

# Observational Notes on Short-Lived and Infrequent Behaviors Displayed by *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

J. G. WHITMAN AND B. T. FORSCHLER<sup>1</sup>

413 Biological Science Building, UGA, 120 Cedar Street, The University of Georgia, Athens, GA 30602

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**ABSTRACT** Behaviors displayed by worker termites, which make up 80–90% of any eastern subterranean termite population, provide the foundation for understanding the details of termite social organization. Individually marked termite workers were placed in an enclosed, darkened arena and videotaped for three 24-h periods. Behaviors observed in detail include ecdysial assistance, gallery excavation, oscillatory movements, worker feeding and adult mating. Primary reproductive pairs were observed to mate, on average, once every 3 d. Ecdysis lasted 43 min and involved multiple allogrooming attendants. Excavating termites manipulated the substrate with their mouthparts and showed fidelity to the site of excavation but not the site of deposition. Four different types of oscillatory movements were characterized with the type IV always ending in defecation. Worker feeding behaviors are described that involve swallowing materials obtained by five different routes and evoke use of the superorganism concept. The relevance of these observations for a better understanding of a variety of topics, including biology, management, and evolution of these economic pests, is included.

**KEY WORDS** subterranean termite, worker behavior, feeding, social interactions, allogrooming

Our understanding of termite biology is impaired by conundrums that would benefit from detailed descriptions of various behaviors (Thorne et al. 1999, Laine and Wright 2003). The majority of published reports were conducted by visual observations made in real time. Descriptive accounts of short-lived or infrequent behaviors, taken as they occur, are complicated by rapid movement or frequency of occurrence. Reviewing film has several advantages, including the opportunity to score events at a later time, detailed examination of short-term or rapid movements, and simultaneous scoring of multiple subjects. Examples of filming in the literature include several studies of termopsid vibratory behavior (Howse 1962, 1965; Stuart 1988) and one each on kalotermitid group interactions (Maistrello and Sbrenna 1996), *Coptotermes* delate behavior (Raina et al. 2003), and feeding of *Coptotermes*, *Reticulitermes*, and *Incisitermes* (Indrayani et al. 2007).

We recently concluded an experiment that required reviewing >400 h of videotape of *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae) that yielded several interesting qualitative observations. In this article, we describe five behaviors not previously discussed in detail such as mating between 18-mo-old kings and queens, ecdysis in the worker caste, “tail-chasing,” feeding, and excavation. In addition, we profile four types of oscillatory movements (OM) and conclude with a discussion of the implications of these observations.

## Materials and Methods

Detailed descriptions of the arena and experimental design can be found in Whitman (2006). Marked termites were videotaped in three-chambered, glass-topped, artificial arenas made of Densite. The narrow confines of a termite gallery were simulated by an arena depth of 2 mm. The three chambers, each measuring 2.3 by 3 cm, were connected by 3-mm-wide openings in the 1-mm-thick walls that defined separate chambers. Chambers were half-filled with  $\alpha$ -cellulose powder and kaolin clay as feeding and tunneling substrates, respectively. Three Jai coefficient of variation-M50IR charge-coupled cameras, two equipped with Navitar 7000 zoom lenses and one with a 35-mm telephoto zoom lens, were placed above the arena to record termite behaviors with one camera per chamber. Recordings used two Panasonic AG-2560 SVHS and one Emerson DA-4 Head brand VCR. AEC Box-18 video time-code generators allowed precise determination of behavioral start and stop times. The VCR’s “extended play” mode enabled 8 h of continuous recording but resulted in a loss of video quality. The movement of small body parts such as the maxillae and maxillary palps were obscured, but there was sufficient clarity to observe movements of the body, antennae, and mandibles.

Six arenas were filmed involving two treatments, laboratory colonies that were 18-mo-old and equal-sized groups of workers randomly selected from field-collected populations. The six replicates, containing

<sup>1</sup> Corresponding author, e-mail: bfor@uga.edu.

≈50 termites per arena, were filmed for 24 consecutive hours on three separate days: days 1, 4, and 7 after placement in the arena. The behaviors described included a review of all 432 h of video on fast-forward to catch the mating, tail-chasing, and ecdysial events reported. Feeding, OMs, and excavation behaviors were scored on six workers per replicate for 12 evenly spaced 15-min intervals over the course of a 24-h day, totaling 3 h per worker per d to obtain the frequency and time-spent data.

**Glossary of Behavioral Terms.** In the interest of discussion, we provide a glossary of terms, listed alphabetically, used in the behavioral descriptions. Video files of selected behaviors can be viewed and downloaded at <http://www.ent.uga.edu/termitebehavior.htm>.

*Allogrooming* is defined as grooming of one individual by another in contrast to self- or autogrooming. Allogrooming was characterized by rhythmic lateral movements of the mandibles while the head capsule remained in contact with various body parts (i.e., the head, antenna, legs, thorax, and abdomen) of another termite. This type of grooming did not physically displace the subject being groomed.

*Chewing* behavior was described by movement of the mouthparts indicative of macerating a substrate before ingestion. Chewing was characterized by rhythmic lateral movement of the mandibles. The substrate being chewed was often clearly visible as it was manipulated by the mouthparts before consumption.

*Consumption* was defined by cessation of chewing with no visible signs of having donated or deposited the macerated material. We divided consumption into two classes (autofeeding and allofeeding) based on how the food was acquired. Trophallaxis, as described by Wheeler in 1918 (Sleigh 2002) and applied in later studies by Wilson (1975), is an exchange of food and information that could be either stomodeal (by mouth-to-mouth contact) or proctodeal (by mouth-to-anus contact). Trophallaxis and feeding on wood was found to be inadequate to describe the distribution of behaviors we term "consumption" by worker termites. Materials that were procured through some action by the consumer are termed *Autofeeding* events and included  $\alpha$ -cellulose obtained from the arena (autofeeding cellulose, assumed analogous to feeding on wood); a bolus formed during the act of allogrooming (autofeeding allogrooming); and a clear, viscous bolus formed after regurgitation (autofeeding regurgitation). *Allofeeding* was defined by consumption of a donation from the anus of a nestmate (allofeeding proctodeal) or the mouth (allofeeding stomodeal).

*Excavation* was the act of extending a gallery through one of the two substrates provided (kaolin clay or cellulose powder) and was accomplished in four distinct phases: pill formation, pill transportation, pill deposition, and return to the excavation site.

Pills were formed by a termite facing the active site of excavation. *Pills* were "barrel-shaped" objects whose width and length were presumably established by the size (width) of the mouthparts and were formed, in our study, of either kaolin clay or  $\alpha$ -cellu-

lose powder. It was assumed that the maxillary and labial palps performed the bulk of shaping the macerated substrate, into a pill, by pressing against the labrum.

*Pill formation* was initiated by a lateral extension of the mandibles followed by forward movement of the body (tarsi remaining stationary) that pushed the head capsule into, as the mandibles closed and cut, the substrate simultaneous with alternating rotations (describing a 45° arc) of the head capsule. The overall impression was that the substrate was "shoveled" into the mouthparts. This distinctive head motion, described by Stuart (1967) as "a characteristic side-to-side motion of the head" and "pulling activity" by Indriyani et al. (2007), was observed in both pill formation and deposition. Pill formation was accompanied by a barely discernible rocking motion of the entire body as the head capsule was pushed into and pulled away from the substrate. The movements described for pill formation were displayed by termites obtaining a bolus of cellulose from the arena but was differentiated from excavation by chewing and consumption—in succession—to encompass the act of autofeeding cellulose. Pill formation is analogous to the three "activities" describing as "feeding behavior" in Indriyani et al. (2007).

*Pill deposition* was characterized by repeated, alternating head capsule rotations combined with a backward movement of the body mimicking pill formation in reverse. Pill transportation and deposition were readily distinguished from chewing by the absence of mandibular movement and characterized by the mandibles being held in an extended or open posture.

*Pumping* is indicative of the hydrostatic swelling of the body during ecdysis before rupture of the ecdysial suture (Chapman 1998). In termite workers, pumping was characterized by peristaltic-like contractions of the body that started at the tip of the abdomen and progressed to the thorax.

*Tail-chasing* is a behavior characterized by two workers chasing the tip of each other's abdomen resulting in tracing circles with their movement.

*Vigorous allogrooming* was strong enough to physically displace or move the subject being groomed, often for a distance of several body lengths.

## Results and Discussion

**Mating.** Three mating events were observed in 18-mo-old *R. flavipes* laboratory cultures that contained primary reproductives, soldiers, larvae, and workers. Eggs were not present in any replicate throughout the experiment. Reproductives from one culture mated twice, on day 1 and day 7, whereas one replicate displayed one mating event, on day 7, and the third pair never mated. The adult females were not physiogastric; thus, we could not differentiate males and females with certainty. Mating events lasted 34–42 s (mean duration, 38.7 ± 4.2 s) and involved three phases: antennation, positioning, and coupling. Adult

behaviors were noted 20 min before and after each mating episode.

Mating events were determined to begin when one adult approached and rapidly antennated the other's head capsule. This behavior was almost instantaneously returned in kind. The rapid antennations by each adult started from the head and proceeded at roughly equal speed to the tip of the abdomen. The forward motion this required, combined with the focus each had on the other's abdomen, caused each reproductive to describe a rough half-circle at which point antennation ceased. The head of the first adult was adjacent to the abdominal tip of the second and vice versa ending the antennation phase which lasted 7–9 s ( $8.7 \pm 1.5$  s).

The positioning phase followed as the adults straightened their bodies and walked forward until the tips of their abdomens were roughly parallel, and, with slight adjustments by both, brought their abdominal tips together. The positioning phase lasted 3–8 s ( $5.0 \pm 2.6$  s) and was followed by coupling, which was maintained for 22–30 s ( $25.0 \pm 4.4$  s). Mating events ended with uncoupling and the adults, after walking a short distance, resumed what they had been doing earlier—standing still.

Afzal (1985) studied mating in >300 reproductive pairs, established and recently paired, of the drywood termite *Bifiditermes besoni* (Gardner). Afzal reported a complex mating ritual involving antennation, head-butting, allogrooming, and “abdominal quivering” reminiscent of OMs, without providing durations. The mating episodes we observed did not involve any obvious preparation beyond the initial antennation.

The pair that was observed to mate twice also performed several episodes of the antennation phase—as had preceded the other mating events—without proceeding to the positioning or coupling phases. There were three displays of rapid antennation behavior observed on day 4 (successful matings were recorded on day 1 and 7 with this pair), with 10 min elapsing between the first and second episodes, whereas 30 min passed between the second and third rapid antennation displays.

Raina et al. (2003) analyzed time-lapse video recordings and determined that *Coptotermes formosanus* (Shiraki) reproductives ( $n = 19$  pairs) mated, on average, 2.2 times in the 48-h immediately after flight and pairing. Raina et al. (2003) noted “extensive grooming” between reproductives immediately before mating, a behavior we did not observe, and that *C. formosanus* “matings” lasting  $27.9 \pm 7.8$  s, which is close to the coupling phase ( $25.0 \pm 4.4$  s), we recorded for *R. flavipes*. *C. formosanus* laid eggs in batches over the course of weeks rather than daily (Raina et al. 2003). It is clear from our 9 d of video that egg laying is not a daily occurrence in *R. flavipes* as assumed by Grube and Forschler (2004) in their population growth model.

This is the first detailed account of mating events in *R. flavipes*. Undoubtedly, primary reproductives mate shortly after flight, as evidenced by the 18-mo-old laboratory cultures we filmed that consisted of work-

ers, soldiers and larvae but no eggs. Matings occurred in our *R. flavipes* study once every 3 d, a figure that agrees with the conclusions of Costa-Leonardo and Patricio (2005) that spermatheca size speaks to multiple matings. The variability of termite behavior is illustrated by the one reproductive pair we observed to mate twice, a second pair mated once, and the third was never observed to mate over 3 d.

**Tail-Chasing.** We observed eight separate pairs of workers performing a tail-chasing behavior similar to the “half-turn” described with adults during the rapid antennation phase of mating. Tail-chasing was initiated by a worker that seemed to solicit a proctodeal exchange by antennation of the abdominal tip followed by the intended donor turning and attempting to solicit the same by antennation. Workers would then describe full circles with the tandem movement as each tried to maintain antennal or head capsule contact with the other's abdominal tip. Chewing by either participant was never observed after a tail-chasing episode. There was no rapid antennation, and tail-chasing did not end with an attempt to bring the abdominal tips together, indicating that this behavior was not a mimic of the mating half-rotation displayed by the adults. Tail-chasing events persisted longer (mean duration  $413 \pm 201$  s, range 157–725 s,  $n = 8$ ) and described numerous complete circles (each revolution taking  $9.6 \pm 1.7$  s, range 8–12 s,  $n = 8$ ) compared with the single half-turn executed by the adults during the antennation phase (mean duration 8.7 s, range 7–10 s,  $n = 3$ ).

Tail-chasing behavior has never been mentioned in subterranean termites. We hypothesize this display is the result of miscommunication of the signal to solicit proctodeal material and suggests antennation of the abdominal tip, alone, does not signal a request for donation. The state of the donor should play a role in allofeeding proctodeal events and the rarity with which this behavior was observed would indicate miscommunication. However, tail-chasing could simply be a high-speed rehearsal for the adult mating half-turn. Unfortunately, we did not identify the sex of the performers and so cannot provide support for the latter hypothesis.

**Ecdysis.** We witnessed two molting events during our experimental observations. The first began around 2:28 p.m. on day 7, lasted 43 min, and included three phases: pumping, ecdysis, and recovery. The subject began pumping with contractions increasing in intensity and duration over 12 min. The ecdysis phase began with the cuticle splitting along the upper dorsal midline of the thorax as the not-yet sclerotized insect emerged from the old cuticle. The time from suture rupture to removal of the old cuticle took  $\approx 10$  min. The recovery phase lasted 20 min and consisted of the transition from a near motionless state (there was periodic leg flexing) in a side-laying recumbent position to the return to an upright posture and self-locomotion.

Pumping attracted the attention of a single nestmate that began allogrooming at 2:40 p.m., and this individual continued allogrooming throughout the ecdysis

event. The number of workers performing allogrooming varied over the course of the ecdysis as the subject was continually allogroomed by as many as five nestmates at any given time. Termites involved in ecdysis-allogrooming displayed various degrees of fidelity, ranging from 30 s to 5 min, and only one individual groomed throughout the entire event. The vigorous allogrooming observed during ecdysis displaced the subject over an area of 3 cm<sup>2</sup>. Allogrooming of a nonecdysing worker never displaced the subject over more than a body length.

The exuvia was constantly attended, during and after the molt, by at least one termite that seemed to be engaged in allogrooming. It was never evident that any termite attempted to consume the exuvia during ecdysis, but once it was completely removed the old cuticle was torn apart and consumed by 16 different termites over the next 28 min. The newly molted subject solicited and procured a proctodeal allofeeding 10 min after the recovery phase ended.

During the course of the first molting event, a second termite undergoing ecdysis was dragged onscreen at 2:45 p.m. by vigorous allogrooming. At the time of appearance, several terminal abdominal segments of the second subject still remained in the old cuticle. The second event proceeded as the first, including the gradual decrease in allogroomers and consumption of exuvia. Comparing the ecdysis timelines, the second ecdysis seemed to have begun  $\approx$ 3 min before the first.

La Fage and Nutting (1978) state that consumption of exuvia by termites is primarily an act of sanitation with the secondary role of nitrogen recycling. Rosenhaus and Traniello (1993), working with *Zootermopsis*, mention the act of nestmate assistance with molting, although their description does not detail the number involved or percentage of molting events that involved assistants. Su and Scheffrahn (1993) likewise mention, without elaboration, that molting is a group event. The molting episodes we described are the first detailed description of ecdysis being an activity that attracts the attention of up to 16 different workers.

Group-assisted molting behavior has implications for a diversity of topics such as toxicant transfer and evolution of social behavior. The benefit of developing a life history dependent on group-assisted growth (molting) would be to hold immatures to a social lifestyle analogous to the dependent larval stage of the social Hymenoptera (Wilson 1971, Nalepa 1994, Shellman-Reeve 1997). The mechanisms used to explain toxin transfer, for example, mention mortality during ecdysis (Su and Scheffrahn 1993, Sheets et al. 2000) without considering the mechanism of group-assisted molting. The "jack-knife" position of workers exposed to a chitin synthesis inhibitor described by Su and Scheffrahn (1993) is analogous to the condition we observed at the beginning of the ecdysis phase—after hydrostatic pumping and suture rupture—while the not-yet sclerotized termites' upper thorax emerge but the lower thorax, legs, and abdomen remain in the old cuticle. We can speculate on a reason for finding termites in this position in those bioassays assuming that allogrooming nestmates assist in ecdysis by com-

pleting the molt through extracting the lower thorax and abdomen. The jack-knife position would occur as a notable event in bioassay as the result of no assistance during molting and possibly indicating a signal that the affected termite is "not normal."

**Excavation.** Termites involved in excavation did not differentiate the food quality of the substrate. It should be noted that termites involved in pill formation were never observed consuming either substrate nor were termites observed to use a body part other than mouthparts during gallery construction. Gallery formation involved excavation in either  $\alpha$ -cellulose powder or kaolin clay ( $n = 136$ ). Excavation was characterized by three stages beginning with obtaining and manipulating the substrate to form a pill (pill formation), which was transported from the excavation site (pill transportation) and placed at another location (pill deposition). Deposition of a pill was usually followed by return to the excavation site to continue the task of gallery construction.

Excavation sites were often attended by more than one termite. The width of a developing gallery allowed a single termite to extend the gallery by pill formation, which forced others intent on excavation to line up or wait at points adjacent to the gallery entrance. The worker that was excavating would form a pill and back out, at which point those waiting would push forward in an attempt to gain access to the developing end of the gallery.

Pills, once formed, were immediately transferred out of the developing gallery and placed along the periphery of one of the chamber walls. A limited number of pills were deposited on the floor near the excavation site before return to the queue or to pill formation at the excavation site. These "errant" pills were later moved to the chamber walls, but it was not determined whether the same termite was involved in the later movement. Chamber walls were lined with pills within the first 24-h in five of the six replicates, with the exception being a replicate where excavation began between days 4 and 7.

Individuals involved in excavation had several locations where pills were deposited with no indication of site fidelity. The route to and from the deposition site was often circuitous especially in chambers where other termites obstructed a direct path. Return to the excavation site was, on occasion, interrupted by a stop at another excavation site but the worker would always leave this "secondary" site and return to the original to form a pill. Individuals were seen at different excavation sites at different observation intervals, but they were never observed to excavate at more than one site during a particular 15-min scoring interval, indicating a degree of excavation site fidelity.

The literature on termiticide efficacy has, for 20 yr (Su and La Fage 1984), endeavored to account for the importance of behavioral reaction to soil treatments (Forschler 1993, Gold et al. 1996, Valles and Woodson 2002), but the details of the mechanics of excavation have yet to be considered (Laine and Wright 2003). Recent studies on termite tunneling have focused on the direction, distance, and angle without examining

the mechanism (Pitts-Singer and Forschler 2000, Cornelius et al. 2002, Su and Puche 2003, Campora and Grace 2004). Fifty years ago, Ebeling and Pence (1957) reported that *R. hesperus* used the head and body to compact sand while small particles were taken into the "buccal cavity" and mixed with a "gluey substance" for placement along gallery walls, whereas larger particles were carried in the mandibles. The use of dried fecal pellets as building blocks and liquid feces as cement has been noted in *Zootermopsis* (Stuart 1967, Howse 1968) and *Macrotermes* (Gerber et al. 1988). However, we observed none of these practices in *R. flavipes*. Whether the differences indicate species-specific repertoires or are merely artifacts of our experimental design will require further experimentation. It is clear from our study that gallery construction through substrates with a small particle size is linked to pill formation. Close examination of shelter tubes suggests that pills are the basic building blocks of subterranean termite construction. We have observed, in structural infestations, shelter tubes composed of pills of earth, polystyrene insulation, and gypsum several feet from the assumed source of those materials. Understanding this aspect of termite behavior would shed light on the potential for movement of toxicants using pill formation/construction behavior. Gallery construction involving extensive manipulation of the substrate using mouthparts would certainly play a role in understanding transfer of substrate-borne termiticides. Our data indicates that 50% of the time spent during gallery construction (pill formation, transportation, deposition, and return to the excavation site;  $n = 136$ ) involves contact with a pill, exposing excavating termites to an oral dose of toxicant.

**Oscillatory Movements.** We recorded four distinctly different OMs but because of the overhead view it was difficult to determine any vertical component. An OM was described by longitudinal oscillations that moved approximately one-quarter of a body length in alternating forward and backward movements that began with a clearly defined forward lunge. The termite's tarsi remained stationary on the substrate while its body was in motion. OMs, from our observations, involved the same movements but were differentiated by speed, duration, and repetition.

The type I OM was brief, lasting  $\approx 0.5$  s from the first oscillation to the last, with the rapid motion blurring the video-taped image ( $n = 390$ ). The type II OM was much slower taking  $\approx 2$  s to complete ( $n = 140$ ). The type III OM type involved a type I followed immediately by a type II ( $n = 269$ ). The type III OM was often performed three times or more in succession. The type IV OM was  $\approx 3$  s in duration and contained the slowest oscillations with each movement clearly visible on video ( $n = 85$ ).

The type IV OM was associated with defecation. Type IV OMs always immediately preceded deposition of visible frass. Assigning clear associations to any of the other OMs was difficult, because we did not detect an apparent precursor—behavior or incident—that triggered an OM, nor could we assign a consistent nestmate response. The type III OM dis-

played as a single event did, on occasion, signal a nestmate to cease grooming or move aside. The type III OMs performed in succession induced no apparent reaction from nestmates. It is worth noting that OMs were never performed by termites waiting for another to vacate an excavation site.

Howse (1962) coined three terms to describe the distinctive jerking or jittery movements observed in termites. The first, called vertical oscillatory movement (VOM), was described as a rapid up-and-down motion of the termite head capsule. The second, termed longitudinal oscillatory movement (LOM), was indicated by a back and forth motion of the entire body, whereas complex oscillatory movement (COM) involved both vertical and horizontal body movements. Oscillatory movements have been described from numerous termite species and referred to as an "alarm reaction" (Howse 1962, 1964, 1965), as evoking attraction (Stuart 1963, Reinhard et al. 1997, Reinhard and Clement 2002), dispersal (Rosengaus et al. 1999a), or eliciting building and repair activities (Stuart 1963, 1967). The occurrence of OMs suggest this behavior has purpose, yet the disparate associated activities clearly indicates additional study is required to fully understand the reason or function of this behavior.

We observed termites being groomed perform a single type III OM at which point the grooming termite immediately ceased. Additionally, termites moving through an arena were observed to perform a single type III OM if their path was blocked by a nestmate, which typically resulted in the obstructing termite moving to create a clear path. This observation agrees with a point by Maistrello and Sbrenna (1996) that *Kaloterms* performing OMs are responding to a short-term need rather than an alarming stimulus. Linking the type IV OM with defecation is the first substantive (100%;  $n = 85$ ) connection between an OM display and a resultant behavior. The type IVs slow, rhythmic mimic of the rapid type I-III OMs allows comparison to the longitudinal body movements involved in pill formation. We hypothesize that the rocking motion that accompanies extraction of a mouthful of substrate during pill formation, also described by Indriyani et al. (2007) for three species feeding on wood, are one and the same and the genesis of the movements involved in OMs.

**Feeding Behavior.** Observations on the mechanics and distribution of feeding behaviors can provide insights into the digestive physiology of subterranean termites. There were five routes by which the workers in our study consumed something: autofeeding cellulose ( $n = 311$ ), autofeeding regurgitated ( $n = 198$ ), autofeeding allogrooming ( $n = 146$ ), allofeeding stomodaeal ( $n = 246$ ) and allofeeding proctodaeal ( $n = 336$ ). The nutritional value of each material was not examined but can be inferred based on the source of the material.

Autofeeding cellulose was assumed to be analogous to termites feeding on wood and was the only source of unaltered cellulose we observed to be consumed by the worker caste. Three routes involved partially digested materials—autofeeding regurgitated and both

allofeedings. Autofeeding allogrooming ( $n = 146$  of 2,983 allogrooming events) has never been reported as a feeding behavior, and the food quality of this material should be examined in future research. Termites autofeeding allogrooming were never solicited for a donation to initiate an allofeeding stomodeal event perhaps, indicating it has little nutritional value. We also observed termites that displayed no previous activity, as confirmed by review of video for 2 min prior, suddenly begin chewing. The material was assumed to be regurgitated because it was thin, clear, and unlike the white pulp typically seen in autofeeding  $\alpha$ -cellulose. This is the first delineation of *R. flavipes* workers "recycling" gut contents by regurgitation and this route represented 16% of the "consumption" or feeding events ( $n = 1237$ ) recorded in our study.

Worker-to-worker allofeeding was observed to involve three phases: initiation, procurement, and chewing. Allofeeding was observed to be a recipient-driven process—100% initiated by antennation followed by eventual mouthpart contact with the donor. Initiation always involved either antennation and/or allogrooming. Approximately 50% of allogrooming events ended in an allofeeding donation. Allogrooming preceded half of the proctodeal and two-thirds of stomodeal allofeedings. The antennation that preceded allofeeding always involved the body part of the donor used in the donation, and it provided equivalent durations proceeding either proctodeal ( $1.5 \pm 0.61$  s) or stomodeal ( $1.1 \pm 0.68$  s) allofeeding. Allofeeding stomodeal was observed to always involve a donor that was already chewing material obtained by four of the aforementioned five routes of food acquisition. Termites autofeeding  $\alpha$ -cellulose were donors in 39.1% of allofeeding stomodeal events. However, allofeeding materials made up the majority of stomodeal donations, with 32.4% taken from termites chewing after allofeeding proctodeal and 27.3% from those chewing allofeeding stomodeal. The remaining 1.3% of allofeeding stomodeal events involved termites autofeeding regurgitation.

Procurement of proctodeal material ( $8.1 \pm 36.5$  s) took more time than stomodeal ( $6.8 \pm 15.6$  s) and was characterized by movement of donor mouthparts in contact with the appropriate recipient body part. Time spent chewing proctodeal donations before consumption was slightly less ( $137 \pm 168$  s; median 72 s) than allofeeding stomodeal ( $167 \pm 188$  s; median 103 s). Autofeeding  $\alpha$ -cellulose powder was the most time-consuming food-procurement activity we recorded ( $184 \pm 169$  s; median 122 s).

The traditional definition of trophallaxis has its observational roots in food exchange between adult and larval Hymenoptera (Wilson 1971). Stomodeal trophallaxis used in reference to termites often assumes a two-way exchange of materials (Nalepa 1994, Cabrera and Rust 1999, Valles and Woodson 2002), although others have defined it as a one-way transfer of alimentary liquids (Suarez and Thorne 2000a, 2000b). In our observations, stomodeal allofeeding seemed to be a one-way transfer, although a two-way exchange would have been difficult to detect because

the donor's behavior did not change (i.e., it was chewing when the event began). However, the literature often does not differentiate, in experiment design, between stomodeal and proctodeal trophallaxis and usually assume a one-way transfer (Rosengaus et al. 1986; Cabrera and Rust 1999; Sheets et al. 2000; Suarez and Thorne 2000a, 2000b; Saran and Rust 2005).

Liquid held in the labial gland reservoirs of *R. santonensis* is taken from sources outside the body and used both to moisten building material and maintain humid conditions within the network of galleries (Grube et al. 1997, Grube and Rudolph 1999). Labial gland secretions also are involved in chemical communication including feeding site selection (Kaib and Ziesmann 1992, Reinhard et al. 1997, Reinhard and Kaib 2001). We propose that our observations support the hypothesis that chewing exudes a feeding-stimulus, perhaps including labial and/or salivary gland contents. The hypothesis of a chemical signal fits the observation that recipient-workers moved directly to a chewing nestmate to initiate an allofeeding stomodeal event. There was little attraction to workers chewing autofeeding regurgitation, which represented 1.3% of all allofeeding events. The rarity with which we observed sharing of regurgitated material cannot be explained by the behavior of the potential donors because all chewing termites acted the same. It seemed that there was simply no interest on the part of the potential recipients—as if termites were not "chewing." It should be reiterated that termites autofeeding allogrooming were never solicited and therefore never shared that material.

It was observed that allofeeding proctodeal recipients often completed the event with a quick and sudden separation of mouthparts from the anus of the donor. Three anecdotal observations further illustrate the "sticky" nature of proctodeal material. On one occasion, a termite backed into another termite's leg and was instantly stuck as evidenced by the lateral movement of the stuck termite for  $\approx 1$  cm before separation. A second involved a termite that seemed to glue itself to the chamber floor by backing into it immediately after a proctodeal donation. The stuck termite struggled for several seconds before it was able to separate from the floor and move freely. We also recorded a worker that obtained proctodeal material from a nestmate, but the separation was not clean. A thin strand of material could be seen connecting the two that quickly became rigid, as evidenced by synchronous movement of the donor's abdomen and the recipient's head for 80 s after the completion of the proctodeal event. Proctodeal transfers have been cited as a mechanism for reestablishing the hindgut fauna lost during molting (Weiss 2006). The first feeding observed by freshly molted termites ( $n = 2$ ) was allofeeding proctodeal and occurred within 10 min of the conclusion of ecdysis. Based on our observations, we propose that the viscous, sticky nature of proctodeal material serves to protect protists during transport through the upper alimentary tract in newly molted individuals.

In conclusion, behavioral observations can address questions affecting numerous aspects of biology and this manuscript illuminated additional avenues of inquiry examining a diversity of topics involving subterranean termite biology. The frequency of mating reported herein corroborates statements by Raina et al. (2003) and Costa-Leonardo and Patricio (2005) that morphology can be used to infer mating frequency. Additional work on the relationship between mating and egg laying also would resolve whether the incidence of mating we observed in three laboratory cultures without eggs is typical of reproductive pairs in that segment of the egg-laying cycle. Clearly egg laying is not a daily occurrence in the rhinotermitids, and models of colony growth should account for this phenomenon.

The conclusion that ecdysis in *Reticulitermes* is a group-assisted event should be acknowledged as important for understanding the evolution of eusociality (Thorne 1997) in addition to partially explaining colony-level immune response as influenced by group size (Rosengaus et al. 1999b, Traniello et al. 2002). The observation that 15 termites consumed the exuvia should increase the accuracy of toxicant-transfer models, including group-assisted molting. Our observations on group-assisted molting suggest recognition of intoxicated workers could be manifested by jackknifed cadavers in bioassay and further work could illuminate how communication of health-status impacts toxin transfer. The literature contains comment on cannibalism in toxicant transfer without quantifying the incidence or impact (Su and Scheffrahn 1993, Sheets et al. 2000, Valles and Woodson 2002). We did not observe cannibalism in the current study, but have noted it in bioassay control groups using paint-marked termites (B.T.F., unpublished data). The cues that lead to cannibalism in subterranean termites also needs further study.

The role that pill formation/deposition plays in gallery construction with its attendant oral manipulation of the substrate opens new approaches to understanding termite foraging behavior. The mechanics of gallery construction through pill formation should be considered when modeling foraging, interpreting termiticide tunneling bioassay data, and explaining movement of soil-based toxicants.

The distribution of feeding behaviors described from this study should be considered in future studies of termite digestive physiology (Inoue et al. 1997, Tokuda et al. 2005, Zhou et al. 2007). Our feeding data, either time-spent or frequency, when viewed on the level of an individual termite was highly variable and not normally distributed (Whitman 2006) matching the variability noted in the literature (Forschler 1996, Thorne 1998). Applying the superorganism concept (Wilson 1971, Wilson and Sober 1989) to the data however, by combining 9 h of video per worker from all 36 workers, illuminates interesting trends. These data indicate that *Reticulitermes* workers, assuming frequency equates to amounts consumed, acquire one-third of their diet from each of three routes: autofeeding cellulose, allofeeding proctodeal, and al-

lofeeding stomodeal. Unaltered consumables (457 autofeeding cellulose and allogrooming of 1,237 consumption events) make up 37% of the worker diet, whereas the remaining 60% is partially digested (both allofeedings and autofeeding regurgitated). Assuming that time spent in the different major feeding categories was indicative of amounts consumed (medians for autofeeding cellulose, 122 s; autofeeding regurgitated, 23 s; allofeeding stomodeal, 103 s; and allofeeding proctodeal, 72 s) easily half the food consumed by worker termites involves substrates subjected to a digestive process before acquisition.

The frequency and type of allofeeding we described also has implications for interpreting toxicant transfer data that often provide equivocal results (Ibrahim et al. 2003, Remmen and Su 2005). Our observations, for example, could explain the "less than expected" transfer of [<sup>14</sup>C]sucrose by termites in a recent study (Saran and Rust 2005). Their experimental design fed donors and recipients in different arenas thereby permitting transfer by two food acquisition schemes: allofeeding proctodeal and autofeeding allogrooming. Two other routes, allofeeding stomodeal from nestmates autofeeding regurgitation and consumption of exuvia, are possible but occurred at such low frequency that their impact would be negligible. The presence of salivary cellulase (Nakashima et al. 2002) and removal of sugars in the alimentary tract supports the hypothesis that the experimental design used by Saran and Rust (2005) would have shown very little "trophallaxis" of [<sup>14</sup>C]sucrose.

Our observations on OMs have described a response to this behavior—that of the type IV with defecation—and detailed the similarity of the movements involved in OMs and feeding/excavation behavior. It is our hope that describing the mechanics and incidence of behaviors obtained in this study will stimulate further research.

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