and additional soldiers that were subsequently used in laboratory tests. Fifth- through seventh-instar workers were used in individual assay replicates.

Preliminary Tests. Preliminary tests were initiated to determine the appropriate concentration of IgG marker to use. Fifteen worker termites were placed in a 3.5-cm-diameter petri dish containing 2.5 g of moist sand:vermiculite mixture (1:1, vol:vol) and allowed to acclimate (i.e., construct galleries throughout the substrate) for 3 d. Five concentrations of technical grade rabbit IgG protein (Sigma, St. Louis, MO) were prepared in distilled water: 0.31, 0.625, 1.25, 2.5, and 5.0 mg IgG/ml. For each treatment, 50 μ l of solution containing the IgG marker was pipetted onto a 3.5-cm-diameter (9.616 cm²) piece of paper towel. The treated paper was placed on top of the sand:vermiculite mixture and away from the termites, which preferred to tunnel at the bottom of the dish. This minimized contact with the treated paper and helped reduce external contamination with the marker. Termites were considered workers if they did not possess any sign of wing buds or distended abdomens, as would be present in pseudergates or nymphs (Buchli 1958). Termites fed on the treated paper ad libitum. After 24 h, all termites were collected into individual vials and frozen (–20°C) until analyzed for the IgG marker by sandwich ELISA (see below). Three replications were performed for each concentration.

In addition, we performed an experiment to test whether termites become externally contaminated with the marker while feeding on the IgG-treated paper. The experiment was set up as above, using the 2.5 mg IgG/ml dose, and replicated three times. After 24 h, all termites were frozen to prevent possible regurgitation of ingested marker during the subsequent washing. Frozen termites were individually washed by submerging in phosphate-buffered saline (PBS) buffer and agitating for 15 s. The washate and the termite bodies were analyzed separately by ELISA.

Uptake and Retention of the IgG Marker. The uptake and retention of the IgG protein marker was examined in groups of 100 worker termites. Suárez and Thorne (2000) found no effect of group size (10 versus 100) on the rate of uptake and retention of radiotracers in *Reticulitermes*. To estimate the daily uptake and accumulation of the marker, 100 workers were placed in a 9.5-cm-diameter petri dish containing 10 g of moist sand:vermiculite mixture and allowed to acclimate for 3 d. Two 3.5-cm-diameter pieces of paper, each treated with 50 μ l of 2.5 mg/ml solution of IgG, were placed on top of the sand. The 2.5 mg IgG/ml concentration was selected based on the results of preliminary tests, which revealed that the increases in optical density were minimal >2.5 mg/ml. Thus, we used the 2.5 mg/ml concentration in all subsequent tests. Fifteen workers were sampled from the dish every 24 h for 3 d and analyzed individually by ELISA. To estimate retention of the marker 100 workers were fed IgG-labeled paper as above for 48 h and transferred to a new dish containing unlabeled food and clean substrate. Fifteen workers were sampled at 1, 2, 4, 6, 8, and 10 d after the transfer to unlabeled food. The experiment was replicated three times.

Trophallactic Exchange of Food—Dish Assays. The transfer of food between workers and from workers to larvae (L2; sensu Buchli 1958) was studied in dish assays. Donor workers were fed paper containing 0.5% Neutral Red for 7 d so they could be identified in subsequent interactions with other workers. Permanently dyed workers were transferred to a dish with untreated paper for 3 d to purge any remaining dye from their digestive system and prevent it from being passed in subsequent trophallactic interactions. Dyed donor workers were placed in a 3.5-cm-diameter dish containing moist sand:vermiculite mixture and two 3.5-cm-diameter pieces of paper towel, each treated with 50 μ l of 2.5 mg IgG/ml rabbit IgG. The donors fed on the paper ad libidum for 48 h. To examine the trophallactic exchange of food, 10 potential donor workers were transferred to a 3.5-cm-diameter dish containing sand:vermiculite mixture and either 15 untreated, undyed workers or larvae selected at random from stock colonies. No additional food was provided. The amount of protein marker acquired by the recipients was determined 24, 48, and 72 h after mixing the donors with the recipients. Five replicates were performed for each time point.

In addition, the ability of soldiers to feed independently of workers and the trophallactic transfer of the marker from workers to soldiers were examined in dish assays. In tests without workers, five soldiers were placed in a 3.5-cm-diameter petri dish containing 2.5 g of moist sand:vermiculite mixture. A single piece of 3.5-cm-diameter paper towel treated with 50 μ l of 2.5 mg IgG/ml water was added, and soldiers were allowed to feed on the paper ad libidum. The uptake of the marker was studied at 24, 48, and 72 h. Tests involving workers were performed as described above except that 15 donor workers were added to each dish containing the soldiers. All soldiers were collected and

analyzed by ELISA, and five replicates were performed for each time-point.

Distribution of Marker in Larger Colony Fragments. To study the uptake and the distribution of the marker in larger colony fragments, we performed laboratory experiments using colonies composed of multiple castes/developmental stages. The colonies were fragments of a larger field colony and consisted of ≈ 600 workers, 40 soldiers, 40 larvae, and 40 nymphs. All colonies were reproductively competent, i.e., they contained numerous secondary reproductives and produced young larvae. All colonies were maintained in 20 by 14 by 9-cm plastic boxes with a 2-cm layer of moist sand:vermiculite mixture on the bottom. Seven premoistened pine blocks (10 by 10 by 0.7 cm) stacked in the center of the box served as food and harborage. Each experimental colony was provided with two pieces of 3.5-cm-diameter paper towel, each treated with 50 μ l of 2.5 mg IgG/ml water. The treated paper was placed on top of the sand:vermiculite substrate and under the stacked wood. We randomly sampled 15 workers, 10 soldiers, 10 larvae, and 10 nymphs after 24, 48, and 72 h of ad libidum feeding on the IgG-labeled paper to determine the amount of protein marker acquired by the different castes and developmental stages. All individuals were frozen in individual tubes and later analyzed by ELISA. Three replicates were performed for each time-point.

ELISA Procedure. Sandwich ELISA was performed on individual termite samples using previously described techniques (Hagler 1997a, Hagler and Jackson 2001). Frozen samples were individually homogenized in 200 μ l PBS (pH = 7.4) using plastic pestles and assayed for the presence of the rabbit immunoglobulin protein. Each well of a 96-well microplate was coated with 100 μ l of anti-rabbit IgG (developed in goat; Sigma) diluted 1:500 in distilled water and incubated for 2 h at 4°C. After incubation, the primary antibody was discarded and 310 μ l of 1% nonfat dry milk (Bio-Rad Laboratories, Hercules, CA) in distilled water was added to each well to block any remaining nonspecific binding sites. After 30-min incubation at 26°C, the milk was discarded. Termite homogenates were vortexed, and a 70- μ l aliquot of sample was added to each well and incubated for 1 h at 26°C. The samples were discarded, and each well was washed three times with PBS Tween 20 (0.05%) and two times with PBS. Anti-rabbit IgG conjugated to horseradish peroxidase (50 μ l; Sigma; developed in goat) diluted 1:1,000 in 1% nonfat milk was added to each well and incubated at 26°C for 1 h. All wells were washed again as above, and 50 μ l of tetramethylbenzidine horseradish peroxidase substrate (BioFX Laboratories, Owings Mills, MD) was added to each well and incubated for 30 min. Samples were analyzed on a Beckman Coulter AD 340 Absorbance Detector set at 620 nm. The mean \pm SE optical density value and the percentage of samples scoring positive for rabbit protein were determined. Six negative controls (workers never exposed to rabbit IgG) and six blanks (PBS buffer only) were run on each plate.

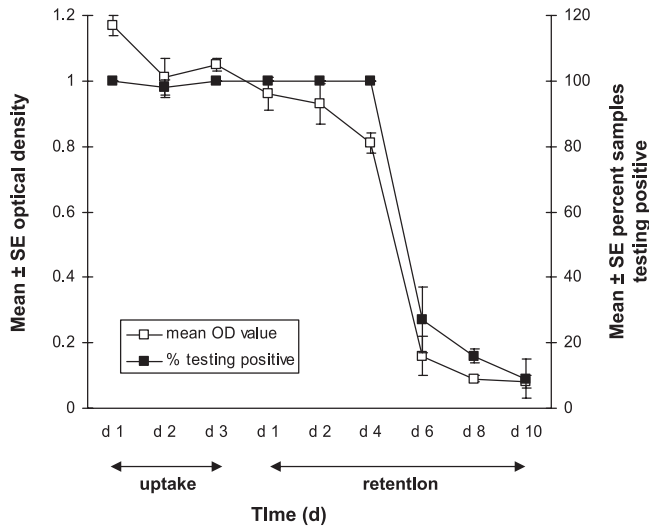


Fig. 1. Uptake and retention of the IgG protein marker by *R. flavipes* workers under laboratory conditions. Mean OD values \pm SE are given ($n = 45$). Mean \pm SE percent of termites testing positive for the marker is also provided.

Statistical Analysis. The samples were scored positive for the presence of the protein marker if the ELISA optical density value exceeded the mean negative control value by 3 SD (Hagler, 1997 a, b). For all experiments, the results are expressed as both the mean number of individuals testing positive and the mean optical density (OD). All mean OD values are an average of all individuals used in the test and not just those testing positive.

To determine whether worker presence affected the uptake of the marker by the soldiers, we used binomial logistic regression. This was accomplished by using the PROC LOGISTIC procedure in SAS 8.1 (SAS Institute 2002). The independent variables had a dichotomous distribution, whereby recipients (soldiers) could either test positive (1) or negative (0). Subsequently, a Wald's χ^2 test was used to test the significance of individual logistic regression coefficients for each independent variable.

Analysis of variance (ANOVA) tests (PROC GLM) were conducted to determine the significance of developmental stage and time on the transfer of marker from donor workers to recipient workers and larvae. The percentage of samples testing positive for the IgG protein was tabulated by first calculating the percent of individuals testing positive within a replicate and then averaging across replicates. Percentage data were arcsine transformed to stabilize the variance (Sokal and Rohlf 1995). ANOVA tests were followed by posthoc Tukey's honestly significant difference (HSD) tests to separate the means.

Results

Uptake and Retention of the IgG Marker. Termite workers rapidly acquired the protein marker after feeding on the IgG-treated paper. Within 24 h, 100 \pm 0% of termites tested positive. At 48 h, 98 \pm 2% of

termites tested positive, and at 72 h, 100 \pm 0% of termites tested positive. This indicates that all workers within the colony either feed directly or receive nutrition from nestmates through trophallaxis. The retention study was designed to determine the length of time the protein marker remained detectable in the termites after ingestion of IgG-treated paper. In laboratory studies, rabbit IgG was readily detectable 4 d after transfer to unlabeled food with 100 \pm 0% of termites testing positive and a mean OD value of 0.81 \pm 0.03 (Fig. 1). After 4 d, however, the amount of IgG retained dropped sharply. On day 6, 27 \pm 10% of termites still tested positive, and the mean OD value decreased to 0.16 \pm 0.06. By 10 d, only 9 \pm 6% of termites still tested positive, and the mean OD value decreased to 0.08 \pm 0.02, which was not significantly different from either negative controls (average OD, 0.07 \pm 0.01; $F = 2.76$, $df = 1$, $P = 0.10$) or blanks (average OD, 0.07 \pm 0.01; $F = 3.56$, $df = 1$, $P = 0.07$).

Trophallactic Exchange of Food—Dish Assays. Preliminary experiments indicated that the termites do not become externally contaminated with the marker while feeding on the IgG-treated paper. No washate samples tested positive for the marker (mean OD value = 0.06 \pm 0; not significantly different from blanks, $F = 3.15$, $df = 1$, $P = 0.09$), whereas 96 \pm 5% of termites from which the washate was obtained tested positive (mean OD value = 1.05 \pm 0.04; $F = 599.95$, $df = 1$, $P < 0.0001$). This suggests that the marker is internally concentrated and transferred to nestmates through trophallaxis and not allogrooming.

The results of the trophallactic flow of food from donor workers to recipient workers or larvae are presented in Fig. 2A and B. Donor workers rapidly acquired the IgG marker, and 100% of donors became positive within 48 h (mean OD value = 0.96 \pm 0.04). Within 24 h, 40 \pm 7% of recipient workers tested

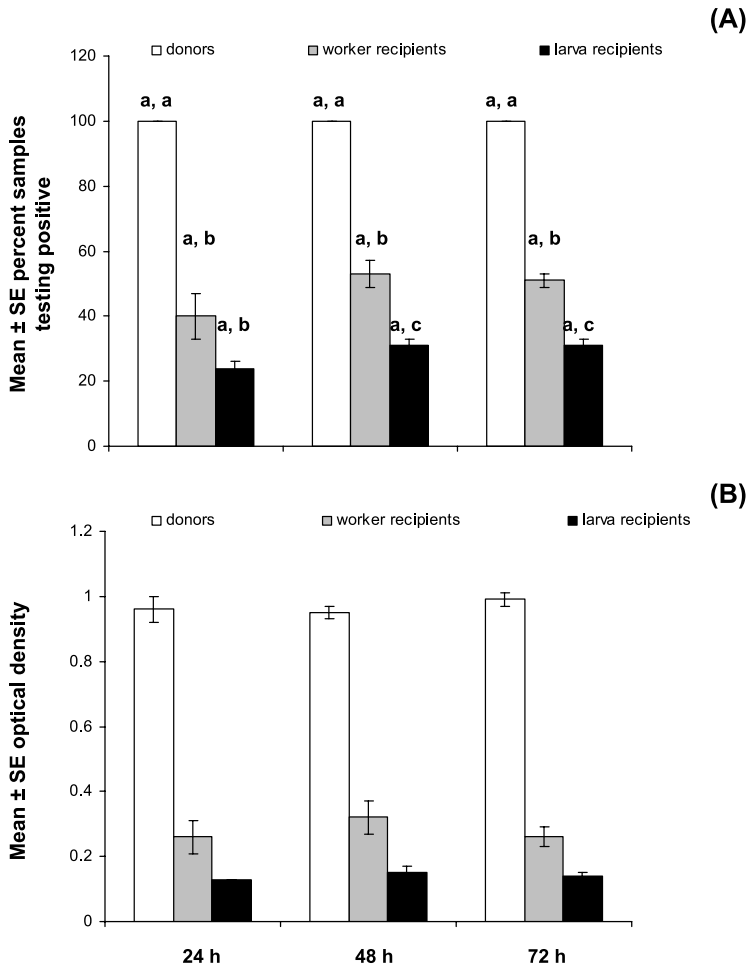


Fig. 2. Transfer of IgG marker from worker donors to worker and larva recipients 24, 48, and 72 h after exposure of recipients to IgG-fed donors. The mean \pm SEM percent of termites testing positive are presented in A and the mean OD values \pm SE are presented in B. For each stage or time, different lowercase letters indicate significantly different values based on Tukey's HSD test ($P \leq 0.05$). First letter indicates comparisons among time periods (24, 48, 72 h) for a given stage (donors, worker recipients, larva recipients), and the second comparison is among the different stages within a given time period.

positive (Fig. 2A). Our data indicate that 18 of 45 (40%) recipients tested positive for the marker, with an average OD value of 0.54 ± 0.06 . The remaining 27 recipients had an average OD value of 0.07 ± 0.01 , which was not significantly different from negative controls ($F = 0.03$, $df = 1$, $P = 0.87$). Based on the mean optical density counts (Fig. 2B), $\approx 27\%$ of marker ingested by the donors was passed on to the recipients in the first 24 h. Relative to the workers, the larvae acquired smaller amounts of the marker. At 24 h, the average OD value for larvae was $0.13 \pm 0\%$, and 11 of 45 larvae (24%) tested positive for the marker (Fig. 2A). Those 11 individuals had an average OD value of 0.32 ± 0.03 , which was significantly lower from the amount of IgG detected in positive testing recipient workers (average OD = 0.54 ± 0.06 ; $F = 8.6$, $df = 1$, $P = 0.006$). Based on the mean optical density counts (Fig. 2B), $\approx 14\%$ of marker ingested by the

donor workers was passed on to the larvae in the first 24 h. Time had no significant effect on the amount of marker acquired by the workers ($F = 3.13$, $df = 2$, $P = 0.15$) or the larvae ($F = 3.05$, $df = 2$, $P = 0.16$).

Soldiers showed no significant uptake of the marker compared with negative controls when isolated from the workers ($F = 0.78$, $df = 1$, $P = 0.38$). Figure 3A shows that a certain percentage of the soldiers (range, 8–16%) tested positive for the marker when isolated from the workers. However, our data indicate that all soldiers classified as positive only slightly exceeded the mean negative control value ± 3 SD. The uptake of marker increased significantly in the soldiers when workers were present (Wald's $\chi^2 = 10.1$, $df = 1$, $P = 0.001$). At 24 h, $28 \pm 8\%$ of soldiers tested positive for the marker, and the percentage testing positive increased to $36 \pm 8\%$ at 48 h and $40 \pm 13\%$ at 72 h. Based on the mean optical density counts (Fig. 3B), $\approx 26\%$ of marker ingested by the

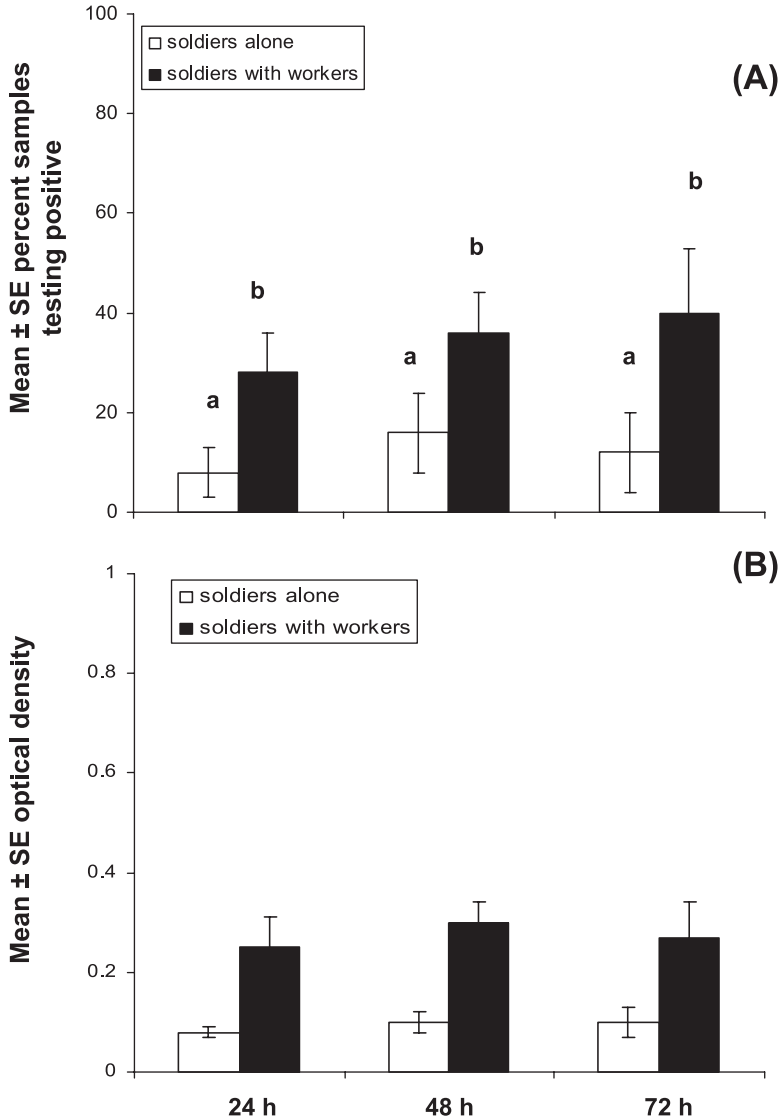


Fig. 3. IgG marker acquisition by *R. flavipes* soldiers when the soldiers are alone or with workers 24, 48, and 72 h after continuous feeding on paper treated with a solution of rabbit IgG protein. The mean \pm SE percent of termites testing positive are presented in A, and the mean OD values \pm SE are in B. For each time, different lowercase letters indicate significantly different values based on Wald's χ^2 test ($P \leq 0.05$).

donor workers was passed on to the soldiers in the first 24 h. Time had no significant effect on the percentage of soldiers testing positive for the marker, either when workers were absent ($F = 2.46$, $df = 1$, $P = 0.07$) or present ($F = 3.92$, $df = 1$, $P = 0.27$).

Distribution of Marker in Larger Colony Fragments. The results of the trophallactic flow of food in larger colony fragments are presented as either mean percentage of termites testing positive for the marker (Table 1) or in histograms displaying the distribution of ELISA results (OD values; Fig. 4). The data in Table 1 are largely quantitative and only indicates the percentage of termites that tested positive, i.e., exceeded a predetermined threshold level. A disadvan-

tage of this approach is that certain individuals may have acquired relatively little marker and only slightly exceeded the threshold level, but still scored positive. Such individuals would not be differentiated from those that ingested substantially larger amounts of the marker. Therefore, in addition to Table 1, we also provide a histogram indicating the frequency distribution of OD values broken into five arbitrary intervals of 0.25 absorbance units each. Workers readily fed on the IgG-treated paper, and $49 \pm 6\%$ of the workers tested positive at 24 h. Workers continued to acquire the marker and $96 \pm 2\%$ of workers tested positive at 72 h. The amount of the IgG marker transferred to the soldiers, nymphs, and larvae was highly variable, both

Table 1. Mean percentage ± SE of termites testing positive for rabbit IgG protein 24, 48, and 72 h after feeding on IgG-treated paper (n = 30–45)

Caste/ developmental stage	Mean percent positive samples		
	24 h	48 h	72 h
Workers	49 ± 6% a,a (0.30 ± 0.05)	82 ± 4% ab,a (0.58 ± 0.12)	96 ± 2% b,a (0.97 ± 0.12)
Soldiers	0 ± 0% b,b (0.09 ± 0.00)	40 ± 6% a,b (0.14 ± 0.02)	57 ± 3% a,b (0.35 ± 0.04)
Nymphs	30 ± 6% a,a (0.16 ± 0.04)	43 ± 15% a,b (0.26 ± 0.08)	53 ± 7% a,b (0.30 ± 0.04)
Larvae	7 ± 3% b,b (0.08 ± 0.01)	50 ± 6% a,ab (0.22 ± 0.01)	43 ± 7% a,b (0.20 ± 0.02)

Mean OD values ± SE are given in parentheses. Means followed by the same letter are not significantly different by Tukey's HSD test (*P* ≤ 0.05). First letter indicates within-row comparisons, the second letter indicates within-column comparisons.

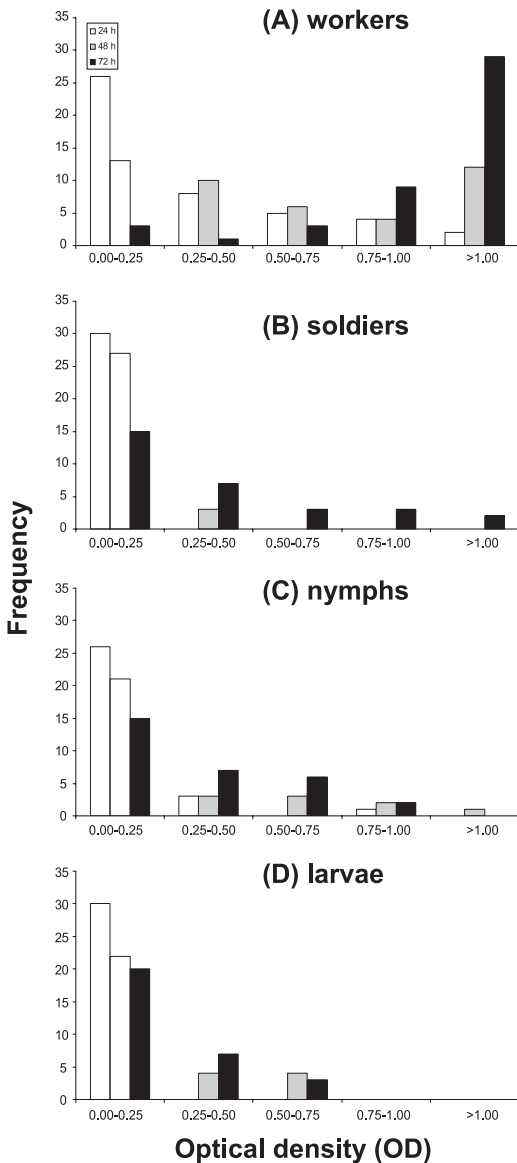


Fig. 4. Histogram of ELISA results (OD values) for (A) workers, (B) soldiers, (C) nymphs, and (D) larvae 24, 48, and 72 h after continuous feeding on paper treated with a solution of rabbit IgG protein.

in terms of the rate of transfer and the absolute amount transferred. Marker acquisition by the soldiers was delayed and no soldiers tested positive within the first 24 h. Subsequently, 40 ± 6% of soldiers tested positive at 48 h, and 57 ± 3% tested positive at 72 h. The nymphs acquired substantial amounts of the marker and 30–53% of nymphs tested positive. The pattern of marker acquisition by the larvae was similar to that observed in the soldiers. Uptake by the larvae was minimal during the first 24 h, followed by more rapid accumulation during the last 48 h.

A histogram of ELISA results (frequency distribution of OD values) for all castes/developmental stages examined is presented in Fig. 4A–D. The distribution of OD values for workers assumed a parabolic shape. At 24 h, the majority of workers had OD values < 0.25, indicating a delay in feeding on the IgG-treated paper. At 48 h, some workers acquired a relatively large amount of marker and others acquired little. At 72 h, the majority of workers (76%) had OD values > 1.0, and 96% of workers tested positive (Table 1). This indicates that almost all workers acquire the marker and the majority receives a relatively large amount. The distribution of the marker to soldiers was delayed, and the majority of soldiers showed low (0.00–0.25) OD values at 24 and 48 h. At 72 h, 57% of soldiers tested positive for the marker (Table 1); however, 50% of soldiers still had OD values < 0.25, suggesting soldiers acquire substantially less marker than workers. The distribution of marker to the nymphs was similar to that observed in the soldiers, with the majority of the nymphs receiving little marker initially, followed by a steady increase over time. Optical density values never exceeded 0.75 U in the larvae, and the majority of larvae had OD values < 0.25, indicating they receive relatively little food. However, given that a larva weighs only ≈ 1/10th of the weight of a worker, the larvae received substantial amounts of the marker on a per weight basis.

Discussion

In small dish assays without access to alternative food, termite workers rapidly acquired the protein marker, and 100% of termite donors became positive within 24 h. In contrast, in tests with larger colony fragments (600 workers and 120 other castes), which had access to alternative food, the uptake of the marker was significantly delayed, and only 49% of the

workers tested positive at 24 h and 96% tested positive at 72 h. Several factors including size of the colony, size of the test arena, access to alternative food, presence of other castes and/or developmental stages, and proximity to the labeled food may have accounted for the difference. While our experimental design did not allow us to single out the primary factor or rank the above factors, colony size may have substantially affected the rate at which the marker spread through the colonies. In studies using radioisotope markers, McMahan (1966) showed that, in *Cryptotermes brevis* (Walker), the average amount of radioactivity per termite decreased as colony density increased and a similar process was observed in ants (Wilson and Eisner 1957). In both cases, the result was attributed to the chain transmission of food, also known as the "trophallactic cascade" as proposed by Suárez and Thorne (2000). In both termites and ants, the chain transmission of food is thought to optimize colony homeostasis, especially when the transfer of gut symbiotes and/or pheromones is involved. Second, termites in the larger colony fragments nested in wood and therefore had access to a natural and unlabeled food source, whereas termites in the dish tests could only feed on IgG-treated paper. Preferential feeding on wood could have reduced the consumption of IgG-treated paper.

Experiments on the distribution of IgG-labeled paper in larger colony fragments composed of multiple castes and developmental stages revealed that it takes ≈ 3 d for the marker to reach all workers in an experimental colony comprised of 600 workers. This contrasts with the social Hymenoptera where the flow of food is much more rapid and usually takes minutes to hours (Wilson and Eisner 1957). Moreover, recent studies using the IgG protein marker to track the flow of food in social Hymenoptera showed that the marker reaches $>90\%$ of workers within 24 h (honey bees: DeGrandi-Hoffman and Hagler 2000, ants: Buczkowski and Bennett 2006). The results of our study support findings of other studies that show that termites distribute food more slowly relative to other social Hymenoptera (Afzal 1984, Rosengaus et al. 1986, Suárez and Thorne 2000). In comparison with the workers, the nymphs acquired substantially less marker, even though the nymphs are thought to be capable of independent feeding (Buchli 1958, Noirot 1990). It remains unknown whether the nymphs obtained the marker directly, by feeding on the IgG-treated paper, or indirectly, by trophallaxis with IgG-fed workers. Even though 53% of the nymphs tested positive at 72 h, the great majority of them contained relatively little marker (Fig. 4C), suggesting the nymphs may indeed receive nutrition indirectly from the workers. However, nymphs may simply consume less food relative to the workers. In assays involving larger colony fragments, the larvae experienced a delay in feeding during the first 24 h. Subsequently, approximately one half of all larvae tested positive for the marker. In contrast, in petri dish experiments, the larvae seemed to acquire the

marker at a faster rate, even though fewer larvae tested positive overall. This suggests that feeding by the larvae may depend on factors such as the proximity of larvae to the workers, the ratio of larvae to the workers, and perhaps even the presence of other castes and/or developmental stages (e.g., soldiers, nymphs), which might affect the rate and amount of the food delivered to the larvae. A comparison between dish assays (Fig. 2) and larger colony assays (Table 1) revealed that the majority of termite workers may acquire the marker by directly feeding on the treated paper and not by trophallaxis with nestmates. In dish assays, only $\approx 50\%$ of all recipient workers tested positive despite close proximity to potential donors. In larger colony assays, where all the workers had continuous access to IgG-labeled food, almost all workers eventually tested positive. This indicates that only a relatively small fraction of the marker consumed by the donors is transferred to the recipients and/or the marker is shared unequally, with some workers receiving substantially more marker than others. Suárez and Thorne (2000) reported similar results and found that, on average, only $\approx 1\text{--}2\%$ of the donor's food load was transferred to the recipient(s). The significant difference in marker acquisition by soldiers alone compared with soldiers in the presence of workers indicates lack of direct feeding by the soldiers and is consistent with other termite species (McMahan 1969, Su and La Fage 1987, Cabrera and Rust 1999, Suárez and Thorne 2000).

The IgG protein seems suitable for conducting studies on the foraging ecology in subterranean termites and may offer a simpler, safer, and more rapid alternative to other tracers such as various radioisotope markers or the nonradioactive rubidium (Rb). The marker seems nontoxic and nonrepellent. Furthermore, the sandwich ELISA procedure is very sensitive and specific, does not generate hazardous waste, and does not require any specialized equipment. Relative to radioisotope markers (e.g., ^{60}Co ; Suárez and Thorne 2000), the IgG protein marker shows two important differences. First, the uptake of the protein marker is more rapid. It took ≈ 1 d for the IgG marker to reach maximum uptake, whereas the ^{60}Co marker took ≈ 14 d for. Second, the protein marker has a much shorter retention time (≈ 4 versus ≈ 11.5 d for ^{60}Co). One the one hand, the shorter retention time can be beneficial because the markers is eliminated faster and does not become internally sequestered, which could result in incorrect estimates of the rate and quantity of the transfer. However, the IgG marker may be less suitable for field studies where long-term tracking may be necessary. The shorter retention time may be caused by differences in the chemical nature of the two markers. The protein marker is organic and could therefore be readily metabolized by the termites. In contrast, the radioisotope marker is inorganic, cannot be metabolized, and might be sequestered within the tissues.

The results of this work may have implications in designing control programs based on baits where the

toxicant is taken back to the colony and shared through trophallaxis with other colony members. Recently, baits have been shown effective for controlling subterranean termites (Su and Scheffrahn 1993, Su et al. 1995, Sheets et al. 2000). The results of our study suggest that toxicants presented in baits may have the potential to reach a relatively large portion of the colony, especially workers, which are the most numerous caste and one responsible for the majority of foraging. Furthermore, liquid termiticides, inadvertently ingested by the termites during routine foraging, cleaning, and/or building activities could be shared in a similar manner.

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