OBSERVATIONS OF BEHAVIORS IN THE WORKER CASTE OF *RETICULITERMES FLAVIPES* (KOLLAR) (ISOPTERA: RHINOTERMITIDAE)

by

JEFFERSON G. WHITMAN

(Under the Direction of Brian Forschler)

ABSTRACT

Termites were placed in an enclosed, darkened arena and videotaped for three 24-h periods. Treatments consisted of laboratory cultured colonies and equally sized randomly selected groups of field-collected worker termites. Specific individuals were scored over 12 15-min periods for a total of 3-h per full day of video-tape. The behavioral repertoire of workers included thirteen behaviors and display of each behavior was highly variable for each individual. Workers spent 72.8% of their time performing no visible activity, 6.3 of their time allogrooming, 4.9 autofeeding cellulose, 4.0 proctodeal trophallaxis, 4.2 stomodeal trophallaxis, 0.5 chewing regurgitated material, 0.3 chewing after allogrooming, 4.5 excavation of substrates and 0.7 autogrooming. Excavation of substrate was the only behavior that consistently displayed a binary pattern of performance. This is the first study to document division of labor in the lower termite *R. flavipes.*

INDEX WORDS: task allocation, subterranean termite, insect behavior

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JEFFERSON G. WHITMAN

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JEFFERSON G. WHITMAN

Major Professor:	Brian Forschler
Committee:	Dan Suiter Duane Jackson

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
Dec 2006

DEDICATION

I would like to dedicate this paper to Dr. Daniel V. Hagan, professor of Biology at Georgia Southern University, whose entomology class sparked my interest in this field.

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CHAPTER 1

INTRODUCTION

The experiment we performed examined, in detail, the behavior over time of specific individual workers of the lower subterranean termite *Reticulitermes flavipes* to determine whether or not this species of termite displays division of labor. It also addressed the question of how well groups of randomly-selected termite workers used in lab bioassay model the behaviors of intact termite colonies found in the field. This work presents the results of this experiment and is divided into five chapters. The second chapter is a literature review which begins by examining the Hymenopteran roots of most insect behavior terms and the biological reasons why these terms may apply differently to the Isopterans and then delves into the behavioral literature on the upper and lower termites. The third chapter deals with behavioral observations obtained during the course of the experiment. These observations include details on activities such as matings between reproductives, molting, gallery construction and excavation and molting. In addition, the complicated process of termite feeding and its behavioral components are examined. The fourth chapter is concerned with the behavioral data we obtained and the statistical analyses performed on it. The behavioral repertoire of the termite worker is examined and the data are examined for evidence for division of labor and groups of randomly-selected workers are compared to intact colonies. Finally, the fifth chapter briefly summarizes our major results.

CHAPTER 2

REVIEW: BEHAVIOR OF THE LOWER TERMITE

2.1 Introduction

The social Hymenoptera have been the subject of observation for thousands of years. It is therefore not surprising, then, that they have been the topic of discussion regarding such diverse topics as entrepreneurial pursuits to social evolution (Wilson 1975, Gauld and Bolton 1988, Alcock 2001). Nearly all of the terms associated with insect behavior have their roots in Hymenopteran research, and termites themselves were commonly referred to as “white ants” until relatively recently (Hagen 1876, Reaumur 1926, Kofoid et al. 1934). Any reasonable review of the Isoptera should mention the work done on the Hymenoptera to define the terminology of social insect behavior and how it applies to termites.

This review is divided into two sections. The first will compare biological and behavioral aspects of the Isoptera and the Hymenoptera and address differences in how pertinent terminology applies to the two groups. The second section will focus on the Isoptera and compare the higher and lower termites while reviewing lower termite behavioral literature.

2.2 The Hymenoptera and Isoptera

Castes. Social Hymenopteran and Isopteran societies are composed of individuals that are categorized into castes based on their size and form. These castes are the result of species-specific developmental options. The entomological definition of caste is "A specialized level in a colony of social insects, such as ants, in which its members, such as workers or soldiers, carry out a specific function" (The American Heritage Dictionary 1985). An insect caste, then, is a group of individuals in a community that are similar in size, shape, and behavior but distinctly different from other individuals in that community. Castes allow for specialization of form and function while increasing the efficient maintenance of an orderly society (Oster and Wilson 1978, Gordon 1989a).

In the Isoptera, the primary castes are larva, worker, nymph, soldier, and reproductive. Workers comprise the bulk of a colony and perform tasks such as feeding, foraging, construction, repair, and grooming. The term “worker” technically refers to a “non-reproductive, non-solder individual of the third […] or later instar that has diverged irreversibly from the imaginal line” (Thorne 1996). This definition is therefore used with the caveats that individuals of some species can leave the imaginal line via a regressive molt and some individuals can become reproductives without ever entering the imaginal line (Laine and Wright 2003). Workers can differentiate into soldiers or reproductives, though the exact mechanism prompting this is not known (Laine and Wright 2003). The percent of soldiers in a population can vary widely among species (from approximately 15% among *Coptotermes* to 1-2% among *Reticulitermes*). Soldiers are much more numerous in the central-nesting higher termites, especially those with an "efficient chemical defense" (Roison 1990).

The reproductive caste produces eggs to increase the population. Primary reproductives are adults that progressed through the imaginal line and conducted a successful mating flight. Neotenics are workers that became sexually mature without entering the imaginal line and can be further divided into replacement and supplementary neotenics. A replacement takes the place of a primary reproductive while a supplementary becomes sexually mature while the primary reproductives are still functional (Thorne 1996; 1997). The Isopteran reproductive caste includes males and females that are long-lived, up to 20 years in the primary stage (Krishna and Weesner 1969).

Castes among the social Hymenoptera include a sterile female worker caste and a reproductive caste with a long-lived female and a male with a shorter, seasonal, lifespan. The sole purpose of the Hymenopteran male is to fertilize the female. They generally do not contribute to maintenance of the social integrity of the colony (Spradberry 1973, Dumpert 1978, Winston 1987). The Formicidae have a soldier caste that defends the nest and nestmates, but among the social bees older workers fulfill this role (Robinson 1992, Gordon 1996). The reasons why an individual develops into a specific caste has been examined in the Hymenoptera (Oster and Wilson 1978) but not in the Isoptera (Krishna and Weesner 1969).

The term "larvae" should be used cautiously when referring to termites because of its association with sexually immature holometabolous insects. As termites are not holometabolous and all termites but the reproductives are immature, this term is technically inaccurate (Thorne 1996). However, it is kept due to convention and is generally applied to first or second instar non-sclerotized termite workers (Laine and Wright 2003).

Development. The organization of Isopteran and Hymenopteran societies are fundamentally dissimilar, primarily due to developmental differences. Termites are paurometabolous, progressing through 5 or more instars, each one showing growth but little morphological change until the final molt. There are many developmental routes that can be taken by a termite on the path to a terminal form (Krishna and Weesner 1969, Thorne 1996). These routes, along with the long-lived immature stages, mean that a given population will contain overlapping generations. During the first two instars of a termite's development they are, like soldiers, dependent on the workers for nourishment (Traniello and Leuthold 2000). Past these instars they are social and independent (Thorne 1997).

Hymenopterans, in contrast, are holometabolous. The larvae are dependent on mature worker caste members for successful development. While only soldiers and the first two instars within a termite colony are considered dependent castes, all immature Hymenopterans - collectively referred to as brood (eggs and immatures) - are dependent. Hymenopteran reproductives, which are usually nest-bound, also rely on the worker caste for nourishment (Dumpert 1978, Winston 1987).

Another major difference between the two orders is that worker sex ratios of the Hymenopterans are entirely female (Gauld and Bolton 1988), while Isopteran societies contain male and female workers in equal proportions (Jones et al. 1988). Workers from both orders, however, perform many of the same tasks, such as nest construction and maintenance.

Nesting. Social insects inhabit some type of communal structure, commonly referred to as a nest. The details of nest architecture, materials, and size vary widely from species to species, but they all provide the stability and efficiency necessary to maintain a social structure. Nest are constructed and modified over time to suit the ever-changing needs of the community (Nielson 1992, Noirot and Darlington 2000).

The social Hymenoptera are generally considered centralized nesters - they have a single nest where the majority of the colony spends most of their time (Spradberry 1973, Dumpert 1978, Jeanne 1986, Winston 1987). The select few that leave the nest do so to forage for food and other resources that are returned to the nest. Isopteran nesting habits can be divided into two broad categories based on the division of lower and higher termites - the majority of the higher termite species favor a central nest, whereas the more primitive lower termites tend towards decentralization (Abe 1987).

The species that do use a central nest clearly display division of labor, with correspondingly less evidence for those species that do not (Traniello and Leuthold 2000). Dedicated foraging groups means less time wasted traveling between tasks and thus increased efficiency. Division of labor is therefore the most efficient means of organizing the foraging tasks required to maintain central-nesting societies (Wilson 1971).

Terminology of behavior. Behavior is defined as "The actions or reactions of persons or things under specified circumstances" and a task is "A piece of work assigned or done as part of one’s duties", with an implication that the assignment comes from a higher authority (The American Heritage Dictionary 1985). The identity of this authority is the topic of much discussion in the insect literature (Robinson 1992, Gordon 1989a; 1989b; 1995, Anderson et al. 2001).

In describing a potential task, one must use specific observed behaviors as building blocks. For example, if one were to describe a man building a brick wall (the task), behaviors such as "retrieving and stacking bricks" would be used. While direct observation is useful in describing what an individual is doing, it alone cannot answer why the observed behavior is being performed. A second type of task could be defined by location (stationary guard position) or a path of travel (patrolling an area in maintenance). This depends on the nature of the activity, which may or may not include observable behaviors. Determining the nature of this task would address the “why” rather than the “what” of the task itself.

Specific behaviors described in termite research include vibratory alarm, guarding, grooming, trophallaxis, brood and queen care, tunnel construction and nest maintenance (Howse 1962; 1964; 1965; 1968, Stuart 1963; 1967; 1988, Jones 1980, Kramm et al. 1982, Badertscher et al. 1982, Traniello and Beshers 1985, Rosengaus et al. 1986, Su and La Fage 1987, Gerber et al. 1988, Iwata et al. 1989; 1999, Grace and Zoberi 1992, Rosengaus and Traniello 1993, Kirchner et al. 1994, Kettler and Leuthold 1995, Boucias 1996, Maistrello and Sbrenna 1996, Myles 1996, Crosland et al. 1997, Crosland and Traniello 1997; 1998, Rohrig et al. 1998, Rosengaus et al. 1999, Connetable et al. 1999, Suarez and Thorne 2000a; 2000b, Shimizu and Yamaji 2003). Most tasks and behaviors described in termites involve direct manipulation of an object using mouthparts, particularly the mandibles, while vibratory alarms consist of moving the entire body and guarding by body position. The social Hymenoptera display all of the aforementioned behaviors, but these are performed by mature sterile female workers while most Isopteran tasks are performed by immature male and female workers (Wilson 1971).

Trophallaxis. Trophallaxis is a behavior by which insects obtain nutritive fluids from a nestmate, most often from the foregut (termed stomodeal) via the mouth but also from the hindgut (termed proctodeal) via the anus. Trophallaxis is common to nearly all social insects (Wilson 1971; 1975, Spradberry 1973, Dumpert 1978, Breed et al. 1982, Winston 1987, Hunt 1991). In the social Hymenoptera, trophallaxis is preceded by antennal stroking or grooming (Wilson 1975, Dumpert 1978, Winston 1987, Hunt 1991) but whether or not this is the case in the Isoptera is unclear. In the Isoptera, proctodeal trophallaxis enables a freshly molted termite to restore gut symbionts lost during the molting process (Nalepa and Bandi 2000).

The overall rate of trophallactic exchange is lower in lower termites than it is in the social Hymenoptera (Suarez and Thorne 2000a). Hymenopterans tend to transfer a higher percentage of gut contents per exchange than do termites (Wilson 1975, Rosengaus et al. 1986, Winston 1987, Suarez and Thorne 2000a). These differences may be due to the vastly different social context within which these exchanges occur. Hymenopteran trophallaxis can be divided into adult-adult and adult-larvae, each involving different types of nutritive material (Wilson 1971, Oster and Wilson 1978, Winston 1987, Hunt 1991). The social Hymenoptera have a dependent larval caste that is integrally involved in nutrient flow within the colony. This arrangement has been referred to as a “social stomach” (Wilson 1971). In contrast, the lower termites nest in and on their food resource, reducing the need to rely on worker to worker trophallaxis as a nutritional necessity, and relegating the task of foraging to location of food resources, followed by recruitment and colonization (Traniello and Leuthold 2000).

Direct observation of trophallaxis is useful in detecting behavioral patterns such as feeding differences between castes or individuals and can also demonstrate the use of one group as a unit of nutrient storage (Wilson 1971). While not providing quantitative data on the amount transferred, it is a tool that can be used to obtain qualitative information on who is feeding whom, how, and when.

Grooming. Grooming among social animals is either a cooperative cleaning act between members of a group (allogrooming) or a self-cleaning act (autogrooming) (McFarland 1981). Grooming in termites is primarily allogrooming (Maistrello and Sbrenna 1996). This is in marked contrast to the Hymenoptera, who readily clean themselves and often have body parts specially adapted to facilitate autogrooming (Wilson 1971, Spradberry 1973, Winston 1987).

Allogrooming is thought to be how harvester ants spread and maintain the "colony scent" among nestmates (Gordon 1999). Whether this is true in the Isoptera is unclear (Bagneres et al. 1991, Maistrello and Sbrenna 1996), but it at least serves to keep the cuticle clear of mycopathogens (Kramm et al. 1982, Iwata et al. 1989; 1999, Grace and Zoberi 1992, Boucias et al. 1996, Myles 1996, Shimizu and Yamaji 2003).

Construction and maintenance. Nest construction and maintenance are easily observed tasks that are essentially the same in the social Hymenoptera and the Isoptera (Spradberry 1973, Dumpert 1978, Winston 1987, Wenzel 1991, Noirot and Darlington 2000). It is clear that, among the social bees and wasps, these tasks are primarily performed by workers of certain ages (Dumpert 1978, Winston 1987, Jeanne 1991). Age specific tasks - termed temporal polyethism - have been demonstrated for the Termitidae (Badertscher et. al. 1982, Hinze and Leuthold 1999, Hinze et. al. 2002), but there is little evidence for this among the other termite families (Howse 1968, Rosengaus and Traniello 1993, Crosland et. al. 1997, Crosland and Traniello 1998).

Division of labor. Division of labor is defined as "the breakdown of labor into its components and their distribution among different persons, groups, or machines to increase productive efficiency" (Merriam-Webster's Online Dictionary). This is perhaps best exemplified in the social Hymenoptera, where division of labor based on age or morphology is well-documented (Wilson 1971, Spradberry 1973, Oster and Wilson 1978, Dumpert 1978, Winston 1987, Gordon 1989b; 1995; 1996, Jeanne 1991).

Temporal polyethism is common in the social Hymenoptera (McMahan 1979, Gordon 1989a, Jeanne 1991, Robinson 1992, Traniello and Rosengaus 1997, Gautrais 2002), where younger individuals tend to perform tasks such as nest maintenance, cleaning, and brood care. Older individuals assume behaviors such as leaving the nest to forage. The assumption is that higher risks are accompanied by decreased life expectancy, so it is most efficient for the older individuals to perform risky but necessary behaviors (Winston 1987, Jeanne 1991, Robinson 1992).

Morphological division of labor in the ants and termites is evident in the size differences between the worker and soldier castes of these orders. The bees and wasps, however, have less clearly defined size-based behavioral roles – large size may increase efficiency in some tasks, but not to the point of precluding other, smaller, individuals from performing those same tasks (Wilson 1971, Jeanne 1991).

Task switching involves altering work patterns to suit changing conditions. A Hymenopteran example would be ants that had been foragers becoming involved with nest repair (Gordon 1989a; 1989b; 1995; 1996; 1999). In one study involving lower termites, Crosland and Traniello (1997) used the term behavioral plasticity to describe what by any other name would be task switching, as they found that medium-sized workers were able to partially compensate for the absence of large workers by altering their behavior patterns towards those displayed by large workers.

Robinson and Page (1989) found evidence for a genetic division of labor in the honey bee, with sub-families consisting of the daughters of different males showing significantly different preferences for tasks such as corpse removal and nest guarding. It was later shown (Robinson and Page 1995) that these preferences were slow to change, suggesting limited plasticity. Whether termites display a similar method of genetic division of labor has not been examined.

Temporal and morphological polyethism can be difficult to separate because the age of a termite worker is not easily determined. Studies have assumed age and size are correlated (Crosland and Traniello 1997, Crosland et al. 1997). Caution must be exercised, however, because termites can undergo supernumerary or regressive molts, making age determination difficult beyond the second instar (Krishna and Weesner 1969).

Summary. Fundamental differences of biology exist between the social Hymenoptera and the Isoptera. However, both the social Hymenoptera and Isoptera are eusocial as defined by Wilson (1971), displaying reproductive division of labor, overlapping generations of laborers, and communal care of the young. It therefore seems reasonable to use Hymenoptera-derived terms while discussing the Isoptera as long as the differences that exist are recognized when describing certain activities appropriately unique to termites.

2.3 The Isoptera - higher versus lower termites

The Isoptera are divided into the higher and lower termites, the dividing factor being the presence of flagellated protists in the lower termite gut which assist in the breakdown and digestion of cellulose. The lower termites (families Rhinotermitidae, Hodotermitidae, Kalotermitidae, and Mastotermitidae) are comparatively primitive, sharing relatively few features with the social Hymenoptera. The higher termites (Termitidae) occupy a social middle ground between the lower termites and social Hymenoptera, sharing behavioral and developmental characteristics with both groups (Wilson 1971, Thorne 1997).

There are no true sterile workers among most of the lower termites, allowing any immature worker to theoretically become a winged adult, soldier, or neotenic (Wilson 1975, Oster and Wilson 1978). Sterile workers are prevalent among higher termites (Traniello and Rosengaus 1997), whereas lower termites display considerable developmental plasticity (Krishna and Weesner 1969). Neotenics are common in lower termites, but this blurring of the reproductive line is relatively rare in higher termites (Thorne 1997). Additionally, while gender often limits the developmental pathway that can be followed by a higher termite, this is not the case in the lower termites (Oster and Wilson 1978).

Nesting strategies vary among the Isoptera, where some higher termites display the central nest associated with the social Hymenoptera (e.g. the fungus-growing *Macrotermes*) (Abe 1987, Shellman-Reeve 1997, Noirot and Darlington 2000). These termites also tend to display obvious division of labor. The most primitive termites are “one piece” nesters. In these, the colony lives inside its food source until it is consumed and then disperses via newly-matured winged adults. The one piece nester termites have not been found to display division of labor, not surprising given the lack of any food-nest separation (Abe 1987, Shellman-Reeve 1997). However, there are lower termites (e.g. Mastotermitidae and most Rhinotermitidae) that use nesting strategies that are somewhere between these two examples. These termites live inside a food source, but forage to expand the colonies repertoire of food sources and shift between more than one food resource to feed and rear the young (Noirot and Darlington 2000). They have no true central nest; rather, they have multiple, independent, feeding/nesting sites (Grace et al. 1989, Forschler 1998). Multiple potential nest sites may add enough complexity to favor division of labor. As discussed above, spatial restrictions can reduce the efficiency of "generic" workers when work sites are widely distributed (Shellman-Reeve 1997, Noirot and Darlington 2000).

The lower termites are not commonly thought to exhibit temporal polyethism, but conclusions on this subject have been mixed (Howse 1968, Rosengaus and Traniello 1993, Crosland et al. 1997, Crosland and Traniello 1997; 1998). There are multiple examples of temporal polyethism among the higher termites, especially among the *Macrotermes* (Jones 1980, Badertscher et al. 1982, Gerber et al. 1988, Hinze and Leuthold 1999, Hinze et al. 2002).

Lower termite alarm reactions. Any hazard faced by an individual within a social insect society could threaten the entire colony. It is therefore of benefit to alert others to the presence of danger to either enlist aid or warn nestmates. Termites are known to communicate distress via two methods: the release of chemical signals and the production of substrate vibrations. Vibrations are produced by an oscillation of the body that typically involves banging the head capsule on the tunnel ceiling. Literature on termite "head-banging" dates back to the 19th century, but the chemicals thought to be used for alarm transmission - mono- and sesquiterpenes - have only recently come to light, in both higher and lower termites (Roisin et al. 1990, Reinhard et al. 2003).

Howse (1962; 1964; 1965) reported, in detail, the oscillatory movements (OMs) of the dampwood termite *Zootermopsis angusticollis (*family Hodotermitidae; a lower termite), introducing the terms "VOM" (vertical oscillatory movement),"LOM" (longitudinal oscillatory movement), and "COM" (complex oscillatory movement). VOMs are movements that produce audible sounds by striking the head capsule vertically against the substrate. LOMs are a "back and forth" or longitudinal movement, whereas COMs involve both vertical and longitudinal movements. He found that VOMs were self-perpetuating, as dampening of the transmission by using a flexible substrate reduced the number of VOMS performed (Howse 1962). LOMs appear to be part of a habituation process; that is, they increase in frequency as VOMs decrease. As LOMs often accompanied contact with another individual, it was thought that they might play a role in communication of avoidance behaviors. Howse felt that COMs are involved in the propagation of the initial alarm signal, as they were only observed during the first few minutes following disturbance (Howse 1962).

Howse (1964) determined that the substrate vibration produced by VOMs, not the airborne sound, caused increased activity in other individuals. Temperature was found to predictably influence the duration and intervals of VOMs (Howse 1964). Physical contact was also found to be important in releasing display of VOM behavior. The response range of the termite subgenual organ to vibrations was found to be tuned to the frequency of vibrations given off by termite OMs, other vibrations being effectively filtered out (Howse 1964). Thus, frequency variance in OMs would be of little use, as anything outside the termite’s narrow detection range would be ignored. It was therefore concluded that the existence of a termite "alarm language" was unlikely (Howse 1964).

Howse later ascertained that LOMs followed from relatively small disturbances such as unfavorable antennal stimuli and COMs from larger ones such as such as light, vibration, or foreign insects (Howse 1965). It was concluded that termite OMs do not communicate specific information and are instead used as generic warnings to nestmates (Howse 1965).

Stuart (1963) examined alarm signals propagated in *Zootermopsis nevadensis* with four experimental designs. In the first design, two groups were placed in separate cushioned containers but connected via a flexible air passage through which a steady air current was maintained. If the termites of one group were alarmed via tapping of their arena, the other group displayed no reaction, showing that long-range chemical signals are not used to confer an alarm condition. The second design used two groups, but this time they were placed in a single container that was divided by wire mesh. When workers on one side displayed VOMs in response to an inserted wire probe, workers on the other group did not react - further evidence for the lack of chemical communication. It was decided that physical contact, or at least close physical proximity, was needed to convey alarm. In a third experiment, Stuart (1963) placed a small (44 individuals) colony into an artificial nest and a single worker was prodded with a wire probe. How long this worker and any nestmates remained within 3 cm of where the worker was prodded was noted. Results indicated that significantly more workers and soldiers were attracted to the 3 cm area after the worker was prodded. This could have been due to the contact with the outside air resulting from insertion of the wire probe, so a fourth experiment was performed. Its design called for two identical probe insertion points to introduce outside air but only one would be used to probe a termite. The location used for probing was found to attract more workers and soldiers than the other. This was taken as evidence for the recruitment of workers and soldiers to an area where a worker was alarmed via physical contact indicating a chemical signal. Stuart (1963) also noted that, during periods of alarm, building activities were enhanced, implying that the alarm reaction was designed (at least partially) to spur construction to counter the typical threat of nest damage or destruction.

Stuart (1967) theorized that low-level alarm reactions are, at least in *Zootermopsis*, linked with recruitment and construction (low-level reactions to stimuli such as foreign materials, light, or air currents). He placed copper gauze into a nest and noted that the first worker that came into contact with it retreated to the center of the nest and began performing OMs. Significantly more termites were recruited to the area around the gauze than to a control area. After a week of construction, there was no difference between the two areas in number of visitations. Stuart (1967) concluded that alarm reactions trigger building activity and that this activity is reduced as the situation that produced the initial alarm reactions is addressed by the builders.

Stuart (1988) revisited sound and vibration alarm signals in termites using small groups of *Z. angusticollis* workers and soldiers in a tiered glass and plastic structure which was constructed so that the floor of the top tier was also the ceiling of the lower tier. VOMs performed by termites on the bottom tier would strike the floor of the upper tier, ensuring transmission of vibrations. Termites in the lower chamber were alarmed via a puff of air and the upper group observed. No evidence for alarm or response to head banging was seen in the upper group leading to the conclusion that vibratory signals alone were not sufficient for alarm transmission. Evans et al. (2005) examined the transmission of vibrations through wood in their study of the lower drywood termite, *Cryptocercus domesticus*, but these vibrations were primarily from the act of chewing, not OMs. It was evident, however, that these vibrations resonated throughout the pieces of wood used in their study, as they appeared to play a role in size-based food source selection.

Termites perform OMs in response to a disturbance, but whether these movements convey anything other than individual unrest is not clear. Entire groups of termites would need to be observed to determine the reason(s) for the rise and decline of OMs throughout the group. Edwards (1928) says that a termitophilous staphylinid was observed to elicit grooming from a *Nasutitermes* worker by mimicking a termite OM, but no other reports of this type of behavior have been reported.

Lower termite trophallaxis. Su and La Fage (1987) examined trophallaxis in *Coptotermes formosanus* by starving groups of workers and soldiers and combining them in Petri dishes with similar nonstarved groups. Trophallactic events between workers and soldiers were periodically scored in real time. They determined that workers with higher hunger levels were more likely to feed soldiers, regardless of the soldier's hunger level, suggesting that worker-to-soldier trophallaxis was initiated by the worker.

Forschler (1996) fed *R. flavipes* workers cellulose mixed with a fluorescent dye, mixed these workers with naïve workers, and tracked the movement of the dye over time. Approximately 8% of naïve workers were found to display the dye at both the 24 and 48 h mark. It was impossible, however, to differentiate between dye transferred via trophallaxis and coprophagy. Neither the naïve nor exposed workers displayed dye after 72 h.

Suarez and Thorne (2000a) looked at rates of trophallactic transfer in the termites *R. flavipes*, *R. virginicus*, and *Z. nevadensis*. Select donor termites were fed cellulose treated with a radioactive tracer for two weeks, then placed within a group of naïve nestmates in a Petri dish. The movement of the tracer was recorded and assumed to have occurred via trophallaxis. Spread of trophallactic material in *R. flavipes* was an order of magnitude more rapid (hours versus days) than had been previously reported (Rosengaus et al. 1986), but this may have been due to the use of higher isotope concentrations and more sophisticated measuring equipment. Suarez and Thorne (2000a) introduced and discussed the term "trophallactic cascade". This term was in reference to how a single trophallactic donor can pass material to a number of individuals. Suarez and Thorne (2000b) found that while food quality had no effect on trophallactic transfer, donor termites that fed on distant food sources transferred more tracer isotope than those that fed on nearby sources. This was hypothesized as a method of communicating food source proximity (Suarez and Thorne 2000b).

Saran and Rust (2005) studied the effect of 11 carbohydrates on *R. hesperus* feeding, using simple and multiple choice tests measuring the consumption of treated versus untreated filter paper. The transfer of a radioactive tracer in sucrose from exposed to naïve nest mates was also tested. Four of the treated filter papers in the simple choice tests – 2% maltose, 2% fructose, 5% ribose, and 3% xylose – showed significantly more feeding than controls. The termites showed significant preferences for 5% ribose and 2% glucose solutions over the controls in the multiple choice tests. Saran and Rust (2005) noted that the radiolabeled sucrose was almost entirely metabolized by termites, with only a small fraction (1.5%) of it being excreted, thus making sharing via trophallaxis – presumably proctodeal - limited. Saran and Rust (2005) concluded that the rapid digestion of carbohydrates and the increased feeding activity associated with them recommended their use in termite baits involving slow-acting insecticides.

Lower termite grooming. Assumptions about termite grooming have primarily been inferred from quantitative analyses involving entomopathogen transfer (Kramm et al. 1982, Boucias et al. 1996, Myles 1996, Shimizu and Yamaji 2003), though direct observations have also been used (Maistrello and Sbrenna 1996, Iwata et al. 1999). Kramm et al. (1982) exposed *Reticulitermes* spp. termites to *Metarhizium anisopliae* spores and introduced them to groups of nestmates until accumulated allogrooming by the nestmates on the exposed termites reached 15s, 1min, and 4min, after which the exposed individuals were removed. Replicates in which total allogrooming on exposed and unexposed individuals over a 10min introduction period was tracked and in which exposed individuals were not removed were also run. Mortality on the nestmates was tracked over 21 days. Kramm et al. (1982) found that exposed individuals were groomed for significantly longer than unexposed individuals.

Boucias et al. (1996) examined grooming in *R. flavipes* by looking at how exposure to imidacloprid affects mortality from the mycopathogen *Beauveria bassiana*. Boucias et al. (1996) dusted sets of 18 naïve termites with *B. bassiana* conidia that had been treated with fluorescein isothiocyanate. These termites were placed with sets of 18 termites that had been marked with Nile Blue A. The digestive tracts of 35 marked termites were dissected after 24 h and 97% of these contained fluorescent conidia. The cuticle of the dusted termites was found to be virtually conidia-free. Boucias et al. (1996) determined that a majority of the conidia (72%) were removed within 2 h via normal allogrooming behavior. Boucias et al. (1996) claimed that grooming among termites plays a large role in containing the spread of entomopathogens. When termites were first exposed to sub-lethal levels of imidacloprid and then dusted with conidia it resulted in significantly increased mortality levels that was attributed to a depression of allogrooming by the insecticide. Isolated termites that were treated with lower levels of mycopathogens resulted in "extensive mycosis" (Boucias et al. 1996). Myles (1996) conducted a study where termites that had sulfluramid-based resinous coatings applied to them were introduced into groups of untreated nestmates. The coating was groomed off and ingested by the nestmates thereby disseminating the toxicant, with resulting high cohort mortality. It was observed that the dried coating on the treated termites seemed to stimulate a grooming response in untreated nestmates (Myles 1996). Maistrello and Sbrenna (1996), using videotapes of *Kalotermes flavicollis* colonies in artificial arenas, scored pseudergates, nymphs, and replacement reproductives for time spent walking, resting, eating, OMs, and allogrooming. In allogrooming, pseudergates scored significantly above nymphs in both time spent and frequency of occurrence. Nymphs performed OMs significantly more frequently and for longer periods of time than pseudergates. Pseudergates tended towards allogrooming the proctodeum of pseudergates and reproductives.

Nymphs preferred to allogroom the abdomen of other nymphs, but performed next to no allogrooming on reproductives. Reproductive allogrooming was concentrated on the abdomen in pseudergates and the mouth and head in nymphs. Maistrello and Sbrenna (1996) concluded that these patterns of allogrooming were indicative of how pheromones are transmitted throughout a colony. Iwata et al. (1999) observed allogrooming between multiple castes in *R. speratus* placed in Petri dishes. They found that workers performed the majority of allogrooming, primarily upon other workers. Each caste received allogrooming, but neither reproductives nor larvae were seen to perform it. Under conditions of increased illumination, incidents of allogrooming were reduced, but the previous ratios remained relatively intact. Shimizu and Yamaji (2003) exposed *R. speratus* to varying concentrations of *M. anisopliae* conidia then maintained them singly and in groups of 10. Mortality was calculated after 7 days. Termites that had been maintained in isolation displayed significantly higher mortality rates than those that had been maintained in groups. It was concluded that the removal of conidia by allogrooming was responsible for this difference in mortality.

Division of labor in the higher termites. Temporal division of labor in the higher termites has been clearly demonstrated, not surprising given their many similarities to the Hymenoptera such as nest strategies and development. Jones (1980) examined gallery construction and grooming habits of *Nasutitermes costalis* workers and found evidence for temporal polyethism. Four behavioral categories were used (investigation, construction, grooming, and locomotion), with five worker castes (three small and two large) as subjects of real-time observation at a nest repair site. Approximately six h of observations of individuals from each caste were recorded, with individual observation periods lasting from 10 to 20 s. Clear differences in behavioral patterns were found; for example, large third instar workers showed significant specialization in grooming, more than the remaining four castes combined.

Gerber et al. (1988) found evidence for temporal polyethism in a study of division of labor in *Macrotermes bellicosus*. This termite forages for plant litter, which is brought back to the nest, consumed, and the resultant feces used to construct a "garden" for the fungus *Termitomyces*. Gerber et al. (1988) knew from work with *Macrotermes subhyalinus* that individuals foraging outside the nest tend to pick up particles of earth in their gut. Termites older than 30 days always had particles of earth in the gut while termites younger than 25 days never did, thus allowing the division of termites into two groups, “old” – which had left the nest - and “young” – which had not. *M. bellicosus* was observed to follow a similar regime, but exact timeframes were unknown. Gerber et al. (1988) recorded the foraging castes performing various tasks such as maintenance of the fungus comb and the queen, carrying water, spontaneous construction, and nest repair. Individuals were divided into major and minor workers and soldiers. Gerber et al. (1988) determined that the foraging and water-carrying force is composed exclusively of old workers. These data also showed that major workers were predominantly foragers, with minor workers being prevalent at the other studied activities (Gerber et al. 1988).

Hinze and Leuthold (1999) confirmed temporal polyethism in *M. bellicosus* in a study that utilized marking methods along with metal and infrared detectors to determine if the transition between working inside the nest to working outside the nest was correlated with age. Detectors were placed at the nest entrance and the queen's chamber. Major and minor workers of known age were marked with metal wires that allowed detection of their passage in or out of either area. The infrared detectors recorded the passage of all termites, marked and unmarked.

Marked workers showed a gradual shift from work inside to outside. This shift occurred 13-25 days after the final molt in major workers and 9-32 days in minor workers. This was clear evidence that, as the workers aged, their tasks changed accordingly.

Hinze et al. (2002) revisited temporal polyethism in *M. bellicosus* by looking at gut contents of workers from foraging sites, fungus combs, and the queen's cell. Termites could be separated into two groups - those that had fed on fungus comb (secondary food) and those that had fed on collected plant litter (primary food). There were significantly more major than minor workers from the fungus comb and queen's cell sites that had fed on primary food. Hinze (2002) concluded that, in this species, food processing is a task primarily conducted by the major workers.

Evans (2006) examined task switching in *Nasutitermes exitiosus* with two approaches involving mark-recapture. Both involved placing multiple food sources around *N. exitiosus* nests as forager sampling sites. In the first approach, termites were collected from a sample site near a nest, marked with Nile blue, and returned. Days later, a second food source was sampled and the nest was intentionally damaged to stimulate nest repair. Over 19 additional days, the sample sites were monitored and the formerly damaged sites were sampled for the presence of blue-stained former foragers. In the second approach, a nest was damaged and then left alone for 3 days. All the termites at the damaged site were then extracted, marked with Nile red, and returned to the damaged site. Over the course of 19 additional days, the nearby food sources were sampled for the presence of red-stained former nest repairers. Evans (2005) did not find a significant numerical drop in the number of foragers and the appearance of stained former foragers in damaged sites, indicating a lack of task switching. This was interpreted as evidence that termites instead employ an inactive reserve force which is mobilized according to need.

Division of labor in the lower termites. Rosengaus and Traniello (1993) studied division of labor in colonies of *Zootermopsis angusticollis*. They sequentially examined 1-2 year old colonies that had been raised in separate Petri dishes. Individuals were removed from the Petri dish and graded for instar according to head capsule width. Instars 3-7 were marked with paint to allow visual identification of the different instars. All individuals were then returned to the Petri dish and left undisturbed for 24 h. The Petri dish was then placed under a fiber-optic light and observed in real time for a single 10min period. In total, 280 individuals from 8 colonies - approximately 40 individuals from each instar - were observed. They placed the observed behaviors into six task categories - individual maintenance, brood care, larval care, social interactions, feeding, and nest maintenance (Rosengaus and Traniello 1993). The behavioral repertoire for each instar was determined as well as which instars tended to specialize in certain tasks. Larger instars (3-7) performed the vast majority of the task-related activities, with no correlation between age and task. This suggested an overall lack of division of labor.

Crosland and Traniello (1997) and Crosland et al. (1997; 1998) conducted studies on temporal polyethism using field-collected *R. fukienensis*. They examined task performance of three sizes of termite workers - termed small, medium, and large - with increased size assumed as an indicator of increased age. Crosland and Traniello (1997) looked at the ability of these three size classes to perform exploration, alarm behavior, burying behavior, and tunnel building. Controls consisted of groups containing 20 larvae and 10 each of the three worker sizes, whereas the experimental groups contained no large workers and 20 medium workers. Crosland and

Traniello (1997) found that in the control, large workers were significantly more able to perform the aforementioned activities than the medium or small workers. The experimental group of medium workers performed significantly differently than the control medium workers, approaching the activity levels shown by control large workers. They concluded that this behavioral plasticity might be common in incipient termite colonies due to lower numbers of large workers. Crosland et al. (1997) conducted eight different experiments, each focused on a different behavior - tunnel building, exploration, care of the brood, queen, and larvae, corpse burying, alarm-giving, and guarding/resting. Observation times varied between experiments, ranging from 16 min over 4 days in the egg care experiment to 60 min over 5 days in the guarding/resting experiment. They found that nearly all tasks could be performed by each size category, with the exception being that small workers were never observed to carry larvae. Large workers had higher frequency rates at all tasks than either medium or small workers. Large workers were also more efficient at nearly all tasks, with efficiency scored as the amount of work done in a set time (Crosland et al. 1997). However, due to the overlap in task performance between castes, they could not support the existence of caste-based division of labor. In a later paper, Crosland et al. (1998) compared the performance of three size classes in tunnel construction, covered gallery construction, nest repair, and feeding activities. These experiments involved placing varying ratios of large, medium, and small termites into Petri dishes and observing the progression of behaviors over the course of the experiment. They found that large workers were more efficient at nearly all observed tasks, though medium workers were able perform all tasks and were the equals of the large workers in gallery repair (Crosland et al. 1998). Small workers performed all tasks comparatively poorly and were unable to construct covered galleries. Crosland et al. (1998) concluded that this discrepancy was indicative of small worker dependency and thus constituted evidence for temporal polyethism. This distribution of labor could not be said to be a 'discretized caste system' as predicted by Oster and Wilson (1978), but it did show that with size, comes efficiency.

There is little evidence for the existence of task allocation by either age or morphotype among workers in the lower termites, though only two species have been examined. A possible explanation for this has been put forward by Rosengaus and Traniello (1993), who argued that many factors interact to determine the degree to which social insects display temporal polyethism. The subject of their studies, *Z. angusticollis*, exists in relatively small colonies with slow growth rates within their primary food source, negating the need to forage outside. This reduces the necessity for specialized workers and likewise the likelihood of task allocation.

Little evidence for division of labor in lower termites has been found, although there is evidence for behavioral plasticity defined as a shift in behavior based on changing conditions (Crosland and Traniello 1997). This can be compared to honey bee behavior, where one worker age group is often capable of compensating for the loss of another by altering behavioral patterns (Robinson 1992, Gordon 1996). It should be noted that termite division of labor data have either been drawn from real-time observations of individuals placed in Petri dishes or from unobserved performance of tasks.

2.4 Conclusions

One of the problems associated with studying termites is their cryptic lifestyle. The inability to easily observe termites in their natural habitat of underground tunnels and inside pieces of wood is one of the reasons few observational studies have been published. Data obtained from direct observation of termites must, by necessity, disturb their natural habitat or take place in an artificial environment, which would arguably impact normal behaviors and must be considered when interpreting data. Another factor that might influence experimental results comes from the need to observe individuals who, under normal conditions, may exist in populations numbering in the 10’s of hundreds.

The scientific literature on behavior in the lower termites is clearly equivocal (Rosengaus and Traniello 1993, Crosland et al. 1997). Placing randomly selected groups from a large population into an observation arena must assume no division of labor to validate sub-sampling. Indeed, the published attempts at examining division of labor within the Rhinotermitidae have relied on such sub-sampling to provide test subjects (Crosland and Traniello 1997; 1998, Crosland et al. 1997), which may bias results if the individuals that are assigned tasks within the larger social context are placed in an experimental situation requiring different tasks. In addition, temporal polyethism was assumed to be based on size of termite workers and behavioral observations that were scored, in real time, on termites in Petri dish arenas (Rosengaus and Traniello 1993, Crosland and Traniello 1997; 1998, Crosland et al. 1997). The use of real-time scoring makes it very difficult, if not impossible, to provide simultaneous data on multiple individuals or to score multiple behaviors. Techniques need to be developed to video-record intact social groups over long observational time frames under more natural conditions. A record of normal termite behaviors could then be scored at the observer's discretion and within a more realistic social context to develop a definitive repertoire of termite tasks and interactions. This could provide, for example, the daily activities and behavior patterns of an “average” lower termite. Also, by scoring many individuals during the same time frame, an idea of how many individuals are performing an activity at any one point and how this proportion changes over time could be obtained.

The demonstration of a genetic basis for Hymenopteran division of labor (Robinson and Page 1989; 1995) suggests that, due to the similarities between the Isopteran and Hymenopteran orders, the Isoptera may also use genetic task allocation. However, the examination of this could be complicated by the prevalence for termites to display secondary reproductives. Also, honey bees have only 16 pairs of chromosomes (Hunt and Page 1995) while Isopterans have, on average, 27 (Seger 1983). Research in this area could provide valuable insight into termite task allocation, especially among those species in which the existence of division of labor is not well documented.

Trophallaxis is an aspect of termite behavior which has received relatively little attention in behavioral studies, despite its importance in termite nutrition (Wilson 1971). The majority of assumptions about this activity are drawn from Hymenopteran research, an order in which adult-adult proctodeal trophallaxis is rare (Wilson 1971). Many have performed experiments that involved this process in termites, but few have actually examined the process itself. Those that have (Rosengaus et al. 1986, Su and La Fage 1987, Suarez and Thorne 2000a; 2000b, Saran and Rust 2005) did not attempt to separate stomodeal and proctodeal trophallaxis. Recent work suggests that stomodeal and proctodeal trophallaxis fulfill very different nutritional and informational needs and do not even typically involve the same materials (Whitman et al. 2006). Therefore, a better understanding of stomodeal and proctodeal trophallaxis in termites is necessary so that feeding bioassays, especially those involving chemical transfer, can be put into proper perspective.

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CHAPTER 3

OBSERVATIONAL NOTES ON *RETICULITERMES FLAVIPES* (KOLLAR)[1]

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[1] Whitman, J. G., B. F. Forschler. 2006. To be submitted to Journal of Economic Entomology

3.1 Introduction

Direct observation of an individual is the essence of the study of animal behavior. However, descriptive accounts of short-lived or infrequent behaviors are difficult to record by real-time observation. 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. The scientific literature includes a few studies of Rhinotermitid and Kalotermitid termites that used film to investigate behavior (Howse 1962, 1965, Stuart 1988, Maistrello and Sbrenna 1996). However, many aspects of termite biology remain that would benefit from a better understanding of their behavior (Thorne et al. 1999, Laine and Wright 2003).

We recently concluded an experiment that required reviewing over 300 h of video-taped termite behavior using *Reticulitermes flavipes* Kollar that yielded several interesting qualitative observations. In this paper we describe five behaviors not previously discussed in detail such as mating events between 18-mo old kings and queens, ecdysis in the worker caste, ‘tail-chasing’, trophallaxis, and excavation. In addition, we profile four types of Oscillatory Movements (OM) and conclude with a discussion on the implications these observations have toward a better understanding of termite behavior, ecology, evolution and management.

3.2 Materials and methods

Detailed descriptions of the arena and experimental design can be found in Chapter 4 of Whitman’s (2006) thesis on termite worker behavior. Marked termites were videotaped in three-chambered, glass-topped, artificial arenas made of Densite to maintain moisture and the narrow confines of a termite gallery were simulated by an arena depth of 2 mm. The three chambers, each measuring 2.3 x 3 cm, were connected by 3 mm wide openings in the 1 mm thick walls that defined the separate chambers. Chambers were half-filled with alpha cellulose powder and kaolin clay that were intended to provide feeding and tunneling substrates, respectively. Three Jai CV-M50IR CCD cameras placed above the arena provided sufficient detail to discern movement of mandibles and antennae. Termites were filmed using two Panasonic AG-2560 SVHS and one Emerson DA-4 Head brand VCR that enabled recording of the exact time and duration of each behavioral event. The VCR’s ‘extended play’ mode enabled 8 h of continuous recording but resulted in a loss of video quality where movements of small body parts such as the maxillae and maxillary palps were obscured while providing sufficient clarity to observe movements of the body, antennae, and mandibles. Six replicates were filmed for 24 consecutive h on three separate days - 1, 3, and 7 d after introduction to the arenas. Behaviors were scored on six termites per replicate for 12 evenly spaced 15-min intervals for a total of 3 h per individual per day. The behaviors detailed in this manuscript were ancillary to the objectives of the original design and were obtained, in part, by review of the entire 24-h of video over the three days of the experiment.

In the interests of discussion we provide a glossary of terms used in the behavioral descriptions. In addition, an appendix outlining the time line of pertinent observations is provided (Table 3.1) and video files of specific behaviors can be viewed and downloaded at http://www.ent.uga.edu/termitebehavior.htm.

3.3 Glossary of behavioral terms

Allogrooming. Defined as grooming of one individual by another. This is 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. Allogrooming was occasionally followed by chewing (4.0%, or 2,941 times in 73, 410 allogrooming events).

Chewing. Chewing behavior is described as movement of the mouthparts indicative of macerating a substrate prior to ingestion. Chewing was characterized by rhythmic lateral movement of the mandibles while the substrate being chewed was often clearly visible as it was manipulated by the mouthparts prior to consumption. Consumption was indicated by a cessation of chewing with no visible signs of having donated or deposited the macerated material. The substrates consumed by workers came from the following sources: alpha cellulose taken from the arena, regurgitated material, trophallactic material or that obtained during allogrooming. The same characteristic shoveling movements described for pill formation were displayed by termites obtaining a bolus of cellulose from the arena which was then followed by chewing and consumption, in succession.

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

Pill Formation. Substrate ‘pills’ were formed by a termite facing the active site of excavation. Formation of pills was initiated with a lateral extension of the mandibles followed by a forward movement of the body (tarsi remaining stationary) pushing the head capsule into, as the mandibles closed and cut, the substrate simultaneous with alternating rotations (describing a 45^{o} arc) of the head capsule. This distinctive head motion accompanied both pill formation and deposition and was described by Stuart (1967) as “a characteristic side-to-side motion of the head”. The motion was repeated leaving the overall impression of the substrate being shoveled into the mouthparts. Pill deposition was characterized by placement of the pill against the floor or a wall followed by repeated rotations of the head capsule combined with a backwards movement of the body mimicking formation in reverse. Pill formation, transportation and deposition were readily distinguished from chewing by the absence of mandibular movement indicated by stationary mandibles held in an extended or open posture. It was assumed that the maxillary and labial palps performed the bulk of shaping the 'barrel-shaped' pill against the labrum.

Pumping. Pumping, or hydrostatic swelling of the body during ecdysis prior to rupture of the ecdysal suture, is characterized by peristaltic-like contractions of the body which, in our termite subjects, started at the tip of the abdomen and progressed to the thorax.

Tail-chasing. A behavior characterized by a worker tandem chasing the tip of each other's abdomen resulting in tracing circles with their movement.

Trophallaxis. Exchange of digestive fluids and/or food that could be either *Stomodeal (*by mouth-to-mouth contact) or *Proctodeal* (observed as mouth-to-anus contact) as characterized by the initiator/recipient displaying chewing after contact. We further characterize trophallaxis as either primary or secondary based on origin of the donation. Primary materials were self-procured by the donor and included cellulose obtained from the arena, regurgitated material, and substrates acquired in allogrooming or coprophagy. Secondary materials included donated substrates that were acquired in a proctodeal or stomodeal exchanges. Secondary trophallaxis can involve donations of materials obtained by either primary or secondary exchanges.

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

3.4 Observations

Mating. Mating events were observed in 18-mo old *R. flavipes* colonies containing primary reproductives and the full complement of soldiers, larvae, and workers. Eggs were not observed throughout the experiment that included 72 h of video involving each of three pairs over three separate days. Reproductives from one colony was observed to mate twice, on Day 1 and Day 7, while the reproductives in another colony mated once on Day 7 and one pair never mated. The adult females were not physogastric and as a result we could not differentiate males and females with certainty. The mating events lasted 34 to 42 s (mean duration ± standard deviation of 38.7 ± 4.2 s) and involved three phases – antennation, positioning and coupling. The mating timelines are summarized in Table 4.1. There were no unusual behaviors observed preceding or succeeding the mating events for 20 min in either direction.

Mating events were determined to began when one adult approached the second 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 meant that they traveled in a rough half-circle at which point antennation ceased. At this point the head of the first adult was adjacent to the abdominal tip of the second and vice versa. The antennation phase lasted 7-9 s (8.7 ± 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 after which, with slight adjustments by both, they brought their abdominal tips together. The positioning phase lasted 3 to 8 s (5.0 ± 2.6 s) and was followed by coupling, which was maintained for 22-30 s (25.0 ± 4.4

s). The abdominal tips uncoupled to end the event and the adults, after walking a short distance, resumed what they had been doing earlier - standing still.

The reproductive 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 of filming (successful matings were recorded on Day 1 and 7 with this pair), with 10 min elapsing between the first and second episodes while 30 min passed between the second and third rapid antennation displays.

We observed eight separate pairs of workers performing a ‘tail-chasing’ maneuver similar to the mating ‘half-turn’ previously described with adults during the rapid antennation phase. Tail-chasing was initiated by a worker soliciting a proctodeal exchange that was followed by the potential donor turning to solicit the same. Workers that displayed tail-chasing continued describing full circles with their tandem movement as each termite tried to maintain antennal or head capsule contact with the other’s abdomen. Chewing by either participant was not observed, indicating a failure to respond to the initial stimulus. There was no rapid antennation, as noted with the adults, and tail-chasing did not end with an attempt to bring the abdominal tips together. Tail-chasing events, as displayed by the workers, persisted longer (range 157-725 s, mean 413 ± 201 s, N=8) and described numerous complete circles (approximately 9.6 ± 1.7 s per revolution, range 8-12 s) compared to the half-rotation observed during the antennation phase (mean 8.7 s) with the adults.

Ecdysis. The molting event, which began around 2:28 P.M. on Day 7 and lasted 43 min, included 3 phases – pumping, ecdysis, and recovery. The subject began pumping with the contractions increasing in intensity and duration over the next 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 approximately 10 min. The recovery phase 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 and lasted 20 min.

Pumping attracted the attention of a single nest mate that began allogrooming at 2:40 PM and this individual continued allogrooming throughout the ecdysis event. The number of individuals performing allogrooming increased to three by 2:42 and five by 2:45. The ecdysing subject was continually allogroomed by as many as five nest mates at any given time. Termites involved in allogrooming displayed various degrees of fidelity, ranging from 30 seconds to 5 min and only one individual groomed throughout the entire event. The allogrooming observed during ecdysis displaced the subject over an area of 3 cm^2 and was, at times, more vigorous than observed during allogrooming of non-ecdysing workers. Allogrooming of a non-ecdysing nest mate 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 appeared to be engaged in allogrooming. It was never evident that any termite attempted to consume the exuvia during ecdysis but once the old cuticle was completely removed it was torn apart and consumed, over the next 45 min, by 16 different individuals. The newly molted subject solicited and procured a proctodeal donation 10 min after the recovery phase ended.

A second termite undergoing ecdysis was dragged onscreen at 2:45 by vigorous allogrooming during the aforementioned filming episode. At the time of appearance, several terminal abdominal segments of the second subject still remained in the old cuticle. This second event proceeded as the first in respect to the gradual decrease in allogroomers and consumption of the exuvia. Comparing the ecdysis timelines, this second individual appeared to have begun approximately 3 min before the first.

Excavation. Gallery formation involved activity in either alpha cellulose powder or kaolin clay. Excavation was characterized by a series of three stages beginning with manipulating the substrate using the mouthparts to form a macerated, barrel-shaped ‘pill’ (pill formation) which was then transported away 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.

Pills were readily formed using either of the two substrates provided in the arenas, although only kaolin clay was expected to be a tunneling/building substrate. It would appear that termites involved in gallery formation did not differentiate the food-quality of the substrate. It should be noted that we never observed termites consuming kaolin clay nor were termites observed to use a body part other than their head capsule and mandibles to push or pack the substrate during gallery construction.

The majority of pills made while excavating were immediately transferred out of the gallery being excavated and placed along the periphery of one of the chamber walls. A small number of pills were deposited on the chamber floor near the excavation site before the termite returned to the queue or to excavating the gallery. These ‘errant’ pills were later moved to the chamber walls, but it was not determined if the same termite was involved in the later movement. Termites that had deposited a pill, in 33% of observed depositions (N = 55), used the same head capsule motions described for pill formation (except the mandibles did not move) as they positioned and pressed/smoothed pills into a single structure – to line a gallery.

The chamber walls were lined with pills within the first 24 h in five of the six replicates of the experiment. The exception was a colony in which excavation did not begin until Day 7 of filming.

Excavation site fidelity. Sites where substrate was being excavated were often attended by more than one termite. The width of a developing gallery only allowed a single termite to extend the gallery forcing others intent on excavation to line up behind the excavating termite 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.

Individuals involved in excavation had several locations where they deposited pills displaying no indication of fidelity to a particular deposition site. The route to and from the deposition site was often circuitous, especially in chambers where the presence of other termites amounted to obstruction. The return to the excavation site might be interrupted by a stop at another excavation site, but the termite would always leave this ‘secondary’ site and return to the original. Individuals were seen at different excavation sites at different observation intervals, but were never observed to excavate at more than one site during a particular 15-min scoring interval, indicating a degree of fidelity to a particular excavation site.

Oscillatory movements. 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. Finally, the complex oscillatory movement (COM) involved both vertical and horizontal body movements.

We recorded four distinctly different oscillatory movements (OMs) but because the cameras were positioned in an overhead view it was difficult to determine the degree of any vertical component and we therefore cannot categorize the behavior beyond that of an OM. The movements are described as longitudinal oscillations involving displacing approximately one-quarter of a body length in alternating forward and backward movements that begin with a clearly defined forward lunge. The termite's tarsi remained stationary on the substrate while its body was in motion. All OMs, from our observations, involved the same movements with variations in speed, duration and repetition.

The Type 1 OM was brief, lasting approximately 0.5 s from the first oscillation to the last, with the rapid motion blurring the video-taped image. The Type 2 OM lasted approximately 2 s and was much slower. The Type 3 OM type appeared to be a Type 1 OM followed immediately by a Type 2. The Type 3 OM was often performed three times or more in succession. The Type 4 OM was approximately 3 s in duration and contained the slowest oscillations.

The Type 4 OM was always associated with defecation – a small droplet would appear behind those individuals. 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 nest mate response. The Type 3 OM, displayed as a single event, did on occasion signal a nest mate to cease grooming or move aside. However, successively repeated Type 3 OMs induced no apparent reaction from their nearby nest mates. It is worth noting that OMs were never performed by termites waiting for another to vacate an excavation site.

Trophallaxis and Feces. The traditional definition of trophallaxis has its observational roots in food exchange between adult and larval social wasps (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). We observed trophallaxis to include three stages: initiation, procurement and chewing. Initiation involved antennation of the donor by the potential recipient, the duration of which was equivalent for both types of trophallaxis (1.5 ± 0.61 s for proctodeal and 1.1 ± 0.68 s for stomodeal). Antennation, when used to signal a request for donation, always involved the body part of the donor involved in the exchange. Allogrooming immediately preceded half of proctodeal transfers and two-thirds of stomodeal transfers without a clear indication of antennation. Procurement of proctodeal material (8.1 ± 36.5 s) took more time to accomplish than stomodeal (6.8 ± 15.6 s) though it was chewed for a briefer period before consumption (147 ± 175 s versus 192 ± 220 s, respectively), while chewing primary alpha cellulose was the most time consuming food-procurement activity we recorded (216 ± 219 s).

The donor was chewing something before a recipient occasioned to initiate a donation by antennation or allogrooming in stomodeal trophallaxis (N=246). It seems likely that this was a one-way transfer, though a two-way exchange cannot be ruled out. If an exchange took place with material transferred from initiator (which was not chewing) to donor we could not detect it, as the donor’s behavior did not change (i.e., it was chewing when the event began). It should also be noted that all of the trophallaxis we observed was worker to worker. We did not score larval or soldier activity and none of the marked workers we scored fed other castes.

Stomodeal trophallaxis involved procuring something from a donor that was chewing one of three main types of material when the donation was solicited. Alpha cellulose powder picked up by the donor from the arena, a primary material, was shared in 39% of all stomodeal exchanges. Secondary materials obtained by the donor in a prior proctodeal exchange were donated in 32% of all stomodeal events while secondary materials obtained by prior stomodeal trophallaxis were shared in 27%. We observed multiple (N=198) events where termites, displaying no recent previous activity, suddenly began chewing. This lack of contact with a visible food source was confirmed by close review of the video tape for one minute before chewing. The material being chewed was thin, clear, and unlike the white pulp typically seen when chewing alpha cellulose and it was assumed to be regurgitated material. A regurgitated bolus was shared in 1.2% of the stomodeal trophallactic events we recorded. The material chewed after allogrooming, a rare event (4.0% of all allogrooming events), was never shared. The rarity with which we observed sharing of regurgitated or allogrooming material cannot be explained by the behavior of the potential donors, as they did not behave differently from those chewing trophallactic material or cellulose. It appeared that there was simply no interest on the part of the potential recipients, as if the donors were not chewing.

It was observed that the recipient of proctodeal trophallaxis often completed the event with a quick and sudden separation of their mouthparts from the anus of the donor termite. This was a puzzling observation because not all proctodeal feedings ended with a ‘snap’. We propose that this was due to the sticky nature of proctodeal material based on three anecdotal observations. On one occasion, a termite backed into another termite’s leg and was instantly glued to the leg. When the first termite began moving forward, it dragged the second termite along for approximately 1 cm before they separated. A second instance involved a termite that appeared 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 nest mate 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 initial separation.

3.5 Discussion

Mating. This is the first detailed account of mating events in *R. flavipes*. It is assumed that lower termite reproductives mate frequently based on long-lived reproductives and spermatheca size (Costa-Leonardo and Patricio 2005). Undoubtedly, primary reproductives mate shortly after the alate flight, as evidenced by the aforementioned 18-mo old colonies in which the matings were filmed that consisted of workers, soldiers and larvae but no eggs. Raina et al. (2003) analyzed time-lapse video recordings and determined that *Coptotermes formosanus* reproductives (N=19 pairs) mated, on average, 2.2 times in the 48 h immediately following flight and pairing. The *C. formosanus* 'matings' lasted 27.9 ± 7.8 s which was close to the 38.7 ± 4.2 s we recorded for *R. flavipes* that included an antennation phase not mentioned by Raina et al. (2003). If we compare our coupling phase, which lasted 30.0 ± 3.0, s to that of Raina et al.'s (2003) the figures are even closer. Raina et al. (2003) noted allogrooming between the reproductives immediately prior to the mating, a behavior we did not observe. Afzal (1985) studied mating in over 300 reproductive pairs of the drywood termite *Bifiditermes beesoni* and reported a relatively complex mating ritual involving antennation, head-butting, allogrooming and "abdominal quivering" reminiscent of OMs, although no durations were stated. Apart from antennation we saw none of these behaviors in *R. flavipes*. It should be noted that the Raina

(2003) study involved 1-y old pairings while Afzal (1985) examined established pairs and newly-paired reproductives, with no observations that mating rituals differed between an established reproductive pair and one that had never mated. We therefore assume that the relative lack of mating ritual among the 18-mo old reproductives we observed is typical of *R. flavipes*. Matings occurred in our *R. flavipes* study an average of once every three days, a figure which agrees reasonably well with the conclusions of Costa-Leonardo and Patricio (2005) working with *Coptotermes gestroi*. It is worth noting that while one of the reproductive pairs we observed mated twice and a second mated once, the third was never observed to mate, illustrating the variability of termite behavior. Raina et al. (2003) also reported that *C. formosanus* laid eggs in batches over the course of weeks rather than daily. It may be possible that mating occurs on a schedule synchronous with egg deposition and is driven by environmental influences as well as innate hormonal signals. It is clear from our 9 d of video (three primary pairs filmed for three days) that egg-laying is not a daily occurrence in *R. flavipes* as assumed by Grube and Forschler (2004) in their population growth model.

Ecdysis. 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. Rosengaus and Traniello (1993), working with *Zootermopsis,* mention the act of nest mate assistance with molting, although their description does not detail the number involved or percent of molting events that involved assistants. Su and Scheffrahn (1993) likewise mention (but do not elaborate on) molting being a group event. The molting event described here is the first detailed description of ecdysis being an activity that attracts the attention of numerous individuals. The knowledge that molting can be a group-assisted endeavor involving as many as 16 different termites could be used in modeling toxicant transfer and evolution of social behavior. The mechanisms used to explain toxin transfer, for example, mention mortality during ecdysis without knowing the implications of dissemination resulting from the grooming and eventual consumption of exuvia described in the present study (Su and Scheffrahn 1993, 1996, Sheets et al. 2000, Su and Puche 2003, Ibrahim et al. 2003). Mortality during a group-assisted molt may also increase the probability that cannibalism plays a role in toxicant transfer regardless of the chemical class and should be examined in more detail. The jack-knife position of molting workers exposed to a chitin synthesis inhibitor mentioned by Su and Scheffrahn (1993) is analogous to the condition we observed at the beginning of the ecdysis phase, after suture rupture and as the not yet sclerotized termite attempted to extract the lower thorax and abdomen from the old cuticle. We speculate that the allogroomers we observed during ecdysis assist in completing the molt beyond extracting the lower thorax and abdomen from the old cuticle and it would interesting to note whether nest mate assistance in their bioassays was absent because of arena design or toxin recognition. Comparing the cost/benefit of group assisted growth (molting) toward developing a social contract in the context of eusocial evolution may indicate this process is analogous to the dependent larval stage of the social Hymenoptera (Wilson 1971, Shellman-Reeve 1997, Nalepa 1994).

Pill formation and proctodeal material. The literature on termiticide efficacy has for 20 years (Su and La Fage 1984) endeavored to account for the importance of behavioral reaction to treatments (Gold 1996, Sheets et al. 2000, Valles and Woodson 2002, Su 2003), but the details of specific behaviors have yet to be elucidated (Laine and Wright 2003). Previous studies on termite tunneling behavior focused on the direction and distance of excavations conducted in sand whose larger particle size does not lend itself to pill formation (Cornelius et al. 2002, Su and Puche 2003, Campora and Grace 2004). Close examination of shelter tubes suggests that the pills described in this paper are the basic building blocks of subterranean termite construction. The use of dried fecal pellets as building blocks has been noted in *Zootermopsis* (Stuart 1967, Howse 1968) and *Macrotermes* (Gerber et al. 1988) and liquid feces used as construction cement has also been reported (Stuart 1967, Howse 1968). However, we observed neither 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. Gallery construction involving extensive manipulation of the substrate using mouthparts would certainly play a role in understanding transfer of substrate-borne termiticides. Our data indicate that gallery construction (pill formation, transportation, deposition and return to the excavation site) includes 50% of the time spent on-task in contact with a pill, which would certainly expose the excavating termite to an oral dose. Termites in our experimental arenas always excavated/improved galleries using the mouthparts to form and shape pills, lending insight into the mechanics of termite gallery construction. We have observed, in structural infestations, shelter tubes composed 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 utilizing the pill formation/construction behavior observed in this study.

OMs. Oscillatory movements have been described from numerous termite species. They have been referred to as an “alarm reaction” (Howse 1962, 1964, 1965), as evoking attraction (Stuart 1963, Reinhard and Clement 2002, Reinhard et al. 2003), dispersal (Rosengaus et al. 1999a), and in eliciting building and repair activities (Stuart 1963, 1967). The occurrence of

OMs indicates 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 propose that OMs can, at times, be little more than a general signal to 'do something else'. We observed, on several occasions, a termite being groomed suddenly perform a single Type 3 OM, at which point the grooming termite immediately ceased. Additionally, termites moving through an arena were sometimes observed to perform a single Type 3 OM if their path was blocked by nest mates. This typically resulted in the obstructing termites moving to create an opening and agrees with a point Maistrello and Sbrenna (1996) made concerning *Kalotermes* that individuals performing OMs might merely be responding to a short-term need rather than an alarming stimulus. Our observation linking the Type 4 OM with defecation is the first substantive (100%, N = 238) link between the physical display of an OM and a resultant behavior. This slower, rhythmic mimic of the more rapid Types 1-3 OMs is similar to the longitudinal body movements involved in pill formation. We hypothesize that this rocking motion is the genesis of the movements involved in producing the defecation-preceding Type 4 OMs as well as the other OMs.

Stomodeal trophallaxis. The literature often does not differentiate between stomodeal and proctodeal trophallaxis in experimental design (Rosengaus et al. 1986, Cabrera and Rust 1999, Sheets et al. 2000, Suarez and Thorne 2000a; 2000b, Saran and Rust 2005). Trophallaxis was common among the termites we observed, with an 'average' termite obtaining food by one of three types of acquisition behaviors: stomodeal trophallaxis (246 events), proctodeal trophallaxis (336 events), and self-obtained alpha cellulose powder (311 events) (Whitman 2006, Chapter 4). However, regurgitated materials were shared rarely (4 times in 198 events) and material obtained during allogrooming (146 events) was never shared. It can be assumed that considerable time and effort must be expended by a termite to acquire a bolus of wood and taking a bit of macerated cellulose saves the effort of extracting. In addition, cellulose is difficult to digest, low in nitrogen and provides low energy returns (Slaytor 2000), a food acquisition scheme that favors food sharing (Thorne 1997). It has recently been demonstrated that lower termites produce their own salivary gland cellulases (Watanabe 1998, Bignell 2000, Nakashima et al. 2002, Scharf et al. 2003). Acquiring macerated cellulose from a nest mate would therefore be doubly attractive – not only is it easily obtained but it is likely to be partially digested.

The 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 climate conditions within the nest (Grube et al. 1997, Grube and Rudolph 1999). Labial gland secretions are involved in chemical communication between nest mates including feeding behavior (Kaib and Zeismann 1992, Reinhard et al. 1997, Reinhard and Kaib 2001). We propose that a termite chewing fresh cellulose could be mixing feeding-stimulating secretions as well as cellulases into the cellulose. The labial gland secretions would alert nest mates to the presence of chewed cellulose and the partially pre-digested state reward them for partaking. This chemical signal fits well with our observation that termites often moved directly to a primary or secondary material-chewing nest mate and initiated a stomodeal transfer. Termites chewing cellulose shared this material 30% of the time, with materials obtained by proctodeal transfer being shared 23% and stomodeal transfer 27%. Regurgitated substrates were exchanged only 2% of the time and material obtained via allogrooming was never shared. It is possible that the allogrooming materials contained cuticular hydrocarbons thought to be used in chemical communication between termites or were related to control of pathogens (Bagneres et al. 1991, Boucias et al. 1996, Myles 1996, Kaib et al. 2002). It was clear that something attracted nest mates toward cellulose or trophallaxis-chewing nest mates but not to those chewing regurgitated or allogroomed material. Sharing regurgitated materials was an infrequent occurrence in our experiments and highlights another area where lower termites differ from the Hymenopteran-derived definition of stomodeal trophallaxis (Wilson 1971).

Proctodeal trophallaxis. Proctodeal transfers have been cited as a mechanism for reestablishing the hindgut fauna lost during molting (Weiss 2006). The first feeding observed in the freshly molted termite we observed was proctodeal trophallaxis, which occurred within 30 min of conclusion of the ecdysis event. It is possible that the viscous nature of the material we observed serves to protect protists during transport through the upper alimentary tract in newly molted individuals.

3.6 Conclusions

Behavioral observations can address questions affecting numerous aspects of termite biology. Our observations demonstrate that the assumption of Grube and Forschler (2004) in their calculation of colony growth rates using a daily egg-laying rate must be re-examined. Understanding the frequency of mating and its correlation with egg laying would corroborate statements by Raina et al. (2003) and Costa and Patricio (2005) of the role that morphology and mating have on reproductive capacity and colony growth in the lower termites. The conclusion that ecdysis in *Reticulitermes* is a group event should be acknowledged as important for understanding the evolution of eusociality (Thorne 1997). The role that group molting plays in colony-level immune response could support observations that group size influences survivorship (Rosengaus et al. 1999b, Traniello et al. 2002). Numerous papers report the possibility that cannibalism is involved in toxicant transfer, but none have examined the potential role of consumption of exuvia (Su and Scheffrahn 1993, Sheets et al. 2000, Valles and Woodson

2002). Modeling toxicant transfer by contact and grooming during group ecdysis and consumption of the exuvia would gain accuracy with information on the frequency and number of termites involved in molting. 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; for instance, the need for moisture during exploration (Grube and Rudolf 1999). Other implications could involve movement of termites between established feeding sites as result of pill deposition. How pill formation/deposition affects termite communication of feeding site selection, direction of gallery construction, and movement of toxicants must consider the mechanics of this construction behavior. The observation that regurgitated material is rarely shared via trophallaxis is significant to understanding food acquisition and complete digestion of a recalcitrant, low energy food source that is more often shared by proctodeal exchanges than stomodeal has implications for understanding bait-toxicant transfer. Examining previous work on trophallaxis our observations support the findings of Saran and Rust (2005) about transfer of C^{14} between nest mates. The methods of transfer available, because of experimental design, would have been accomplished by consumption of proctodeal material and material obtained during allogrooming. We suggest most of the C^{14} sucrose was transferred by incorporation into cuticular components and obtained thru allogrooming or consumption of exuvia after molting. Our observations on OMs have described two new responses to this behavior: that of the Type 4 with defecation and a ‘move out of the way’ or ‘stop grooming’ with the single Type 3.

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Table 3.1 Timelines of mating and ecdysal events

Mating event one (seen in reproductive pair one on Day 7, began 10:32:49 A.M.)

10:32:49 to 10:32:56	Antennation phase
10:32:56 to 10:33:00	Positioning
10:33:00 to 10:33:23	Coupling

Mating event two (seen in reproductive pair two on Day 1, began 7:35:00 A.M.)

7:35:00 to 7:35:09	Antennation phase
7:35:09 to 7:35:12	Positioning
7:35:12 to 7:35:42	Coupling

Mating event three (seen reproductive pair two on Day 7, began 1:58:44 A.M.)

1:58:44 to 1:58:54	Antennation phase
1:58:54 to 1:59:02	Positioning
1:59:02 to 1:59:24	Coupling

Tail chasing (seen in all colonies, start and stop times are total elapsed time that day)
0:02:34 to 0:10:08, total duration 7:34 or 454 seconds
13:36:05 to 13:48:10, total duration 12:05 or 725 seconds
13:50:18 to 13:53:34, total duration 3:16 or 216 seconds
18:52:38 to 19:03:30, total duration 10:52 or 652 seconds
19:05:05 to 19:10:53, total duration 5:48 or 348 seconds
1:27:16 to 1:34:56, total duration 7:40or 460 seconds
4:14:03 to 4:16:40, total duration 2:37 or 157 seconds
1:51:35 to 1:56:25, total duration 4:50 or 290 seconds

Ecdysis (seen in colony six on Day 7, began 2:23 P.M.)

2:23	Subject ceases locomotion
2:28	Light pumping begins
2:37	Pumping increases
2:40	One termite allogrooming
2:42	Three termites allogrooming, vigorous allogrooming begins
2:43	Subject exiting exuvia, head capsule deflexed against ventral thorax
2:45	Five termites allogrooming
2:50	Head begins to relax from deflexed posture
2:51	Exuvia removed
3:02	Three termites allogrooming
3:07	Subject's head in typical posture
3:11	One termite allogrooming, subject assumes normal posture, end of vigorous allogrooming
3:21	First post-edcysal feeding (proctodeal material)

CHAPTER 4

DIRECT OBSERVATION OF DIVISION OF LABOR IN THE LOWER TERMITE *RETICULITERMES FLAVIPES* (KOLLAR)[2]

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[2] Whitman, J. G., B. F. Forschler. 2006. To be submitted to Animal Behavior.

4.1 Introduction

The worker caste in *Reticulitermes* constitutes between 80-90% of the individuals in a subterranean termite colony (Krishna and Weesner 1969). The term used to describe this caste is indicative of their role in performing the tasks required to maintain the colony as a functional social unit. Workers care for and feed the dependent castes and forage for food resources. The repertoire of behaviors displayed by worker termites has been examined in three genera of lower termites (Rosengaus and Traniello 1993, Maistrello and Sbrenna 1996, Crosland et al. 1997, Crosland et al. 1998, Iwata et al. 1999). The list of behaviors examined included grooming, building activity, oscillatory movements, cellulose consumption and trophallaxis. The primary focus of these works has been on intercaste interactions such as worker-nymph and worker-soldier, though intracaste worker interactions have also been examined.

It seems unlikely that a cohesive social structure can be maintained without some degree of task differentiation (Wilson 1971, Oster and Wilson 1978), however, task allocation has not been demonstrated for termites from the genus *Reticulitermes*. The work published on higher termites (see in part Jones 1980, Gerber et al. 1988, Hinze and Leuthold 1999, Hinze et al. 2001) have few points for comparison with the basal termite families because of differences in social structure and food acquisition strategies (Krishna and Weesner 1969, Wilson 1971). Evidence for temporal polyethism in Rhinotermitidid's was provided in a series of studies that showed larger - and presumably older - workers performed tasks such as tunnel construction more efficiently than smaller workers (Crosland et al. 1997; 1998, Crosland and Traniello 1997). Iwata et al. (1999), working with *R. speratus*, reported that larger workers were most often involved in carrying eggs or larval termites. However, Rosengaus and Traniello (1993) found no evidence for temporal polyethism in the genus *Zootermopsis* and reported the time spent feeding matched what Maistrello and Sbrenna (1996) found in *Kalotermes.*

Laboratory tests involving *Reticulitermes* typically use, for the sake of efficiency, randomly selected individuals chosen from a larger population (Smythe and Carter 1970, Behr et al. 1972, Grace 1991, Oi et al. 1996, Forschler and Townsend 1996, Gold et al. 1996, Smith and Rust 1993). The random selection process is predicated on the assumption that all worker termites display the same suite of behaviors and/or the ability to switch tasks within the context of the experimental design. The presence of division of labor combined with the random selection process could influence bioassays results especially if certain tasks are performed by a low percentage of the population. The variation common in wood preference studies (McMahan 1966, Smythe and Carter 1969; 1970, Behr et al. 1972, Carter et al. 1972, Su and La Fage 1984, Delaplane and La Fage 1989a; 1989b, Su and Scheffrahn 1993, Cornelius 2001) could be explained if tasks such as gallery construction or brood care were allocated to 40% of the workers within a population and these showed no predisposition for feeding. In such an instance, it is easy to understand why certain randomly-selected replicates would feed more, or less, than others.

We report, for the first time, evidence for task allocation in workers of *R. flavipes* (Kollar) from a study that examined the incidence of and time spent on a suite of observable worker/worker behaviors including autogrooming, allogrooming, feeding and excavation. The null hypothesis that groups of randomly selected workers and comparably sized intact colonies would display similar behavioral patterns was tested by analyzing data collected from reviewing time-coded video tape of 36 individual worker termites for a total of nine hours per termite. The data are discussed in relation to the behavioral repertoire of worker termites, the time spent on various activities and task specificity.

4.2 Materials and methods

Experimental design. Two treatments were tested, intact colonies or randomly selected workers. The first contained an entire laboratory-reared colony (LC) that included the following castes: primary reproductives, soldiers, larvae and workers. The second treatment consisted of randomly selected workers (RW) taken from a larger population of field-collected termites. A replicate consisted of a cohort of termites within an arena and three replicates per treatment were scored. Data recorded for 24 consecutive hours on three separate days included the occurrence and duration of the following behavior groups: grooming, excavation, oscillatory movements, performing no visible activity, and feeding. Six randomly selected termites from each replicate were scored for all behaviors during 12 separate 15-min periods equally spaced through the 24 h of video tape for each of the three days of filming.

Termites. All termites were *R. flavipes* (Kollar) identified using soldier and/or alate characters (Scheffrahn and Su 1994). Three replicates were drawn from 18-mo old laboratory colonies (LC) maintained as described in Grube and Forschler (2004). The remaining 3 replicates consisting of randomly selected workers (RW) taken from laboratory cultures maintained as described in Forschler and Townsend (1996) that were collected one to two months prior to inclusion in the experiment. Two LC replicates (LC1, LC2) contained approximately 50 termites and one (LC3) had 15 termites. The RW replicates were size-matched to the laboratory-reared colonies with one (RW3) containing 15 workers and the other two 50.

Marking. All termites were marked with paint pens to aid in tracking individuals during observation. Termites were marked using a Decocolor (Uchida of America, Extra-Fine hard tip) paint pen whose plastic tip gave better flow and marking control than soft felt tips. Termites were positioned underneath a binocular dissecting microscope to enhance marking precision and this was accomplished on a piece of wet filter paper to provide traction for termites to resist sticking to the pen.

Two colors of paint - red and black - were used to allow sufficient variation to readily distinguish individual termites. Grooming did not affect the marks, which were only lost during molting throughout the seven days of these experiments.

Experimental Arena. Observation arenas were formed as a puck (11.4 cm ID) by moistening 200 ml (dry volume) of Densite industrial plaster (Georgia Pacific Gypsum Corporation) with 90 ml of water in a 11.4-cm round baking pan to a depth of 25 mm. Pucks were removed from the pan when dry and the bottom was made flat by hand-sanding with 220-grit sandpaper.

A Dremel rotary tool mounted in a model 330 Dremel hand router with a tile grouting bit (3.175 mm diameter) was used to carve three 6.99-cm^2 rectangular chambers (Feeding Chambers, FC 1-3) that measured 2.33 x 3 cm by 2 mm deep into each Densite puck. Chambers were separated by 1-mm wide Densite walls and connected by 2-mm wide openings to allow termites to pass from chamber to chamber, thus creating two dead-end chambers (FCs 1 and 3) with a single connecting chamber (FC 2). A small (4x4 mm) notch was drilled from the edge of the puck into FC1. This notch allowed for placement, underneath the glass cover, of a piece of 4.76-mm OD Tygon tubing which acted as the termite introduction pathway (Figure 4.1).

The Densite puck was saturated with distilled water and all chambers half-filled with separate, equal deposits (approximately 0.5 g) of Kaolin clay and water-saturated alpha cellulose slurry intended as tunneling and food substrates, respectively (Figure 4.2). Kaolin clay was placed in the arena as a dry substrate but it drew moisture from the Densite puck and became visibly wet within minutes. Connections between chambers remained open and unobstructed to allow termites free access from one to the next. Care was taken to keep the Kaolin and cellulose to a depth of 2 mm, equivalent to the top of the Densite puck.

The substrate-filled puck was placed in the bottom of a 140 mm diameter petri dish (Falcon®). The water-filled petri dish base allowed moisture to be maintained in the arena as Densite can retain 25% of its weight in water. A piece of standard 0.3175-cm thick window glass served as the cover-plate and it was secured to the puck using metal binder clips (Office Depot, 2.54 cm). A 20 cm length of 4.76-mm OD Tygon tubing was placed into the notch in the puck's exterior rim and inserted into a 4 mm ID glass funnel from which termites were tapped into the tubing. The end of the tubing was then clamped shut with a metal binder clip (Office Depot, 8-mm) and termites allowed to enter the arena after which the clamp was moved to exclude termites from the tube and confine them to the arenas.

Cameras and optics. Red lighting was used to reduce light intensity for filming and was accomplished by placing two 0.3175-cm discs of red plastic transparency film on the articulated fiber-optic lights of a 150W microscope light (model LS86-110, Fiberoptic Specialties, Inc). Illumination produced at the light's lowest setting was sufficient for the three Jai CV-M50IR CCD's cameras. Two of cameras were equipped with Navitar 7000 zoom lenses and one with a 35mm telephoto zoom lens. Cameras were mounted on tripods clustered above the arena with one camera focused on one of the three chambers.

Recording. Two Panasonic AG-2560 SVHS and one Emerson DA-4 Head brand VCRs were used to produce videotapes of termites in the arenas. Sony brand tapes (T-160 Standard

Quality) on extended play provided 8-h of recording per tape. Tapes were reviewed on a Panasonic AG-5710 Desktop Editor.

The extended play mode, while enabling a long period of continuous recording, resulted in the loss of video quality. Fine details such as the movements of the maxillae and maxillary palps were obscure, but gross movements of the body, antennal and mandibles were clear and obvious.

Time code. Video lines passed through a separate AEC Box-18 video time-code generator adding a time code stamp to the video signal for accurate measurement of time.

Filming. Filming was maintained for 24-h for each of three days per replicate. Day One began when the termites were placed into the introduction tube. Filming was repeated on Day Four and Day Seven, resulting in a total of 72-h of filmed behavior per replicate.

Scoring. A single termite was chosen from the RW3 replicate and its entire behavioral repertoire was scored for two full 4-h periods - the first on Day One and the second on Day Four. These data were divided into 1-min, 5-min, 15-min and 30-min blocks and analyzed to determine the most efficient time frame or combinations of time frames needed to encompass a complete behavioral repertoire.

Six individuals with easily distinguished marks were selected from each replicate for observation and assigned a unique number for continuous tracking between the three tapes used within a day of filming and between days of filming. The start and stop time for each behavior by individual was recorded using the Observer 5.0 computer program (Noldus 2003). The six selected termites within each replicate were scored for all behaviors exhibited during 12 evenly spaced 15-min intervals (the first 15 min of every other hour) within a continuous 24-h period resulting in 9-h of observation per individual over three days. Individual termites moved into the arena at their own rate on Day 1 and the first scoring interval for each subject could not begin until they were visible. As a result, the scoring intervals were not perfectly spaced on Day 1, though they were spaced as evenly as possible. A single replicate provided 54-h of scored behaviors from all 6 individuals combining the 3 days of filming. Two of the RW replicates had 16-h of video due to VCR operational error with RW1 missing the final 8-h of Day One and RW3 missing the first 8-h of Day Seven.

A sheet of plastic transparency film (3M) was placed on the video monitor and the pen-mark that distinguished that individual was traced to aid in tracking specific individuals over time between tapes and days. A pair of scorers was used to facilitate the time-consuming process of scoring behaviors. Side-by-side training was conducted and comparisons of shared observations made to ensure that both scorers were able to score consistently.

Statistical analyses. Analysis of the continuous 4-h data sets were accomplished in Excel (Microsoft Office 2003) and involved dividing the 4-h data set into 1, 4, 10, 15 and 30-min blocks, then randomizing their order 30 times and determining how many blocks were needed to detect all behaviors. This analysis included behaviors that occupied more than 5 min out of 9 h of video from the experimental group to exclude infrequently observed categories. The behaviors scored were allogrooming, autofeeding of cellulose from the arena, proctodeal and stomodeal trophallaxis, excavation and no visible activity (NVA).

Statistical analyses were run with SAS 9.1 (SAS 2004) and SPSS 14.0 (SPSS 2005). Analysis was conducted using two behavioral datasets that included the time spent performing a behavior and the frequency of occurrence. The time-spent data were obtained by combining the times, by respective behavior, from the 12 15-min filming episodes over a 24-h period into a single number, hereafter termed ‘termite-day’. The frequency data was likewise obtained by combining the occurrences from all 180 min, by subject, into a single termite-day number. Both datasets were analyzed by comparing individuals within replicates, replicates within treatments and treatments against each other.

The termite-day time data were analyzed using Proc Discrim in SAS 9.1 (SAS 2004). Predictor behaviors, when used, were selected by stepwise analysis with an entry value of 0.03 and retention value of 0.05. Analysis of variance via Proc GLM was used to recommend the use of a pooled or unpooled covariance matrix for each analysis. The time data was conservatively adjusted to correct for the scoring intervals missing from the RW1 and RW3 replicates by imputing absent values using imputed means (Huberty 1994), with means taken from the entire data set rather than the replicates with missing values.

Chi-Square crosstabs analysis was conducted using SPSS (2005) and analyzed frequency data by termite day. The frequency data were placed into categories with interval widths designed to maximize and equalize cell counts. Allogrooming and autogrooming frequency categories were 0-14 occurrences, category 1; 14-30, 2; 31+, 3. Autofeeding cellulose, proctodeal and stomodeal trophallaxis and Type 1 OM frequency categories were 0 occurrences, category 1; 1-3, 2; 4+, 3. Categories for chewing regurgitated material were 0 occurrences, category 1; 1 occurrence, 2; 2+, 3. Chewing after allogrooming, excavation and OM types 2-4 categories were defined as 0 occurrences, category 1; 1+, 2. Termite-days with missing frequency values (RW1 and RW3) were conservatively adjusted by imputed means to correct for the missing data before assignment to category.

Behavioral definitions. Detailed descriptions of the mechanics of each behavior can be found in Chapter 3 of Whitman (2006). Grooming is either cooperative cleaning between members of a social group (allogrooming) or a self-cleaning act (autogrooming) (McFarland

1981). We define *allogrooming* as sustained contact of another individual's body with the mouthparts. The time spent allogrooming started when the mouthparts of one termite made and sustained contact with any body part of a nest mate and ended when the contact ceased. We noted that termites performing allogrooming would switch between body parts or subjects. If time spent switching to another target was less than 10 s it was scored as a single ongoing allogrooming event. *Autogrooming* was limited in all instances to cleaning the antennae and scored separately from allogrooming, with start and stop times beginning with contact between the mouthparts and the antenna and ending with release.

Feeding behaviors were defined by the display exhibited by a characteristic, rhythmic motion of the mandibles (chewing) and was divided into autofeeding or allofeeding. Autofeeding was indicated when a worker displayed chewing after picking up alpha cellulose powder from the arena, allogrooming or regurgitated material. *Chewing regurgitated material* was defined by rhythmic mandibular movements that were not preceded by either trophallaxis or picking up alpha cellulose powder. *Chewing after allogrooming* was noted by rhythmic mandibular movement following allogrooming. The time spent autofeeding had different starting points; autofeeding on cellulose included the time involved in obtaining a bolus of food and continued through the time spent chewing while both feeding on materials obtained by allogrooming or regurgitation was started and ended with the chewing phase.

Trophallaxis is a behavior defined as sharing of digestive fluids and food obtained as either stomodeal from the foregut, via the mouth, or proctodeal from the hindgut, taken from the anus of a nest mate (Wilson 1971; 1975, Spradberry 1973, Dumpert 1978, Breed et al. 1982, Winston 1987, Hunt 1991). We included both types of trophallaxis as allofeeding with a *stomodeal trophallaxis* identified when a termite began chewing, after a mouthpart-to-mouthpart encounter with a nest mate. Similarly, we defined an event as *proctodeal trophallaxis* when a termite began chewing, after a mouthpart-to-anus encounter with a nest mate. Allofeeding times included the time spent procuring and chewing the donated bolus.

Excavation of substrate, representing gallery formation and modification, was identified by the formation of a ‘pill’ of either Kaolin clay or alpha cellulose using the mouthparts, subsequent transport of the pill, and its deposition elsewhere (Whitman 2006, Chapter 3). Start and stop times of excavation behavior were recorded to include pill formation, transport, deposition and return to the excavation site.

Oscillatory Movements. OMs were divided into four types as described in Chapter 3 of Whitman (2006). The time spent on this behavior was started with the first movement indicative of that type of OM and ended when the subject returned to a normal posture. Start and stop times were measured by the second for the Type 1 and 2 while repeated Type 3 times included any multiple iterations of the same movement and the slower Type 4 stopped when the act of defecation was noted.

No visible activity (NVA). The NVA behavioral category was defined by the subject performing no actions that could be placed in any of the aforementioned behavioral displays. This category included resting and walking while the time spent in this ‘activity’ was delineated by the cessation of any of the previously described behaviors followed by the resumption.

4.3 Results

The behavioral repertoire of a *R. flavipes* worker, as gleaned from nine hours of scored behaviors for each of 36 termites over three separate days, included no visible activity (resting and walking), auto and allogrooming, excavation, OMs, and two types of feeding behavior – autofeeding (food obtained from the arena, from allogrooming or regurgitation) and allofeeding (food obtained from a nest mate through either stomodeal or proctodeal trophallaxis) (Whitman 2006, Chapter 3).

The interval test demonstrated that it required 24 ± 14 1-min, 11 ± 6 4-min, 5 ± 2 15-min and 5.5 ± 1.6 30-min blocks of observation for detection of tested behaviors for a termite worker on Day 1 (the day termites were introduced into the arena). On Day 4 it required 93 ± 53 1-min, 28 ± 15 4-min, 8 ± 4 15-min and 5.5 ± 1.6 30-min intervals to observe the behaviors. A comparison of mean durations of behaviors scored from the 4-h blocks against 15-min scoring intervals of the appropriate day shows overlapping means, verifying that the composition of our termite-day sampling routine encompassed a robust data set (Table 4.1).

Variability was the overriding theme of this behavioral data set. Individual termites spent various amounts of time on behaviors that persisted for longer than 5 s and performed them at various times with variable frequency within and between days. Means provided high standard deviations, regardless of the data set (Tables 4.1, 4.2). Variability in frequency and duration of an individual termite's performance of allogrooming, autofeeding cellulose, trophallaxis, and excavation are clearly illustrated in ethograms showing these behaviors as they were scored in the continuous 4-h blocks used for the interval test (Figures 4.3-4.4). Ethograms of a single Day 1 15-min scoring interval, each bar representing an individual scored simultaneously with others in its replicate, show that individual patterns for these behaviors varied widely, even during the same period of time (Figures 4.5-4.6). However, all behaviors except excavation, stomodeal trophallaxis and OMs were displayed by all termites in nine hours of observation (Table 4.3).

The experimental data involved the entire behavioral repertoire recorded during 12 15-min intervals from 24-h of video which, unless otherwise stated, were combined to produce 'termite-day' data sets consisting of time spent in a behavior and frequency of occurrence. The amount of time spent in any 15-min scoring segment was variable for all behaviors (Tables 4.1-4.2). The data failed a test of normality (SPSS, Wilks-Shapiro W, p-values for all behaviors >0.001), which recommended use of nonparametric methods. We chose to use discriminant analysis for the time data and crosstabs chi-square analysis for frequency categories.

The average termite worker, represented by all 36 individuals regardless of treatment regime combining all three days, spent most of its time in the NVA category (standing/walking) as expressed by percent time of 9-h (all three termite-days) - 72.8% (Table 4.4). Percent time spent allogrooming was 6.3% while autofeeding cellulose took up 4.9% of 9-h and procuring and chewing proctodeal material and stomodeal material accounted for 4.0 and 4.2%, respectively (Table 4.4). Chewing regurgitated material took up 0.5% and chewing material obtained during allogrooming 0.3% of 9-h (Table 4.4). Worker termites spent 4.5% percent of 9-h excavating substrates, autogrooming accounted for 0.7% of 9-h while all OMs combined accounted for approximately 0.1% (Table 4.4)

We did not statistically compare percent times per termite-day spent on various behaviors between treatments, but we did note some interesting numerical trends. We saw that the RW treatment had higher percent times of 9-h (three termite-days) in allogrooming, proctodeal trophallaxis, chewing regurgitated material, chewing after allogrooming, excavation and autogrooming (Table 4.5). The LC treatment had a higher percent time in stomodeal trophallaxis. Percent time spent in the NVA category was 77.3% in the LC treatment and 68.3% in the RW.

Excavation was an activity that was consistently performed by certain individuals and not others, therefore we calculated percent time of 9-h (three termite-days) for both excavating and non-excavating termites (Table 4.6). Most non-feeding behaviors were numerically similar, but excavating termites had higher percent times in autofeeding, cellulose and proctodeal trophallaxis while non-excavating termites had a higher percent time in stomodeal trophallaxis. Time spent walking and resting accounted for 67.9% of an excavating termite's time and 79.6% of one that did not excavate. Excavators spent 7.7% of 9-h excavating, obviously taking that time from the NVA category.

Comparing the three termite-days of scored video on all behaviors between days indicated a decrease in activity as the experiment progressed (Table 4.7). During a comparison of days of filming using discriminant analysis of time termite-days from all activities (i.e. excluding NVA), stepwise selection indicated that all activities were predictors of the day in bioassay except allogrooming, proctodeal transfers, and Type 4 OMs, suggesting that time spent on the non-selected behaviors did not change between days. For the unfamiliar, discriminant analyses matrices are read by comparing the number of correct classifications in the groups being compared, with a high classification rate indicating that the groups were easily distinguishable. A chi-square test run on the correct classifications indicates whether they differ significantly from what could be expected by chance. The classification rate of the analysis was obtained by adding the number of termite-days correctly classified and dividing by the total number of termite-days classified. This returned a classification rate of 98.1% (chi-square value of 192.9, p-value >0.001), clearly illustrating that the days differed in activity levels (Table 4.8). A second discriminant test using NVA as a predictor demonstrated similar results, with a 55.8% correct classification rate of day of filming (chi-square value of 26.8, p-value >0.001) (Table 4.9).

Due to the change in behaviors over time, we performed our treatment comparisons by day of the experiment, using time termite-day for discriminant analysis and frequency termite-day for chi-square crosstabs analysis. Discriminant classification of allogrooming by the LC and RW treatments gave a Day 1 correct classification rate of 63.9% (chi-square value and p-value of

1.44, 0.229) (Table 4.10). Days 4 and 7 were found to have 63.9% (1.89, 0.169) and 72.2% (3.78, 0.052) correct classification rates. Crosstabs analysis of allogrooming frequency categories revealed no significant differences (Table 4.11)

Discriminant classification results for time spent in autofeeding cellulose by LC and RW treatments indicated correct classification rates of 69.4% (3.22, 0.073), 75.0% (5.89, 0.015) and 86.1% (9.89, 0.002) on Days 1, 4, and 7 while there were no significant differences in frequency category (Tables 4.10 and 4.11). Discriminant analysis of time spent in proctodeal transfers by LC and RW treatments gave correct classification rates of 75.0% (5, 0.025) on Day 1, 61.1% (1.78, 0.0182) on Day 4 and 77.8% on Day 7 (5.56, 0.018) and a difference in frequency category was found on Day 4 (chi-square and p-value of 7.156, 0.028) (Tables 4.10 and 4.11). Time spent in stomodeal exchanges by LC and RW treatments returned correct classification rates of 83.3% (10, 0.002) on Day 1, 69.4% (4.11, 0.043) on Day 4 and 77.8% (7.56, 0.006) on Day 7, while a significant difference was found in frequency category on Day 1 (19.70, >0.001) (Tables 4.10 and 4.11).

Discriminant classification results of time spent chewing regurgitated material by LC and RW treatments gave a Day 1 correct classification rate of 50% (.22, .637), a Day 4 of rate 61.1% (2.89, 0.089) and a Day 7 rate of 88.9% (11.11, 0.001) while no differences were found in frequency category (Tables 4.10 and 4.11). Comparisons of time spent chewing after allogrooming by LC and RW treatments revealed correct classifications of 75.0% (9, 0.003) on Day 1, 75.0% (5, 0.025) on Day 4 and 61.1% (1.78, .182) on Day 7 while no significant differences in frequency category were found (Tables 4.10 and 4.11).

Time spent in excavation of substrate by LC and RW treatments gave correct classification rates of 83.3% (8.22, 0.004) on Day 1, 52.8% on Day 4 (16.11, >0.001) and 58.3%

(13.0, >0.001) on Day 7 and a difference existed in frequency categories on Day 1 (chi-square and p-value 7.2, 0.007) (Tables 4.10 and 4.11).

Time spent performing Type 1 OMs by LC and RW treatments returned correct classification rates on Days 1, 4, and 7 of 75% (4.56, 0.003), 61.1% (1.78, .182) and 58.3% (1, .317) respectively while categorical frequency of occurrence was significantly different on Day 4 (chi-square and p-value 12.77, 0.002) (Tables 4.10 and 4.11). Discriminant analysis of time spent performing Type 2 OMs by LC and RW treatments returned correct classification rates of 33.3% (2, .157) on Day 1, 75.0% (5.89, 0.015) on Day 4 and 77.8% (6.44, 0.011) on Day 7 while categorical frequency of occurrence was significantly different on day 4 (chi-square and p-value 9.03, 0.003) (Tables 4.10 and 4.11).

Examination of time spent performing Type 3 OMs by LC and RW treatments returned correct classification rates on Days 1, 4, and 7 of 69.4% (3.22, 0.073), 86.1% (9.89, 0.002) and 86.1% (9.89, 0.002) respectively while categorical frequency of occurrence was significantly different on Day 4 (chi-square and p-value 5.6, 0.018) (Tables 4.10 and 4.11). Time spent performing Type 4 OMs by LC and RW treatments returned correct classification rates of 66.7% (5.56, 0.018) on Days 1 and 4 and 61.1% (6.44, 0.011) on Day 7 while there were no significant differences in category of frequency of occurrence (Tables 4.10 and 4.11).

Examination of time spent autogrooming by LC and RW treatments showed correct classifications on Days 1, 4, and 7 of 80.6% (6.78, 0.009), 77.8% (4.56, 0.033) and 72.2% (11.11, 0.001), respectively with no significant differences in frequency categories (Tables 4.10 and 4.11).

4.4 Discussion

The data accumulated in this experiment provides the first reasonable approximation of how the average *Reticulitermes* worker occupies its time as determined from an accumulated 9-h of video per termite using six workers scored simultaneously from six replicates over three separate days. The entire data set therefore provides 108 separate measures, termed a termite-day, composed of 3-h per subject representing the sum of 12 separate 15-min blocks of time within a 24-h period. Allogrooming, the most consistently observed activity, was performed in all 108 termite-days (Table 4.2). Feeding behaviors, however, exhibit much less consistency. When time spent in each feeding behavior on each day of filming is expressed as a percent of total time spent feeding, we see there were three out of 108 possible termite-days where an individual termite was not observed to eat anything by any of the five measures we scored (autofeeding cellulose, proctodeal and stomodeal trophallaxis and chewing regurgitated and allogrooming material) (Table 4.12). Excluding those that did not eat for an entire day, there were 26, 20, 25, 31 and 39 termite-days when an individual was not observed autofeeding cellulose, performing proctodeal or stomodeal trophallaxis, chewing regurgitated material, and chewing after allogrooming, respectively (Table 4.12). The inconsistency in feeding episodes need further examination and certainly adds to the variability at the individual level of the time data (Tables 4.1 - 4.3, Table 4.12). These data demonstrate that individual termites spend differing amounts of time engaging in any particular activity, providing a partial explanation for the erratic feeding behavior seen in previous work (Smythe and Carter 1969; 1970, Forschler 1996, Oi et al. 1996). The ethograms clearly show that individual worker termites had disparate behavior patterns (Figures 4.3-4.6). Determining biological significance from statistical comparisons of time spent on a behavior was frustrated by the lack of consistency at the level of the individual worker (Tables 4.2-4.3, 4.12).

The literature is filled with termite behavioral studies that, because of differences in design, are not comparable. Stuart (1963; 1967; 1988) studied OMs in *Zootermopsis* using real-time observations and sound-recording equipment. Howse (1962, 1965) filmed *Zootermopsis* OMs and was the first to separate OMs by bodily movement using real-time visual scoring with unspecified intervals and durations to obtain caste frequencies of OMs, building activity, and trophallaxis. Jones (1980) spent 31-h of live observations in 20-s increments studying building activities of *Nasutitermes costalis.* Su and La Fage (1987) used real-time observations of *Coptotermes formosanus* workers and soldiers, each observation lasting 10-s and taken at 5-min intervals for 2-h, noting that starvation increased feeding frequency and starved workers fed soldiers while satiated workers did not. Rosengaus and Traniello (1993) used 47-h of 10-min observation intervals of individuals in real-time with *Z. angusticollis,* scoring frequency of autogrooming, allogrooming, building activities, OMs, chewing, and walking/resting. Maistrello and Sbrenna (1996) video taped *Kalotermes flavicollis* for three 6-min periods scoring allogrooming, OMs, and walking/resting behaviors. Crosland et al. (1997) used 3, 4, and 20 1-min real-time observation periods per day with *R. fukienensis* scoring frequency of OMs and grooming of eggs, brood, and queens. Iwata et al. (1999) used 200-h of live scoring, unspecified duration and interval, to record frequencies of allogrooming, stomodeal and proctodeal trophallaxis, cellulose sharing and carrying of larvae.

The use of time-coded video-tape allowed us to simultaneously score time and record the frequency of allogrooming, autogrooming, chewing after allogrooming, chewing of cellulose, regurgitated, proctodeal and stomodeal materials, four different types of OMs, excavation of substrates, and walking/resting. Our data, drawn from randomly-selected workers and intact 18-mo colonies, allowed statistical comparison of worker-worker interactions comparing a natural social structure to an experimental population. Our arena design was a reasonable approximation of the natural environment of a subterranean termite to facilitate normal, natural behavior. This is also the first study to score termite behavior for a continuous 4-h period and use that data to estimate the time-frame sequences needed to capture an entire worker behavioral repertoire. This study indicates that there are no observational shortcuts for obtaining a full catalogue of an individual termite's behavioral repertoire. Intuitively, the number of observation time-blocks needed varies depending on the behavior being observed - a behavior that occurs frequently or for long periods would have a correspondingly higher detection rate and would thus require fewer samples to ensure detection.

The termites we observed spent the majority (72.8%) of their day performing no obvious work – standing or moving about the arena, matching well with Maistrello and Sbrenna (1996) who reported pseudergates spent 86.3% of their time performing NVA (Table 4.4). High levels of inactivity are consistent with an animal that displays a low metabolic rate and feeds on a nutrient-poor resource (Nunes et al. 1997, Shelton and Appel 2001). In our study, *R. flavipes* spent 13.6% of their time chewing food, though only one-third of that time was spent autofeeding cellulose. The remaining two-thirds was spent chewing food that was either regurgitated or obtained from a nestmate via trophallaxis or allogrooming (Table 4.4). It should be noted that our experiments used alpha cellulose powder as a food source, which may have influenced feeding behavior. The percent time obtained by Maistrello and Sbrenna (1996) for pseudergate feeding on wood (4.12%) and allogrooming (4.69%) is similar to the percentages we obtained combining treatments for time autofeeding cellulose and allogrooming (4.9% and 6.3%, respectively) (Table 4.4). Maistrello and Sbrenna (1996) reported a higher mean percentage of time spent performing OMs among pseudergates (4.84%). The *Reticulitermes* in our study spent less than 1% of their time performing OMs (Table 4.4). Rosengaus and Traniello (1993) found feeding percentages among *Zootermopsis angusticollis* workers instars III-VII averaged 21.3 ± 5.3%, but it must be noted that their definition of feeding included coprophagy. Rosengaus and Traniello (1993) also found construction/excavation percentages averaged 8.4% which is similar to our mean of 7.7% (Table 4.6). Su and La Fage (1987) described feeding in *C. formosanus* as being donor-driven in worker-soldier and worker-worker interactions. Our observations only support communication of hunger or need by the recipient because all worker-to-worker trophallaxis we filmed using *Reticulitermes* was initiated by the recipient and we never observed a donor solicit a trophallactic exchange (Whitman 2006, Chapter 3).

Chewing after allogrooming and chewing regurgitated material have not, to our knowledge, been detailed as separate behaviors. Allogrooming has been linked to removal of fungal conidia (Myles 1996, Boucias 1996) and is generally described as maintaining a disease-free habitat, so seeing post-allogrooming chewing is not surprising. The low percentage of allogrooming events that were followed by chewing (4.0%) indicates allogrooming also serves to communicate additional information; for example, allogrooming preceded trophallaxis approximately 50% of the time in our subjects (Whitman 200, Chapter 3). Allogrooming could play a role in the spread of the cuticular hydrocarbons that have been found to play a role in interspecies and nest mate recognition in termites (Bagneres et al. 1990, 1991, Takahashi and Gassa 1995, Kaib et al. 2002).

Our observations on five feeding behaviors – autofeeding cellulose, chewing regurgitated or allogrooming material and stomodeal and proctodeal trophallaxis- confirm the erratic individual feeding episodes indicated by Forschler (1996) working with dyed cellulose (Tables 4.2-4.3, Figures 4.3-4.6). Our data indicate workers spend roughly equal amounts of time chewing food acquired by autofeeding cellulose, proctodeal and stomodeal trophallaxis, with chewing regurgitated or allogrooming materials taking less time despite having similar frequencies (Tables 4.4, 4.7). When time spent by all termites out of 9-h in the five feeding behaviors are expressed as percentages of total time spent feeding, autofeeding cellulose occupies 35.4% of time spent feeding, proctodeal trophallaxis 28.6%, stomodeal trophallaxis 30.4%, chewing regurgitated material 3.7% and chewing after allogrooming 1.8%. It is clear that consumption of wood represents only a fraction of *R. flavipes* worker feeding.

More than half (59%) of the donors in the 254 examples of stomodeal trophallaxis we scored were workers that had taken food from a nest mate either autofeeding cellulose or chewing material obtained by prior proctodeal or stomodeal trophallaxis. Donors autofeeding cellulose comprised 39% of the 254 examples and donors chewing regurgitated materials, 1% (Whitman 2006, Chapter 3). Another interesting revelation from this study is that the majority of stomodeal food sharing (66%) was of food in the process of maceration before ingestion; i.e. not proctodeal in nature (Whitman 2006, Chapter 3). One possible explanation is that termite food acquisition involves the labor-saving behavior of removing macerated food from a nest mate's mouth rather than extracting it from a piece of wood. The cellulases in termite salivary glands (Nakashima et al. 2002) would make chewed cellulose even more attractive. If we assume the time spent chewing is equivalent to the amount chewed, termites acquire up to one-third of their food partially pre-digested and the other one-third from proctodeal trophallaxis - quite digested. Therefore, termite feeding on wood represents a small percentage of the daily caloric intake of the average worker. Studies with wood as the source of cellulose could verify the proportions observed in this experiment that used alpha cellulose powder.

Little definitive is known about the cause and effect of termite OMs. In our observations defecation always followed a Type 4 OM, but the meaning and reasons behind OM Types 1-3 remain unclear. Maistrello and Sbrenna (1996) proposed that OMs can be a means to communicate individual short-term needs and our observations suggest that the Type 3 OMs can, at times, signal nest mates to ‘do something else’ (Whitman 2006, Chapter 3). Over 9-h, RW termites performed one and a half times as many Type 3 OMs as LC termites (165 versus 104), with a corresponding increase over that period in total time spent (656 seconds versus 435), suggesting a higher rate of communication between individuals in the RW treatments (Table 4.6).

A task is something that is relegated to certain individuals in a society. Therefore, evidence for task allocation can be attributed to consistent evidence that specific individuals performed behaviors not observed in other individuals. Stomodeal trophallaxis and excavation were the only two activities which could reasonably be called ‘tasks’ that were not performed by all 36 termites (Tables 4.2, 4.3). There were 23 individuals that did not perform one or more types of OM, but given their ephemeral and sporadic nature this was not surprising, as results from the 1, 5, 10, 15, and 30-min analysis of the 4-h blocks tell us that brief or infrequent behaviors are harder to detect. The two individuals that did not display stomodeal trophallaxis came from the least ‘active’ replicate, LC2, as measured by total time or frequency of all behaviors (Table 4.3). This, combined with the variability observed in feeding behaviors, allows us to discount the two workers that were not observed obtaining food by stomodeal trophallaxis.

There were 15 of the 36 workers we scored that did not perform excavation and these were spread across all replicates. The difference in mean duration of excavation between the Day 1 4-h continuously scored block (840.8 ± 995.0 s) and Day 1 15-min intervals (424.6 ± 314.4 s) was due to the tendency of excavation to persist for periods longer than 15-min (Table 4.1). Excavation was the only task-related activity that was performed by specific termites in our data set and was a behavior category that involved a mean duration over all three days of 358.8 ± 315.2 s. This could not have been overlooked, given the time frame of video scored, because allogrooming, autofeeding cellulose, proctodeal trophallaxis and NVA all provided smaller mean durations while being behaviors displayed by all individuals (Table 4.1). It was clear from our data that individuals either did or did not perform excavation, which we believe to be the first strong case for the existence of task allocation in *R. flavipes.*

General behavioral trends involving excavation can be seen when we examine the data on the percent of 9-h spent on behaviors by excavators and non-excavators (Table 4.6). It is clear that while a termite was busy excavating it took time away stomodeal trophallaxis and walking/resting (Table 4.6). The general trend of slowing down over time is reflected in both groups, although excavators remained more active as the days progressed. Why certain termites answered the demand for excavation is a question that will require further research to address, as is whether the fidelity we recorded to excavation was the result of task-switching as suggested by Crosland and Traniello (1997) or 'programmed' task allocation as suggested by Evans (2006) in his study of *Nasutitermes exitiosus*. How the role of 'excavation' as observed in our study relates to foraging for new food resources (essentially excavating galleries in soil) and feeding on a newly discovered food resource (excavating galleries in piece of wood) are tasked to individual termites in the field also needs to be examined in more detail. We observed excavation in both the food (alpha cellulose) and tunneling (kaolin clay) substrates. Yet, those termites involved in excavation manipulated both substrates in the same manner – pill formation and deposition (Whitman 2006, Chapter 3) – and cellulose chewing behavior was not observed to originate from an active alpha-cellulose excavation site. We are therefore confident that the excavation reported in this study is analogous to foraging in field populations and that this task is assigned to specific termites in nature.

Discriminant analysis of days of filming using termite-days of behaviors shows the day of filming were clearly different in activity levels (Table 4.8, 4.9). Termite-days of NVA were often misclassified to the right (i.e. to a later day), but rarely to the left (to an earlier day) (Table 4.9). This illustrates that, while individual termites might have had low activity levels on an early day of filming, they rarely had high activity levels on a later day of filming. This point is further illustrated by comparing the number of Day 4 NVA termite-days that were misclassified to the right with the number from Day 1. Further details on the general trend can be obtained by examining the data on percent time of 9-h spent on behaviors by all individuals (Table 4.4) and total time spent and frequency of occurrence out of 9-h observation intervals of all individuals (Table 4.7). Excavation in particular decreases sharply after Day One (Tables 4.4, 4.7). We feel confident that this slowdown was not due to a lack of tasks to perform, as only two of the three chambers in the experimental arena showed extensive excavation activity. Reduced levels of activity with increased time in bioassay have been reported from termite tunneling (Tucker et al. 2004) and feeding studies (Su and La Fage 1984). Understanding which termite activities decrease with increasing time in bioassay and which are exhibited at a higher frequency are critical to designing and interpreting behavioral bioassays.

Comparing treatments, the differences between termite-days of time spent and categorical frequency represent quantitative differences rather than qualitative ones, as there were no behaviors scored in either treatment that were not also seen in the other (Tables 4.10, 4.11). The high degree of variability evidenced by individuals within both treatments of our data set should not be forgotten (Table 4.1-4.3). We required parallel statistical significant differences in both data sets before we assumed a statistical difference between treatments to have biological significance. Two behavioral groups met the criteria – trophallaxis (proctodeal and stomodeal) and excavation. Clearly, these two areas of behavior merit further investigation.

However, the discriminant and crosstabs analyses do not provide the entire picture, with examination of trends in the data on total time and frequency of occurrence out of 9-h providing valuable insight. The LC termites spent less time allogrooming as the days in bioassay increased while the RWs remained consistently higher (Table 4.7). A similar trend in the allogrooming data is seen in frequency between treatments within days (Table 4.7). The total time spent in each behavior by day shows that the LC treatments were similar on Day One and Four with a 50% decrease (from 10,000 to 5,000 s per termite-day) by Day Seven, whereas the RW termites spent between 10,000-15,000 s per termite-day performing allogrooming on all three days (Table 4.7). Chewing after allogrooming was correspondingly higher in time and frequency in RWs than the LCs on all three days (Table 4.7). RW termites also spent more time autogrooming, maintaining values one and a half times that of the LCs in both time spent and frequency (Table 4.7).

Stomodeal chewing showed a similar trend between treatments, where the LC termites engaged in longer and more frequent episodes of stomodeal chewing than the RWs on the first day (Table 4.7). Over time, the LCs performed fewer and shorter stomodeal chewing episodes, whereas the RWs increased the frequency and duration of their involvement with stomodeal chewing (Table 4.7). A look at the total time spent on proctodeal chewing shows a distinct and steady decline in both treatments, though the RW treatment consistently remained 2,000-3,000 s per termite-day higher than the LCs (Table 4.7). Examining mean percent time spent on the five feeding behaviors as expressed as a percentage of all time spent feeding by individuals within both treatments, LCs spent the most time in stomodeal trophallaxis on Day 1, followed by proctodeal trophallaxis on Day 4 and autofeeding cellulose on Day 7. Interestingly the RWs exhibited the reverse of this, spending more time on Day 1 in autofeeding cellulose, in proctodeal trophallaxis on Day 4, and finally stomodeal trophallaxis on Day 7 (Table 4.13). The mean of all three days shows that percent time spent at the individual level on proctodeal and stomodeal trophallaxis were mirrored between the LC and RW treatments (Table 4.13).

Examining total time spent on excavation also provides some interesting revelations (Table 4.7). The total time spent on excavation clearly shows that the majority of that ‘work’ was accomplished on the first day, regardless of treatment, and that the RW termites spent, on the first day, nearly six times the amount of time on that task than the LC termites (Table 4.7). The excavation data are interesting because it is the only behavior that if a termite did not perform on Day One, it was never observed performing that behavior (Table 4.7). The exception to that statement was in Replicate LC2, where excavation was performed by only one individual, one time, on Day Seven (Table 4.2, 4.3). The data on time spent on excavation out of 9-h clearly show that while the RW termites were more active excavators, both in time spent and frequency, excavation decreased in both areas as time in bioassay increased (Table 4.7). The LC termites, while displaying a reduction in activity after Day One, were relatively more consistent in both time spent (7,405 s to 783 s to 971 s) and incidence (29 to 8 to 8) for the duration of the experiment (Table 4.7).

The number of the six randomly-selected termites that displayed excavation behavior varied by replicate, with more termites in the RW replicates (four, four and six) involved in excavation compared to the LC replicates of three, one and three (replicates 1-3 respectively) (Table 4.2, 4.3). The RW treatments were simply much ‘busier’ in regard to the ‘work’ of excavation compared to their LC counterparts (Tables 4.5, 4.7).

The biological significance of the aforementioned treatment differences is difficult to assess. The distribution of individuals within the arena chambers tended to be clumped, with the vast majority of the termites in one or two of the chambers at any one time. Each replicate was sampled twice on each day of filming and in only three of these samples was there an even distribution of individuals (fewer than 50% of the replicate in any single chamber, N=36). Intuitively, an even distribution of individuals between all three arena chambers would indicate a crowded condition and the fact that the termites in our study confined their activity, as a group, to a limited area demonstrates that activities were not space-limited.

Worker age could not be determined in our experiment, especially in the RW treatments, and worker size also varied between treatments. The longevity of the worker caste in *Reticulitermes* is estimated between 2-4 y (Grube and Forschler 2004). The LC workers in our study, from 18-mo old colonies, could have been older than 1 y given the growth rates reported for laboratory reared colonies, assuming the larger workers we scored were from the first batch of eggs laid during the nuptial chamber stage (Weesner 1956, Grube and Forschler 2004). The RW workers were larger than the LC in our study, as reported in Grube and Forschler (2004) in their comparison of laboratory reared and field collected *R. flavipes*. This difference in the size and/or age of the workers in our study could have affected the data relative to efficiencies of size reported in *R. fukienensis* excavation (Crosland et al. 1997, 1998, Crosland and Traniello 1997). Despite the worker size difference between treatments, the greater number and involvement of RW termites in excavation could be explained by the random selection process with an allocated-task status for excavation. The treatment differences were exacerbated but not caused by the lack of excavation in one replicate (LC2), where excavation was not even attempted until Day 7. The inactive state of LC2 highlights the role that vigor plays in subterranean termite bioassay data (Arquette 2006) and is mentioned as a caveat to interpreting our data.

We propose that our data validate using randomly selected termites for bioassay because the entire range of worker-to-worker behaviors we scored were performed by both treatments with generally equal frequency and time spent on behavior. There were some obvious trends that would demonstrate treatment differences with certain behaviors. These behaviors can be related to social communication between nest mates.

In behaviors thought to be associated with chemical communication - grooming and trophallaxis (Wilson 1971, Bagneres et al. 1991, Nalepa 1994, Thorne 1998) - trends emerge that suggests that the RW replicates devoted considerable effort towards re-establishing their social hierarchy, warranting speculation concerning the role that allogrooming and trophallaxis play in maintaining a cohesive social organization in *Reticulitermes*. The behavioral patterns seen in these behaviors could be explained by the RW termites attempting to gather information on caste proportions and social composition within their new, randomly-selected social group, as compared to the socially-intact LC replicates. The RW termites spent more time allogrooming and autogrooming on all three days and maintained a higher frequency on Days 4 and 7, suggesting the importance of this behavior in communication between nest mates via cuticular hydrocarbons (Bagneres et al. 1991, Takahashi and Gassa 1995, Boucias et al. 1996, Myles 1996, Kaib et al. 2002). (Table 4.7) The RWs chewed proctodeal material more frequently and for longer on Days 1, 4 and 7 and the total time spent data for this behavior showed a near two-fold decrease in time spent by the LC termites for each of the three days, while the RW termites showed that same near two-fold decrease only by Day Seven (Table 4.7). These data could indicate the role that proctodeal exchange has in communicating social-context information between nest mates in that the randomly selected RW termites would have the need to 'sort-out' their position their within the 'new' group longer than the LC intact colonies, once placed into the new environment of a bioassay arena. Stomodeal chewing is less convincing as a predictor for a realistic biological trend in regard to social communication given the nature of the exchange we observed (food sharing versus regurgitated fluid exchange) and can be more easily explained as the result of variables not associated with social communication because of the lack of any clear trends in the time spent data (Table 4.8).

We believe these behavioral trends on the part of the RW replicates illustrate a slow re-establishment of social structure and the corresponding resumption of the more 'typical' work-load seen in the LCs. However, it is apparent from the statistical analyses (Tables 4.10 – 4.11) that the social hierarchy was not completely re-established during the course of our experiment. Determining the duration of this process will require further research.

4.5 Conclusions

This study of worker-to-worker interactions illustrated aspects *Reticulitermes* biology important in understanding bioassay assumptions, digestive physiology and social communication and treatment strategies. The assumption that randomly-selected workers will perform in bioassay in a manner indicative of field populations appears to be sound. Our data indicate that the same repertoire of behaviors was displayed by both treatments although the length and frequency of certain behaviors varied. The behaviors exhibiting differences – allogrooming and trophallaxis - have been linked to social communication and despite increased frequency over the first few days in bioassay, the other behaviors should provide data comparable with intact populations (Wilson 1971, Bagneres et al. 1991, Nalepa 1994, Thorne 1998, Suarez and Thorne 2000a). In addition, these data will assist in defining the contribution and importance that allogrooming and trophallaxis behaviors play in toxicant transfer toward attempts to develop biologically-based control strategies (Ibrahim et al. 2003, Saran and Rust 2005, Rust and Saran 2006, Shelton et al. 2006. The random selection process may initially contain individuals with different roles within the social group, yet by 96 h (i.e. 4 d) the repertoire of behaviors, in both quantity and quality, should sort-out to reach a level expected in a non-random population. This validates the use of randomly selected workers when conducting bioassay of behaviors like feeding rates and termiticide efficacy. Caution, however, should be exercised when designing behavioral tests to provide ample time for acclimatization.

Termite workers spent most of their time in an inactive state (72.8%) and, although indicative of an organism with a low metabolism, feeding activities occupied the greatest part of the average termite-day (13.6%) (Table 4.4). The time *R. flavipes* workers spent feeding is similar to percentages provided in studies of *Zootermopsis* and *Kalotermes* (Rosengaus and Traniello 1993, Maistrello and Sbrenna 1996). The division of feeding into two avenues - auto and allo - and five major forms of acquisition – autofeeding cellulose, proctodeal and stomodeal trophallaxis and chewing of regurgitated and allogroomed materials - indicate that individual termites feed in proportions outside of the population mean (Table 4.2-4.3, 4.12; Figures 4.5-4.6). The worker population indicates equitable distribution of food intake between all four routes (Tables 4.4, 4.7). The realization that subterranean termites take one-third (36.1%) of their caloric intake as unadulterated cellulose while the other two-thirds (63.9%) consists of partially digested cellulose is important to understanding bait toxicant efficacy, the worker energy budget, and helps to explain feeding rate variability.

These data provide a realistic assessment of amount of daily contact between nest mates for understanding the role of allogrooming and trophallaxis in toxicant transfer as well as indicating their importance in social communication. The amount of time workers spent in allogrooming and food sharing occupied a considerable part of the average workers daily activities (Table 4.4). The differences seen in these social communication behaviors, especially prevalent on Day 1, indicate their role in establishing a social hierarchy.

Finally, our data indicate that the excavation of substrates is a task not performed by all *R. flavipes* individuals and, as such, seems liable to fall under some form of division of labor. The existence of task allocation in any social insect should not be surprising given its importance in the maintenance of a social structure (Wilson 1971, Oster and Wilson 1978). These findings raise questions about the nature of task switching in the subterranean termite (Crosland and Traniello 1997, Evans 2006). In addition, the allocation of excavation to specific individuals is important to understanding the role foraging plays within field populations.

4.6 References

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Table 4.1. Mean duration (± SD), in seconds, of behaviors in 4-h blocks and 15-min scoring intervals of individual termites regardless of laboratory-reared (LC) and randomly-selected (RW) treatment, by day of filming

Interval	**Day**	**Allo-grooming**	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Excavation**	**NVA**
15-min block	1	28.8±42.7	216.8±220.9	199.4±194.1	231.4±236.4	424.6±314.4	246.5±288.2
	4	25.3±30.7	166.3±195	123±142.1	210.1±153.3	77.6±67.1	343.1±319.9
	All days	24.8±39.8	205.4±195.5	147.6±168.9	204.8±204	358.8±315.2	332.5±314.1
4-h block	1	18.7±18.4	70±86.6	171.2±130.9	134±116.1	840.8±995	86.5±120.8
	4	18±28.2	260.9±488.4	122.7±107.4	76.8±60.7	25.2±0	58.4±64.3
	All days	18.2±26.2	108.2±227.5	131.5±110.3	99.7±88.1	738.8±0	66.1±84.3

Table 4.2. Mean duration (±SD), in seconds, of behaviors in 15-min scoring intervals of individual termites regardless of laboratory-reared (LC) and randomly-selected (RW) treatment, by day of filming

Day 1						
Subject	**Allo-grooming**	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Excavation**	**NVA**
LC1-1	25.8±17.5	276.4±288.1	147±184.5	477±410.2	0±0	192.5±118.7
LC1-2	37.7±27.1	282.3±23.3	264.2±436.5	517.2±333.2	0±0	126.1±115.6
LC1-3	20.2±21	182.2±264.9	335.6±264.2	36.9±8.5	462±273.1	85.2±63.7
LC1-4	31±22.1	436.9±285.8	73.5±86	223.1±253.8	136.9±156.9	86.7±62.6
LC1-5	25.6±14.4	398.8±345.1	178.3±105.9	174±219.6	0±0	136.9±140
LC1-6	10.4±14.6	267.3±111	130.8±110.5	288.5±303.1	274.8±314	146±119.9
LC2-1	14.4±8.6	30.7±21.3	0±0	0±0	0±0	505.1±317.7
LC2-2	32.2±33.5	0±0	0±0	90±0	0±0	709.7±293.1
LC2-3	45.8±0	0±0	0±0	0±0	0±0	717.6±273.5
LC2-4	7.7±0	0±0	0±0	0±0	0±0	862.2±131
LC2-5	11.2±1.7	530±0	439.7±0	0±0	0±0	690.1±326.9
LC2-6	7.2±2.9	337.3±0	0±0	0±0	0±0	585.2±301.2
LC3-1	19.5±9.1	38±48.2	228.5±239.1	286.3±205.1	0±0	200.3±298.8
LC3-2	29.2±21.6	375.8±287	0±0	326.8±326.1	0±0	308.8±289.4
LC3-3	9.3±9.7	278±65.4	111.9±139.7	304.1±229.8	107.1±136.9	170.9±146.5
LC3-4	24.3±17.1	208±314.8	129.4±106.5	289.2±157.9	338±0	48.9±23.3
LC3-5	25.6±19.5	246.7±272.1	156.8±109.6	138.8±102.2	189.3±147.3	56.7±31.4
LC3-6	14.1±15.8	339±254.6	191.6±147.9	332.8±186.1	0±0	139.4±92.9
RW1-1	29.6±27.2	197.4±159.4	355.8±276.1	58±0	355.5±123.8	101.4±90.9
RW1-2	9.8±6.7	69.6±42.2	234±0	126.3±0	364.6±345.3	76.7±82.8
RW1-3	7.3±8.1	292.3±363.3	313.9±311.6	138.9±103.9	400.7±377	93.9±87.6
RW1-4	64.1±71.1	80.6±74.1	118.4±73.3	39±29.5	461.6±403.1	116.8±125.3
RW1-5	28.2±20.9	288.5±0	203.8±135.2	106.5±95.2	0±0	95.1±72.2
RW1-6	107.4±156.9	195.5±112.1	359.6±349.4	27.5±0	0±0	128.1±134.4
RW2-1	1.7±2	10±0	10.8±0	0±0	588.9±278.7	65.2±46.6
RW2-2	5.3±0	113.2±108.3	443.5±305.9	18.6±0	507.5±339.4	113.8±121.3
RW2-3	72.5±51.2	52.5±34.9	50.3±0	600±0	653.4±301.3	135.6±92.1
RW2-4	36.2±34.8	174±298.7	168±183.9	0±0	471.7±409.7	357.3±280.3
RW2-5	53.4±62	0±0	0±0	44.6±0	0±0	707±253.4
RW2-6	82.7±76.9	223.3±268.3	0±0	262.6±0	0±0	368.4±269.1
RW3-1	34.6±19	334.2±0	196.4±177.7	174.9±0	372.9±372.2	126.2±103.5
RW3-2	30.9±14.4	349.7±180.9	204.1±98.6	103.4±46.1	579.6±493.4	77±46.1
RW3-3	22.3±11.8	100.8±90.6	0±0	549±0	661.7±244.5	154±151.2
RW3-4	18.3±14	125.1±47.3	0±0	154.4±0	388.9±311.6	91.7±40.9
RW3-5	19.8±13.2	221.4±90.1	143.8±54.1	24.3±0	210.8±307.3	93.3±61.2
RW3-6	18.5±14.3	152.2±123.8	18.7±0	22.9±12.4	437.9±334.3	108.2±102.5

Table 4.2. Mean duration (±SD), in seconds, of behaviors in 15-min scoring intervals of individual termites regardless of laboratory-reared (LC) and randomly-selected (RW) treatment, by day of filming

Day 4						
Subject	**Allo-grooming**	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Excavation**	**NVA**
LC1-1	9.2±9.5	114.4±132.7	97.5±54.4	189.4±62.7	0±0	419.8±239.2
LC1-2	18.5±17.1	0±0	62.5±0	10.9±0	0±0	601.7±380.2
LC1-3	30±30.1	141.6±0	160.8±163.7	109.7±75.1	98.8±129	138.7±105.4
LC1-4	20.2±18.4	70.7±0	223±0	0±0	0±0	273.3±308.6
LC1-5	28.8±20.6	39.5±0	128.6±2.2	109.6±0.4	0±0	256.2±319.3
LC1-6	12.3±8.7	397.6±26.9	71.1±52.7	173±0	0±0	129.1±61.3
LC2-1	31.4±26.7	0±0	81.1±0	0±0	0±0	682.1±319.4
LC2-2	10.6±11.8	0±0	31.4±0	0±0	0±0	534±321.8
LC2-3	29.3±17.8	85.7±31.9	38.5±0	188.6±0	0±0	465.8±319.1
LC2-4	42.9±43.1	80.2±28.9	167.6±232.1	0±0	0±0	216±103.3
LC2-5	2.2±2.7	135.7±76.9	49.1±50.5	0±0	0±0	573.3±354.9
LC2-6	9.1±5.7	0±0	113.5±111.8	405.2±0	0±0	313.2±324.5
LC3-1	38.4±38.1	244±2.3	71.6±61.6	453.4±367.2	0±0	221.2±237.4
LC3-2	23.1±19.8	103.9±0	457.4±0	558.5±0	0±0	500.6±318.8
LC3-3	5.4±5	0±0	192.6±170.8	140.6±41.4	0±0	471.5±347.6
LC3-4	38±22.5	378.4±0	93.3±50.7	179.2±0	0±0	463±388.2
LC3-5	60.1±153.6	55.4±22.6	48.5±51.8	31.2±0	0±0	329.4±275.9
LC3-6	8.8±10.7	0±0	113.4±56.9	414.5±350.2	0±0	325.1±244.2
RW1-1	13.6±8	0±0	119.1±159.1	0±0	0±0	621.2±351
RW1-2	25.8±9.9	0±0	0±0	0±0	0±0	640.9±323.6
RW1-3	101.4±146.9	0±0	0±0	156.1±69.2	0±0	577.2±348.4
RW1-4	20.5±15.9	0±0	90.5±93.3	0±0	0±0	420.2±320.9
RW1-5	49.9±66.2	56.1±0	70.6±54.4	0±0	0±0	287.4±230.9
RW1-6	21.9±23.9	0±0	463.6±0	0±0	0±0	561.1±302.9
RW2-1	3.8±2.5	337.3±342.7	39.8±48	104.8±0	34.7±20.4	137.5±100
RW2-2	10.2±8.2	285.7±223.8	315.2±297.4	56.2±0	0±0	194.5±142.4
RW2-3	58.1±41.5	0±0	200.1±303.9	329.2±332.3	0±0	303.5±232
RW2-4	36.4±30.5	260.4±115.8	76.5±42.6	190.5±0	0±0	163.1±98
RW2-5	26.7±32.5	110.2±0	93.9±134.7	153.1±146.2	0±0	362.2±332.7
RW2-6	40.3±33.8	0±0	125.9±133.7	299.7±200.7	0±0	164.2±111.5
RW3-1	39.5±53.2	196.2±55.4	94.5±87.5	61.3±37.3	39.8±24.3	72.4±65.7
RW3-2	10.8±8.1	0±0	0±0	164.1±129.9	0±0	455.5±345.2
RW3-3	18.5±19.6	178.1±6.9	71.6±68.5	35.3±0	0±0	250.7±242.5
RW3-4	12.6±13.5	95.8±70.1	170.8±269.7	208.7±145.3	94.3±0	48.4±23.7
RW3-5	19.6±22.3	115.4±1.9	59.2±77.6	138.4±127.2	0±0	81.4±25.7
RW3-6	7.5±6.3	149.9±142.4	83.5±74.8	153.4±124.4	110.4±132.2	94.2±43.9

Table 4.2. Mean duration (±SD), in seconds, of behaviors in 15-min scoring intervals of individual termites regardless of laboratory-reared (LC) and randomly-selected (RW) treatment, by day of filming

Day 7						
Subject	**Allo-grooming**	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Excavation**	**NVA**
LC1-1	33±25.8	71.6±0	0±0	59.8±0	0±0	689.3±265
LC1-2	18.9±9.7	286.9±344.5	70.8±60.7	15.5±0	0±0	548.4±379.7
LC1-3	5.4±5	85.1±0	40.1±38.4	631.1±0	138.2±74.7	418.7±308.9
LC1-4	11.6±13.3	0±0	152.6±148	0±0	0±0	415.6±368.6
LC1-5	16±10.5	0±0	22.5±0	121.8±0	0±0	571.8±305.5
LC1-6	18.2±14.4	0±0	146.6±137.9	87.7±0	0±0	329.4±286.3
LC2-1	11.5±4.2	350.6±0	0±0	31.1±0	0±0	570.8±252.1
LC2-2	13.5±20.4	292.1±234.1	94±18.2	0±0	0±0	609.1±309.9
LC2-3	14.4±8	161.2±41.4	0±0	0±0	112.2±0	447.8±301.7
LC2-4	28.3±14.8	229.5±101.7	70.7±28.8	0±0	0±0	412.5±364.2
LC2-5	17.6±19.3	257.5±130.5	114.8±0	0±0	0±0	572±330.5
LC2-6	3.2±2.7	185.1±30.7	151.7±0	150.6±52.9	0±0	508.5±308.1
LC3-1	32.6±19.2	378.5±358.9	80.9±90.8	168.3±74.8	0±0	229.2±233
LC3-2	11.8±8.4	229.5±273.7	57.7±0	318.3±0	0±0	386.9±329.5
LC3-3	5.5±5.5	630.1±235.8	100.8±77.5	353.5±341.4	44.7±0	226.6±228
LC3-4	15.2±16.8	485.8±0	76.9±23	316.9±280.5	0±0	609.2±332.2
LC3-5	3.6±2.2	533.6±0	25.4±9.4	175.2±179.2	0±0	238.1±104.8
LC3-6	13.2±11.8	199.8±90.7	76±43.3	174.7±0	0±0	241.5±231.6
RW1-1	24.9±18.3	0±0	0±0	0±0	0±0	510.2±299.7
RW1-2	33.6±17.8	0±0	0±0	0±0	0±0	599.8±325.6
RW1-3	33.4±16.9	0±0	0±0	18.2±0	0±0	477.6±303.8
RW1-4	41.5±0	0±0	0±0	0±0	0±0	642.5±335.1
RW1-5	27.2±20.9	86.6±1.1	250.9±360.9	0±0	0±0	224.1±120.9
RW1-6	27.1±16.4	90.4±0	0±0	0±0	0±0	406±253.7
RW2-1	16.9±10.4	106.6±127.9	16.6±8.8	73±85	0±0	140±244.3
RW2-2	11.7±12	138.6±169.5	134.9±148.4	136.5±132.2	0±0	397.9±339.8
RW2-3	26.7±19	42.2±0	288.9±291.9	179.7±0	0±0	274.1±224.4
RW2-4	19.8±27.9	130.9±34.8	158.5±63.6	185.3±80.5	0±0	297.2±286.3
RW2-5	22.1±25.8	0±0	200.6±49.1	158.7±93.4	0±0	379.3±319.9
RW2-6	29.3±31	264.8±178.8	0±0	137±102.8	0±0	478.8±289.1
RW3-1	26.3±18.3	0±0	159.1±251.8	132.1±86.6	0±0	114±57
RW3-2	4±0	0±0	0±0	0±0	0±0	821±180.2
RW3-3	7.7±6.1	0±0	192.9±96.2	173.3±82.8	0±0	294.9±230.3
RW3-4	10.6±10.5	154±83.2	55.6±51.2	108.8±143.6	28.8±11.1	71.8±54.2
RW3-5	52.5±116.2	126.7±110.5	158.2±144.6	114.7±57.3	46.2±26.1	67.7±74.7
RW3-6	7.6±7.2	48±11.3	154.9±206.2	167.4±204.9	0±0	395.4±299.1

Table 4.3. Total time spent, in seconds, out of 3 termite-days (9-h) on behaviors by termites in laboratory-reared (LC) and randomly-selected (RW) replicates

Subject	Allo-grooming	Autofeeding cellulose	Proctodeal trophallaxis	Stomodeal trophallaxis	Chewing regurgitated material	Chewing after allogrooming
LC1-1	707, N=34	1477, N=8	933, N=7	2087, N=8	240, N=6	22, N=1
LC1-2	2837, N=90	1437, N=5	997, N=6	3416, N=9	122, N=3	25, N=2
LC1-3	2504, N=82	1255, N=9	2019, N=10	1034, N=8	127, N=4	55, N=3
LC1-4	3999, N=158	1818, N=5	1365, N=14	1465, N=8	211, N=6	109, N=4
LC1-5	3771, N=131	2117, N=7	636, N=5	1347, N=10	42, N=2	70, N=4
LC1-6	1457, N=115	1597, N=5	1954, N=19	2280, N=9	443, N=6	9, N=2
LC2-1	629, N=40	443, N=4	81, N=1	31, N=1	67, N=3	6, N=1
LC2-2	378, N=21	584, N=2	219, N=3	90, N=1	58, N=4	10, N=1
LC2-3	474, N=18	1439, N=14	39, N=1	189, N=1	96, N=3	12, N=1
LC2-4	1747, N=45	1615, N=15	1256, N=10	0, N=0	26, N=1	67, N=2
LC2-5	221, N=16	1806, N=9	702, N=5	0, N=0	60, N=4	4, N=1
LC2-6	488, N=59	708, N=3	606, N=5	1262, N=5	133, N=8	5, N=1
LC3-1	2894, N=102	1605, N=8	952, N=9	6952, N=25	58, N=1	137, N=6
LC3-2	1553, N=66	2100, N=9	515, N=2	2252, N=7	63, N=3	40, N=2
LC3-3	540, N=65	3941, N=9	1657, N=13	3216, N=11	60, N=2	57, N=2
LC3-4	3215, N=107	1770, N=7	1153, N=11	2384, N=8	21, N=1	29, N=1
LC3-5	1672, N=88	2675, N=12	1466, N=14	1503, N=11	70, N=3	39, N=2
LC3-6	1676, N=103	2456, N=10	1588, N=14	3136, N=10	14, N=1	119, N=4
RW1-1	1931, N=66	1263, N=7	950, N=4	58, N=1	24, N=1	20, N=1
RW1-2	768, N=34	803, N=9	234, N=1	126, N=1	103, N=5	114, N=4
RW1-3	1643, N=59	1610, N=6	1728, N=6	1222, N=10	172, N=6	23, N=4
RW1-4	3253, N=80	322, N=4	1891, N=16	117, N=3	59, N=5	114, N=7
RW1-5	3911, N=117	518, N=6	3336, N=22	320, N=3	396, N=14	251, N=15
RW1-6	3381, N=72	1496, N=10	2262, N=6	28, N=1	25, N=1	44, N=2
RW2-1	1800, N=139	1088, N=7	278, N=12	495, N=5	597, N=26	147, N=16
RW2-2	606, N=52	2572, N=12	3114, N=11	348, N=4	538, N=10	20, N=1
RW2-3	2245, N=55	494, N=8	2057, N=10	1438, N=4	191, N=8	121, N=5
RW2-4	1795, N=61	3078, N=17	1185, N=10	1307, N=7	440, N=3	72, N=6
RW2-5	1999, N=63	110, N=1	942, N=7	1722, N=11	112, N=3	56, N=2
RW2-6	2976, N=76	2183, N=10	252, N=2	2163, N=10	17, N=1	132, N=4
RW3-1	5226, N=168	1588, N=8	2903, N=22	755, N=7	163, N=6	340, N=13
RW3-2	1491, N=61	699, N=2	736, N=4	1354, N=10	89, N=2	42, N=3
RW3-3	1048, N=64	1019, N=7	1854, N=14	1451, N=7	170, N=6	91, N=3
RW3-4	3242, N=213	2401, N=18	1936, N=17	1569, N=10	481, N=16	103, N=4
RW3-5	4600, N=199	2981, N=19	1509, N=11	1239, N=11	328, N=14	141, N=8
RW3-6	736, N=64	2621, N=19	1185, N=12	1176, N=9	284, N=10	294, N=8

Table 4.3. Total time spent, in seconds, out of 3 termite-days (9-h) on behaviors by termites in laboratory-reared (LC) and randomly-selected (RW) replicates

Subject	Excavation	Type 1 OM	Type 2 OM	Type 3 OM	Type 4 OM	Autogrooming	NVA
LC1-1	0, N=0	0, N=0	19, N=5	5, N=2	3, N=1	137, N=43	26799,N=86
LC1-2	0, N=0	12, N=29	13, N=3	64, N=17	9, N=4	137, N=60	23239,N=124
LC1-3	4881, N=21	27, N=35	5, N=4	97, N=14	17, N=5	216, N=89	19982,N=149
LC1-4	274, N=2	20, N=55	11, N=6	179, N=39	12, N=6	115, N=102	22592,N=196
LC1-5	0, N=0	0, N=0	5, N=2	7, N=2	7, N=2	97, N=71	24203,N=171
LC1-6	1099, N=4	6, N=5	17, N=6	3, N=1	57, N=16	278, N=100	23121,N=171
LC2-1	0, N=0	6, N=5	15, N=4	7, N=1	0, N=0	116, N=28	30826,N=85
LC2-2	0, N=0	7, N=6	4, N=2	2, N=1	0, N=0	44, N=18	30713,N=69
LC2-3	224, N=2	8, N=8	5, N=4	0, N=0	4, N=2	87, N=24	28961,N=83
LC2-4	0, N=0	0, N=0	41, N=15	3, N=2	0, N=0	38, N=31	27153,N=100
LC2-5	0, N=0	0, N=0	4, N=3	0, N=0	2, N=1	96, N=21	29247,N=74
LC2-6	0, N=0	8, N=4	0, N=0	0, N=0	2, N=1	136, N=41	29036,N=109
LC3-1	0, N=0	6, N=18	80, N=15	24, N=10	3, N=1	301, N=100	19027,N=150
LC3-2	0, N=0	2, N=5	0, N=0	5, N=2	0, N=0	233, N=65	25670,N=109
LC3-3	463, N=4	5, N=1	0, N=0	3, N=1	2, N=1	121, N=54	22122,N=124
LC3-4	676, N=2	2, N=3	2, N=1	7, N=2	3, N=2	328, N=108	22779,N=156
LC3-5	1542, N=10	2, N=4	3, N=2	5, N=2	4, N=2	295, N=102	22602,N=170
LC3-6	0, N=0	0, N=0	13, N=5	25, N=8	19, N=6	371, N=117	22662,N=149
RW1-1	2653, N=8	1, N=3	1, N=1	11, N=2	0, N=0	278, N=77	24670,N=111
RW1-2	4624, N=15	2, N=36	0, N=0	146, N=35	0, N=0	204, N=62	24386,N=101
RW1-3	1677, N=6	2, N=1	0, N=0	0, N=0	0, N=0	297, N=78	23367,N=107
RW1-4	1824, N=7	1, N=1	13, N=3	4, N=1	7, N=3	325, N=90	23920,N=134
RW1-5	0, N=0	1, N=2	0, N=0	3, N=1	15, N=2	438, N=135	22581,N=170
RW1-6	0, N=0	4, N=9	0, N=0	15, N=5	0, N=0	395, N=80	24247,N=109
RW2-1	6278, N=15	3, N=5	2, N=2	1, N=1	0, N=0	314, N=152	20630,N=250
RW2-2	5423, N=12	1, N=3	0, N=0	9, N=5	11, N=4	108, N=57	19359,N=121
RW2-3	3920, N=6	0, N=0	1, N=1	0, N=0	0, N=0	203, N=58	21510,N=116
RW2-4	1432, N=4	0, N=0	0, N=0	4, N=1	0, N=0	266, N=68	22786,N=117
RW2-5	0, N=0	5, N=17	0, N=0	23, N=12	0, N=0	95, N=61	27166,N=99
RW2-6	0, N=0	8, N=4	0, N=0	15, N=4	0, N=0	136, N=54	24476,N=120
RW3-1	1689, N=9	2, N=2	15, N=5	4, N=2	12, N=4	343, N=137	18082,N=219
RW3-2	1749, N=4	9, N=6	23, N=8	44, N=6	6, N=3	161, N=62	24665,N=105
RW3-3	3044, N=5	2, N=9	2, N=2	55, N=7	8, N=4	292, N=68	21333,N=132
RW3-4	3645, N=15	34, N=36	56, N=19	76, N=19	5, N=3	274, N=203	16709,N=307
RW3-5	1719, N=12	5, N=21	60, N=18	47, N=15	11, N=8	207, N=180	17859,N=283
RW3-6	3764, N=12	9, N=57	8, N=4	198, N=49	20, N=4	189, N=78	20385,N=167

Table 4.4. Percent of 3 termite-days (9-h) spent on behaviors by all termites, by day of filming

	Allo-grooming	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Chewing regurgitated material**	**Chewing after allogrooming**
Day 1	7.0	7.2	5.4	6.1	0.6	0.2
Day 4	7.1	3.6	4.0	3.3	0.6	0.3
Day 7	4.8	4.0	2.6	3.4	0.4	0.2
All days	6.3	4.9	4.0	4.2	0.5	0.3
	Excavation	**Type 1 OM**	**Type 2 OM**	**Type 3 OM**	**Type 4 OM**	**Autogrooming**
Day 1	12.8	0.0	0.0	0.1	0.0	1.0
Day 4	0.5	0.0	0.1	0.1	0.0	0.6
Day 7	0.3	0.0	0.0	0.1	0.0	0.4
All days	4.5	0.0	0.0	0.1	0.0	0.7
	NVA				**NVA**	
Day 1	56.6			Day 7	82.2	
Day 4	79.5			All days	72.8	

Table 4.5. Percent of 3 termite-days (9-h) spent on behaviors by laboratory-reared (LC) and randomly-selected (RW) replicates, by day of filming

		Allo-grooming	Autofeeding cellulose	Proctodeal trophallaxis	Stomodeal trophallaxis	Chewing regurgitated material	Chewing after allogrooming
Day 1	LC	6.7	7.7	4.7	10.2	0.6	0.1
	RW	7.3	6.8	6.1	2.1	0.6	0.4
Day 4	LC	6.1	2.6	2.9	3.5	0.2	0.2
	RW	8.1	4.5	5.1	3.0	0.9	0.4
Day 7	LC	3.1	5.6	1.7	3.2	0.2	0.1
	RW	6.5	2.5	3.4	3.5	0.6	0.3
All days	LC	5.3	5.3	3.1	5.6	0.3	0.1
	RW	7.3	4.6	4.9	2.9	0.7	0.4
		Excavation	**Type 1 OM**	**Type 2 OM**	**Type 3 OM**	**Type 4 OM**	**Autogrooming**
Day 1	LC	3.8	0.0	0.0	0.1	0.0	0.8
	RW	21.8	0.0	0.0	0.1	0.0	1.1
Day 4	LC	0.4	0.0	0.1	0.1	0.0	0.5
	RW	0.5	0.0	0.0	0.1	0.0	0.7
Day 7	LC	0.5	0.0	0.0	0.0	0.0	0.4
	RW	0.1	0.0	0.0	0.2	0.0	0.5
All days	LC	1.6	0.0	0.0	0.1	0.0	0.5
	RW	7.4	0.0	0.0	0.1	0.0	0.8
		NVA				**NVA**	
Day 1	LC	64.3		Day 7	LC	84.6	
	RW	48.9			RW	79.9	
Day 4	LC	82.9		All days	LC	77.3	
	RW	76.0			RW	68.3	

Table 4.6. Percent of 3 termite-days (9-h) spent on behaviors by excavator and non-excavating termites, by day of filming

	Excavator (Y/N)	Allo-grooming	Autofeeding cellulose	Proctodeal trophallaxis	Stomodeal trophallaxis	Chewing regurgitated material	Chewing after allogrooming
Day 1	N	7.4	6.3	4.7	8.2	0.3	0.2
	Y	6.6	7.9	5.9	4.7	0.8	0.3
Day 4	N	6.7	1.8	3.0	4.8	0.3	0.2
	Y	7.4	4.8	4.8	2.1	0.8	0.3
Day 7	N	3.9	4.7	1.8	2.3	0.3	0.2
	Y	5.4	3.6	3.1	4.1	0.5	0.2
All days	N	6.0	4.2	3.1	5.1	0.3	0.2
	Y	6.5	5.4	4.6	3.6	0.7	0.3
		Excavation	**Type 1 OM**	**Type 2 OM**	**Type 3 OM**	**Type 4 OM**	**Autogrooming**
Day 1	N	0.0	0.0	0.0	0.0	0.0	0.8
	Y	21.9	0.0	0.0	0.1	0.0	1.0
Day 4	N	0.0	0.0	0.1	0.1	0.0	0.4
	Y	0.8	0.0	0.0	0.1	0.0	0.7
Day 7	N	0.0	0.0	0.0	0.0	0.0	0.4
	Y	0.5	0.0	0.0	0.1	0.0	0.4
All days	N	0.0	0.0	0.0	0.0	0.0	0.6
	Y	7.7	0.0	0.0	0.1	0.0	0.7
		NVA				**NVA**	
Day 1	N	71.4		Day 7	N	85.0	
	Y	46.1			Y	80.3	
Day 4	N	82.6		All days	N	79.6	
	Y	77.3			Y	67.9	

Table 4.7. Total time, in seconds, spent on all behaviors out of 3 termite-days (9-h) by laboratory-reared (LC) and randomly-selected (RW) replicates, by day of filming

		Allo-grooming	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Chewing regurgitated material**	**Chewing after allogrooming**
Day 1	LC	12931 N=504	14954 N=60	9046 N=58	19743 N=77	1125 N=31	218 N=14
	RW	14175 N=425	13203 N=84	11814 N=62	4113 N=31	1160 N=40	753 N=35
Day 4	LC	11886 N=500	5084 N=42	5702 N=52	6750 N=25	437 N=17	359 N=17
	RW	15742 N=636	8724 N=47	9999 N=79	5898 N=34	1801 N=53	746 N=32
Day 7	LC	5945 N=336	10804 N=39	3388 N=39	6150 N=30	349 N=13	237 N=9
	RW	12731 N=582	4919 N=39	6537 N=46	6877 N=49	1229 N=44	627 N=39
All days	LC	30762 N=1340	30842 N=141	18136 N=149	32643 N=132	1911 N=61	814 N=40
	RW	42648 N=1643	26846 N=170	28350 N=187	16887 N=114	4189 N=137	2127 N=106
		Excavation	**Type 1 OM**	**Type 2 OM**	**Type 3 OM**	**Type 4 OM**	**Autogrooming**
Day 1	LC	7405 N=29	36 N=55	41 N=16	147 N=34	63 N=23	1542 N=532
	RW	42317 N=113	21 N=62	80 N=29	144 N=47	38 N=11	2158 N=560
Day 4	LC	783 N=8	46 N=92	186 N=54	237 N=57	57 N=18	905 N=382
	RW	974 N=13	40 N=68	67 N=17	187 N=46	35 N=15	1337 N=553
Day 7	LC	971 N=8	29 N=31	9 N=7	52 N=13	22 N=9	696 N=260
	RW	150 N=4	15 N=82	35 N=17	325 N=72	22 N=9	1032 N=587
All days	LC	9159 N=45	111 N=178	236 N=77	435 N=104	143 N=50	3143 N=1174
	RW	43441 N=130	76 N=212	181 N=63	656 N=165	95 N=35	4527 N=1700
		NVA				**NVA**	
Day 1	LC	125027 N=841		Day 7	LC	164508 N=634	
	RW	95043 N=873			RW	155262 N=894	
Day 4	LC	161196 N=800		All days	LC	450731 N=2275	
	RW	147825 N=1001			RW	398130 N=2768	

Table 4.8. Discriminant analysis matrix classifying days of filming using termite-days of autofeeding cellulose, stomodeal trophallaxis, chewing of regurgitated material, chewing after allogrooming, excavation, OM Types 1-3, and autogrooming

	Observed Day 1	**Observed Day 4**	**Observed Day 7**	**Observed Other**	**% Correct classification**	**Chi-Square value**	**p-value**
Predicted Day 1	35	0	1	1	98.1	192.9	>0.001
Predicted Day 4	0	34	1	1			
Predicted Day 7	1	0	34	1			

Table 4.9. Discriminant analysis matrix classifying days of filming using termite-days of NVA

	Observed Day 1	**Observed Day 4**	**Observed Day 7**	**Observed Other**	**% Correct classification**	**Chi-Square value**	**p-value**
Predicted Day 1	24	4	8	0	55.8	26.8	>0.001
Predicted Day 4	2	9	24	1			
Predicted Day 7	1	7	25	3			

Table 4.10. Discriminant analysis matrices classifying termite-days of total time spent out of 9-h by lab-reared (LC) and randomly-selected (RW) replicates, by behavior and day of filming

Behavior	Day of filming	Predicted	Observed LC	Observed RW	Observed Other	% Correct classification	Chi-Square value	p-value
Allogrooming	1	LC	12	3	3	63.9	1.44	0.229
		RW	4	11	3			
	4	LC	10	4	4	63.9	1.89	0.169
		RW	3	13	2			
	7	LC	14	3	1	72.2	3.78	0.052
		RW	5	12	1			
Autofeeding cellulose	1	LC	14	0	4	69.4	3.22	0.073
		RW	2	11	5			
	4	LC	11	6	1	75.0	5.89	0.015
		RW	1	16	1			
	7	LC	14	3	1	86.1	9.89	0.002
		RW	0	17	1			
Proctodeal Trophallaxis	1	LC	15	3		75.0	5	0.025
		RW	6	12				
	4	LC	13	5		61.1	1.78	0.182
		RW	9	9				
	7	LC	14	4		77.8	5.56	0.018
		RW	4	14				
Stomodeal trophallaxis	1	LC	12	6		83.3	10	0.002
		RW	0	18				
	4	LC	10	8		69.4	4.11	0.043
		RW	3	15				
	7	LC	11	7		77.8	7.56	0.006
		RW	1	17				
Chewing regurgitated material	1	LC	8	10		50.0	0.22	0.637
		RW	8	10				
	4	LC	14	4		61.1	2.89	0.089
		RW	10	8				
	7	LC	17	1		88.9	11.11	0.001
		RW	3	15				
Chewing after allogrooming	1	LC	18	0		75.0	9	0.003
		RW	9	9				
	4	LC	15	3		75.0	5	0.025
		RW	6	12				
	7	LC	13	5		61.1	1.78	0.182
		RW	9	9				

Table 4.10. Discriminant analysis matrices classifying termite-days of total time spent out of 9-h by lab-reared (LC) and randomly-selected (RW) replicates, by behavior and day of filming

Behavior	Day of filming	Predicted	Observed LC	Observed RW	Observed Other	% Correct classification	Chi-Square value	p-value
Excavation	1	LC	16	2		83.3	8.22	0.004
		RW	4	14				
	4	LC	1	17		52.8	16.11	>0.001
		RW	0	18				
	7	LC	3	15		58.3	13	>0.001
		RW	0	18				
Type 1 OM	1	LC	14	3	1	75.0	4.56	0.033
		RW	4	13	1			
	4	LC	9	7	2	61.1	1.78	0.182
		RW	2	13	3			
	7	LC	12	5	1	58.3	1	0.317
		RW	8	9	1			
Type 2 OM	1	LC	6	0	12	33.3	2	0.157
		RW	0	6	12			
	4	LC	11	5	2	75.0	5.89	0.015
		RW	0	16	2			
	7	LC	16	1	1	77.8	6.44	0.011
		RW	5	12	1			
Type 3 OM	1	LC	14	1	3	69.4	3.22	0.073
		RW	4	11	3			
	4	LC	14	4	0	86.1	9.89	0.002
		RW	1	17	0			
	7	LC	17	1	0	86.1	9.89	0.002
		RW	4	14	0			
Type 4 OM	1	LC	8	9	1	66.7	5.56	0.018
		RW	1	16	1			
	4	LC	8	10	0	66.7	5.56	0.018
		RW	2	16	0			
	7	LC	16	1	1	61.1	6.44	0.011
		RW	11	6	1			
Autogrooming	1	LC	14	4	0	83.3	6.78	0.009
		RW	3	15	0			
	4	LC	14	0	4	75.0	4.56	0.033
		RW	1	13	4			
	7	LC	17	0	1	88.9	11.11	0.001
		RW	1	15	2			

Table 4.11. Chi-square comparison of termite-days of behavioral categorical frequency of lab-reared (LC) to randomly-selected (RW) replicates by behavior and by day of filming

Behavior	Day of filming	Category (frequency)	LC	RW	Pearson Chi-Square	df	Asymp. Sig. (2-sided)
Allogrooming	1	1 (0-14)	5	8	1.385	2	0.500
		2 (14-30)	5	5			
		3 (31+)	8	5			
	4	1	6	3	2.091	2	0.352
		2	6	5			
		3	6	10			
	7	1	9	8	.150	2	0.928
		2	5	6			
		3	4	4			
Autofeeding cellulose	1	1 (0)	3	1	1.551	2	0.461
		2 (1-3)	7	6			
		3 (4+)	8	11			
	4	1	6	8	4.886	2	0.087
		2	9	3			
		3	3	7			
	7	1	3	8	3.304	2	0.192
		2	11	7			
		3	4	3			
Proctodeal trophallaxis	1	1 (0)	6	4	1.285	2	0.526
		2 (1-3)	4	7			
		3 (4+)	8	7			
	4	1	0	3	7.156	2	0.028
		2	11	4			
		3	7	11			
	7	1	3	7	4.250	2	0.119
		2	11	5			
		3	4	6			
Stomodeal trophallaxis	1	1 (0)	5	2	19.695	2	>0.001
		2 (1-3)	1	14			
		3 (4+)	12	2			
	4	1	5	5	2.229	2	0.328
		2	12	9			
		3	1	4			
	7	1	5	6	4.935	2	0.085
		2	10	4			
		3	3	8			
Chewing regurgitated material	1	1 (0)	5	3	.650	2	0.723
		2 (1)	5	6			
		3 (2+)	8	9			
	4	1	9	5	1.886	2	0.390
		2	3	4			
		3	6	9			
	7	1	9	6	3.273	2	0.195
		2	6	4			
		3	3	8			

Table 4.11. Chi-square comparison of termite-days of behavioral categorical frequency of lab-reared (LC) to randomly-selected (RW) replicates by behavior and by day of filming

Behavior	Day of filming	Category (frequency)	Non-excavators	Excavators	Pearson Chi-Square	df	Asymp. Sig. (2-sided)
Chewing after allogrooming	1	1 (0)	8	6	.468	1	0.494
		2 (1+)	10	12			
	4	1	9	5	1.870	1	0.171
		2	9	13			
	7	1	11	8	1.003	1	0.317
		2	7	10			
Excavation	1	1 (0)	12	4	7.200	1	0.007
		2 (1+)	6	14			
	4	1	17	14	2.090	1	0.148
		2	1	4			
	7	1	15	16	.232	1	0.630
		2	3	2			
Type 1 OM	1	1 (0)	8	6	.495	2	0.781
		2 (1-3)	7	8			
		3 (4+)	3	4			
	4	1	1	10	12.774	2	0.002
		2	10	2			
		3	7	6			
	7	1	8	10	2.322	2	0.313
		2	7	3			
		3	3	5			
Type 2 OM	1	1 (0)	9	9	.000	1	1.000
		2 (1+)	9	9			
	4	1	5	14	9.028	1	0.003
		2	13	4			
	7	1	15	11	2.215	1	0.137
		2	3	7			
Type 3 OM	1	1 (0)	11	8	1.003	1	0.317
		2 (1+)	7	10			
	4	1	4	11	5.600	1	0.018
		2	14	7			
	7	1	15	10	3.273	1	0.070
		2	3	8			
Type 4 OM		1 (0)	8	11	1.003	1	0.317
		2 (1+)	10	7			
		1	10	11	.114	1	0.735
		2	8	7			
		1	14	11	1.178	1	0.278
		2	4	7			
Autogrooming	1	1 (0-14)	6	4	.900	2	0.638
		2 (14-30)	3	5			
		3 (31+)	9	9			
	4	1	8	2	5.018	2	0.081
		2	6	9			
		3	4	7			
	7	1	11	7	2.766	2	0.251
		2	6	7			
		3	1	4			

Table 4.12. Percent time spent in five feeding behaviors out of all time spent feeding per 3-h termite-day by individual termites regardless of laboratory-reared (LC) and randomly-selected (RW) treatment, by day of filming

Day 1					
Subject	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Chewing Regurgitated material**	**Chewing after allogrooming**
LC1-1	31.8, N=4	21.3, N=5	42, N=4	4.3, N=2	0.6, N=1
LC1-2	17, N=3	15.6, N=3	66.8, N=7	0.4, N=1	0.2, N=1
LC1-3	36.2, N=7	56.4, N=5	6.5, N=5	0, N=0	0.9, N=2
LC1-4	45.3, N=4	13.8, N=9	38, N=8	2.6, N=3	0.3, N=1
LC1-5	60.2, N=6	10.3, N=2	29.2, N=7	0, N=0	0.3, N=1
LC1-6	18.8, N=3	23.6, N=9	47.3, N=7	10.4, N=6	0, N=0
LC2-1	55.7, N=3	0, N=0	0, N=0	40.4, N=3	3.9, N=1
LC2-2	0, N=0	0, N=0	73.5, N=1	26.5, N=2	0, N=0
LC2-3	0, N=0	0, N=0	0, N=0	100, N=2	0, N=0
LC2-4	NF, N=0	NF, N=0	NF, N=0	NF, N=0	NF, N=0
LC2-5	52.5, N=1	43.5, N=1	0, N=0	3.5, N=2	0.4, N=1
LC2-6	77.6, N=1	0, N=0	0, N=0	22.4, N=5	0, N=0
LC3-1	2.6, N=3	13.4, N=4	82.9, N=13	0, N=0	1, N=2
LC3-2	34.5, N=2	0, N=0	63.1, N=5	1.1, N=1	1.4, N=1
LC3-3	32.7, N=4	21.1, N=6	44.7, N=5	1, N=1	0.5, N=1
LC3-4	33.6, N=5	19.2, N=4	46.5, N=4	0.8, N=1	0, N=0
LC3-5	51.6, N=9	30.2, N=7	17.6, N=5	0.4, N=1	0.1, N=1
LC3-6	38.4, N=5	15.2, N=3	45.3, N=6	0.4, N=1	0.7, N=1
RW1-1	60.8, N=7	34.3, N=2	2.8, N=1	1.1, N=1	1, N=1
RW1-2	59.1, N=9	17.2, N=1	9.3, N=1	5.9, N=4	8.4, N=4
RW1-3	37.1, N=6	39.8, N=6	20.6, N=6	2.1, N=3	0.4, N=3
RW1-4	17.7, N=4	71.5, N=11	6.4, N=3	1.1, N=2	3.2, N=3
RW1-5	9.5, N=1	75.9, N=14	10.5, N=3	0.4, N=1	3.7, N=7
RW1-6	43.5, N=8	55.6, N=5	0.9, N=1	0, N=0	0, N=0
RW2-1	4.2, N=1	4.6, N=1	0, N=0	76.1, N=8	15, N=3
RW2-2	25.8, N=3	55.1, N=2	1.2, N=1	17.9, N=3	0, N=0
RW2-3	36.2, N=7	8.1, N=2	48.1, N=1	2.1, N=1	5.5, N=3
RW2-4	71, N=8	26.9, N=3	0, N=0	1.2, N=1	0.9, N=1
RW2-5	0, N=0	0, N=0	66.5, N=1	33.5, N=1	0, N=0
RW2-6	77.7, N=6	0, N=0	18.2, N=1	0, N=0	4.1, N=1
RW3-1	21.6, N=1	55.3, N=5	11.3, N=1	5.4, N=3	6.4, N=2
RW3-2	32.5, N=2	34.2, N=4	32.4, N=6	0, N=0	0.9, N=1
RW3-3	43.8, N=4	0, N=0	49.1, N=1	7, N=4	0, N=0
RW3-4	63.3, N=3	0, N=0	23.9, N=1	12.7, N=4	0, N=0
RW3-5	59.5, N=6	31.9, N=5	1, N=1	4.5, N=3	3.1, N=4
RW3-6	86.9, N=8	1.4, N=1	3.4, N=2	3.5, N=1	4.8, N=2

Table 4.12. Percent time spent in five feeding behaviors out of all time spent feeding per 3-h termite-day by individual termites regardless of laboratory-reared (LC) and randomly-selected (RW) treatment, by day of filming

Day 4					
Subject	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Chewing Regurgitated material**	**Chewing after allogrooming**
LC1-1	21.8, N=2	18.6, N=2	54.1, N=3	5.5, N=3	0, N=0
LC1-2	0, N=0	85.1, N=1	14.9, N=1	0, N=0	0, N=0
LC1-3	17.5, N=1	39.8, N=2	27.2, N=2	11.9, N=3	3.6, N=1
LC1-4	18.3, N=1	57.6, N=1	0, N=0	20.9, N=2	3.2, N=1
LC1-5	6.4, N=1	41.6, N=2	35.5, N=2	6.9, N=2	9.7, N=3
LC1-6	59.4, N=2	27, N=6	12.9, N=1	0, N=0	0.7, N=2
LC2-1	0, N=0	100, N=1	0, N=0	0, N=0	0, N=0
LC2-2	0, N=0	69.8, N=1	0, N=0	30.2, N=1	0, N=0
LC2-3	76.9, N=10	3.7, N=1	18.2, N=1	0, N=0	1.2, N=1
LC2-4	47.3, N=12	51.4, N=6	0, N=0	1.3, N=1	0, N=0
LC2-5	83.8, N=6	16.2, N=3	0, N=0	0, N=0	0, N=0
LC2-6	0, N=0	35.8, N=4	63.9, N=2	0, N=0	0.4, N=1
LC3-1	22.3, N=3	6.5, N=3	69, N=5	0, N=0	2.2, N=3
LC3-2	9, N=1	39.5, N=1	48.2, N=1	3.4, N=2	0, N=0
LC3-3	0, N=0	64.7, N=4	28.6, N=2	2.6, N=1	4.1, N=1
LC3-4	35.4, N=1	45.1, N=5	16.8, N=1	0, N=0	2.7, N=1
LC3-5	28.4, N=2	49.7, N=4	8, N=1	13.9, N=2	0, N=0
LC3-6	0, N=0	28.9, N=5	66.3, N=3	0, N=0	4.8, N=3
RW1-1	0, N=0	100, N=2	0, N=0	0, N=0	0, N=0
RW1-2	NF, N=0	NF, N=0	NF, N=0	NF, N=0	NF, N=0
RW1-3	0, N=0	0, N=0	78.1, N=2	20.1, N=3	1.9, N=1
RW1-4	0, N=0	87.4, N=5	0, N=0	4.4, N=1	8.2, N=4
RW1-5	10.1, N=1	50.9, N=4	0, N=0	28.8, N=4	10.1, N=2
RW1-6	0, N=0	94.9, N=1	0, N=0	5.1, N=1	0, N=0
RW2-1	56.8, N=4	10.5, N=4	6.9, N=1	24.7, N=14	1.2, N=2
RW2-2	48.3, N=7	50.3, N=7	1.4, N=1	0, N=0	0, N=0
RW2-3	0, N=0	51.4, N=4	42.3, N=2	5.5, N=4	0.8, N=1
RW2-4	60.7, N=6	12, N=4	13.6, N=2	13.1, N=1	0.5, N=1
RW2-5	7.5, N=1	25.6, N=4	60.7, N=5	6.1, N=2	0, N=0
RW2-6	0, N=0	16.3, N=2	78.9, N=4	1.1, N=1	3.7, N=2
RW3-1	41.4, N=7	46.5, N=13	6.1, N=3	1.3, N=2	4.7, N=5
RW3-2	0, N=0	0, N=0	90, N=4	6.8, N=1	3.2, N=2
RW3-3	44, N=3	50.9, N=7	2.9, N=1	0, N=0	2.2, N=1
RW3-4	17.5, N=5	56.5, N=12	13.8, N=2	8.8, N=7	3.4, N=4
RW3-5	33.8, N=4	8.7, N=2	38.8, N=4	13.6, N=9	5, N=4
RW3-6	49.9, N=9	25.9, N=8	17, N=3	1.2, N=3	6, N=3

Table 4.12. Percent time spent in five feeding behaviors out of all time spent feeding per 3-h termite-day by individual termites regardless of laboratory-reared (LC) and randomly-selected (RW) treatment, by day of filming

Day 7					
Subject	**Autofeeding cellulose**	**Proctodeal trophallaxis**	**Stomodeal trophallaxis**	**Chewing Regurgitated material**	**Chewing after allogrooming**
LC1-1	60.8, N=2	0, N=0	25.4, N=1	13.8, N=1	0, N=0
LC1-2	67.8, N=2	16.7, N=2	1.8, N=1	11.9, N=2	1.7, N=1
LC1-3	10.1, N=1	11.1, N=3	75.1, N=1	3.7, N=1	0, N=0
LC1-4	0, N=0	84.6, N=4	0, N=0	4, N=1	11.5, N=2
LC1-5	0, N=0	15.6, N=1	84.4, N=1	0, N=0	0, N=0
LC1-6	0, N=0	87, N=4	13, N=1	0, N=0	0, N=0
LC2-1	91.9, N=1	0, N=0	8.1, N=1	0, N=0	0, N=0
LC2-2	73.6, N=2	23.7, N=2	0, N=0	1.5, N=1	1.2, N=1
LC2-3	96.1, N=4	0, N=0	0, N=0	3.9, N=1	0, N=0
LC2-4	68.5, N=3	24.9, N=4	0, N=0	0, N=0	6.6, N=2
LC2-5	78.7, N=2	17.5, N=1	0, N=0	3.8, N=2	0, N=0
LC2-6	36.7, N=2	15, N=1	44.8, N=3	3.5, N=3	0, N=0
LC3-1	35.8, N=2	7.7, N=2	52.8, N=7	2.7, N=1	1, N=1
LC3-2	76.3, N=6	3.5, N=1	19.5, N=1	0, N=0	0.6, N=1
LC3-3	62.2, N=5	6.7, N=3	31.1, N=4	0, N=0	0, N=0
LC3-4	30.5, N=1	9.7, N=2	59.8, N=3	0, N=0	0, N=0
LC3-5	37.4, N=1	5.8, N=3	54.5, N=5	0, N=0	2.3, N=1
LC3-6	60.8, N=5	28.6, N=6	10.6, N=1	0, N=0	0, N=0
RW1-1	NF, N=0	NF, N=0	NF, N=0	NF, N=0	NF, N=0
RW1-2	0, N=0	0, N=0	0, N=0	100, N=1	0, N=0
RW1-3	0, N=0	0, N=0	100, N=2	0, N=0	0, N=0
RW1-4	0, N=0	0, N=0	0, N=0	100, N=2	0, N=0
RW1-5	14, N=4	61, N=4	0, N=0	18.1, N=9	6.8, N=6
RW1-6	67.4, N=2	0, N=0	0, N=0	0, N=0	32.6, N=2
RW2-1	25.2, N=2	12.8, N=7	46.1, N=4	4.9, N=4	11, N=11
RW2-2	25.4, N=2	24.8, N=2	25.1, N=2	22.9, N=7	1.8, N=1
RW2-3	2.8, N=1	77.2, N=4	12, N=1	5.3, N=3	2.7, N=1
RW2-4	20.7, N=3	25.1, N=3	48.8, N=5	3, N=1	2.4, N=4
RW2-5	0, N=0	40.2, N=3	55.8, N=5	0, N=0	4, N=2
RW2-6	60.2, N=4	0, N=0	38.9, N=5	0, N=0	0.9, N=1
RW3-1	0, N=0	54.4, N=4	33.9, N=3	3.3, N=1	8.4, N=6
RW3-2	0, N=0	0, N=0	0, N=0	100, N=1	0, N=0
RW3-3	0, N=0	54.8, N=7	38.3, N=5	4, N=2	2.9, N=2
RW3-4	51.8, N=10	8.2, N=5	35.3, N=7	4.7, N=5	0, N=0
RW3-5	45, N=9	25.6, N=4	27.9, N=6	1.5, N=2	0, N=0
RW3-6	6.4, N=2	30.9, N=3	44.5, N=4	13.7, N=6	4.5, N=3

Table 4.13. Mean percent time (± SD) spent in five feeding behaviors out of all time spent feeding per 3-h termite-day by individuals in laboratory-reared (LC) and randomly-selected (RW) treatments, by day of filming

Subject		Autofeeding cellulose	Proctodeal trophallaxis	Stomodeal trophallaxis	Chewing Regurgitated material	Chewing after allogrooming
Day 1	LC	39.2 ± 18.8	23.6 ± 13.7	46.4 ± 21.6	15.3 ± 27.3	0.9 ± 1
	RW	44.1 ± 24.2	36.6 ± 23.9	19.1 ± 20.1	11.6 ± 19.9	4.4 ± 4
Day 4	LC	35.5 ± 25.8	43.4 ± 25.7	35.7 ± 22.1	10.7 ± 9.6	3.3 ± 2.7
	RW	37 ± 19.2	45.9 ± 30.2	34.7 ± 32.3	10 ± 8.9	3.9 ± 2.9
Day 7	LC	59.1 ± 24.3	23.9 ± 26.2	37 ± 26.9	5.4 ± 4.3	3.6 ± 4
	RW	31.9 ± 22.8	37.7 ± 21.7	42.2 ± 21.6	29.3 ± 40.8	7.1 ± 9
All days	LC	45.3 ± 24.8	31.6 ± 24.8	39.7 ± 23.5	11.2 ± 19	2.3 ± 2.8
	RW	38.9 ± 22.6	40.4 ± 25.6	30.8 ± 26.3	16.6 ± 26.9	5 ± 5.7
All days	Combined	42.3 ± 23.8	35.7 ± 25.4	35.1 ± 25.2	14.3 ± 23.8	3.9 ± 4.8

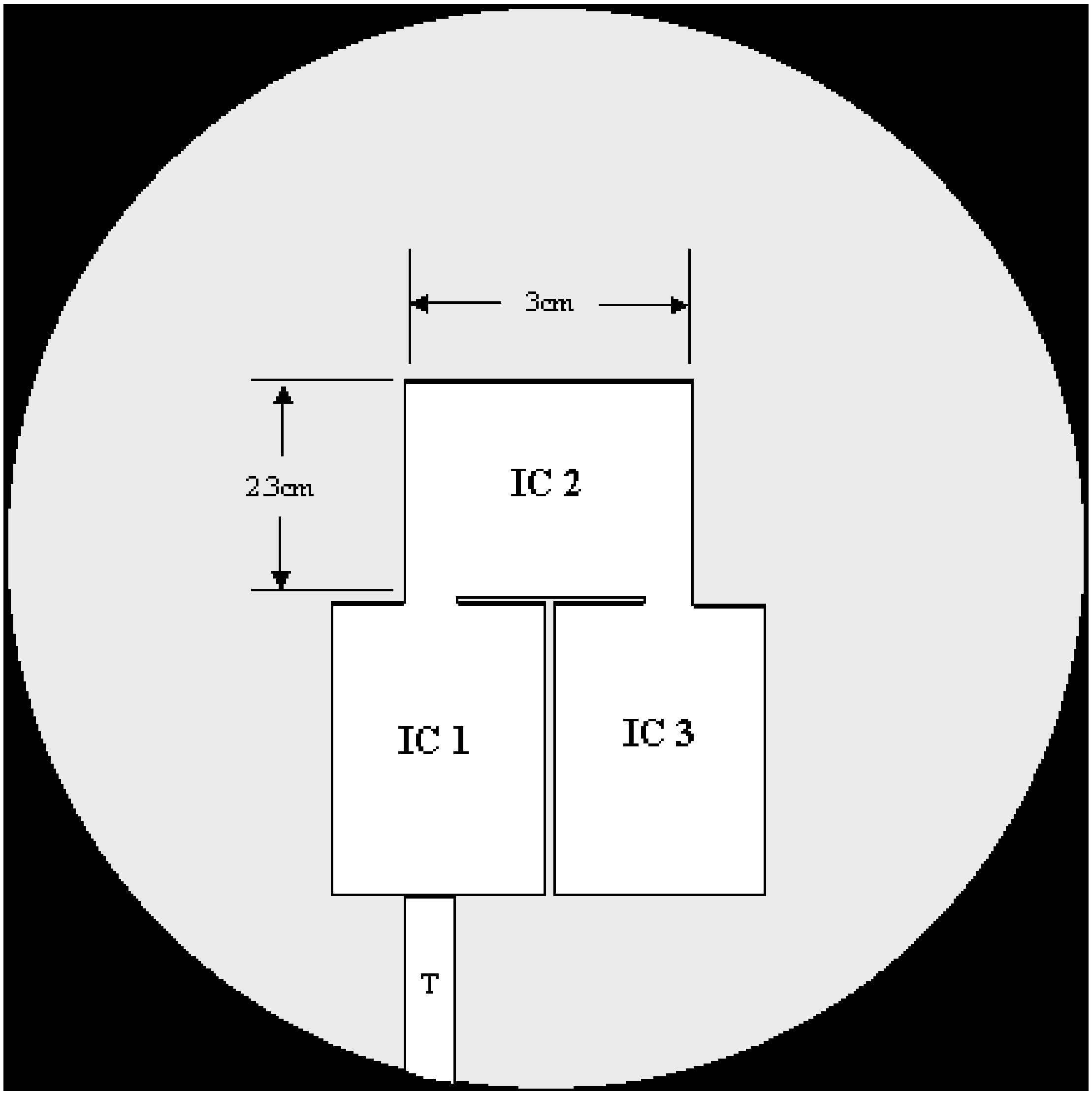

Figure 4.1. Arena detail showing chamber (IC) size, orientation, and placement of channel cut for Tygon tubing (T).

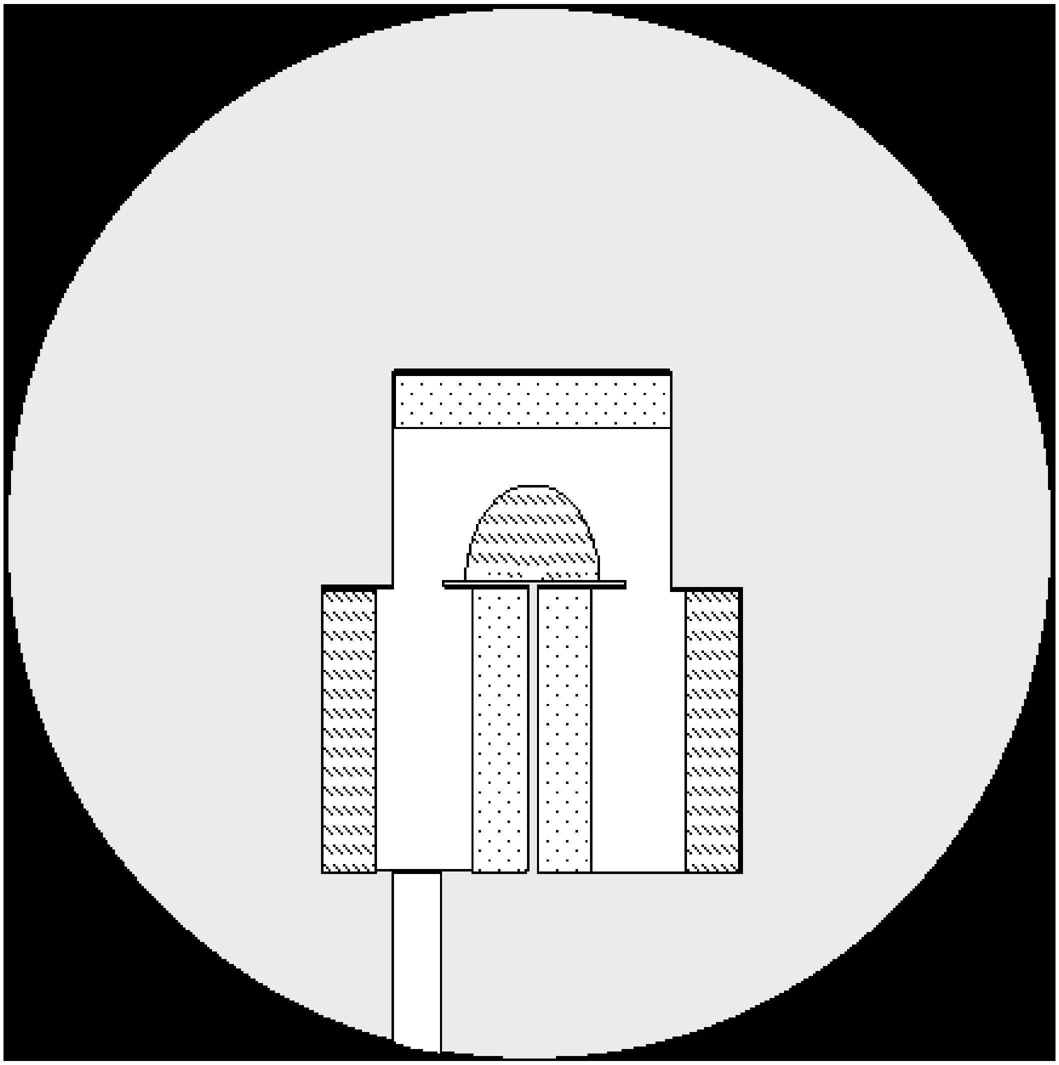

Figure 4.2. Arena detail showing placement of kaolin clay (diagonal pattern) and Alpha cellulose powder (dot pattern).

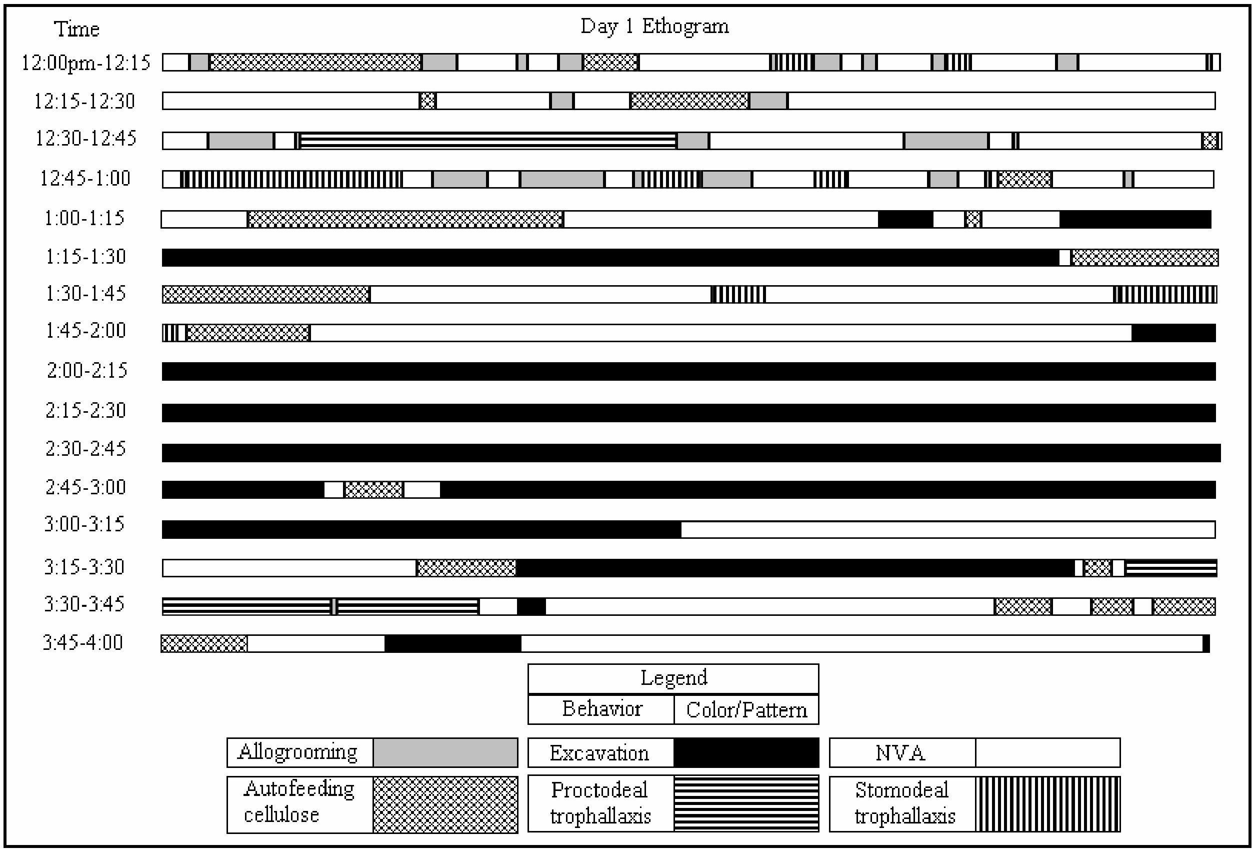

Figure 4.3. Ethogram of a single termite scored for 4 consecutive hours on Day 1 of filming, each bar representing a contiguous 15-min interval

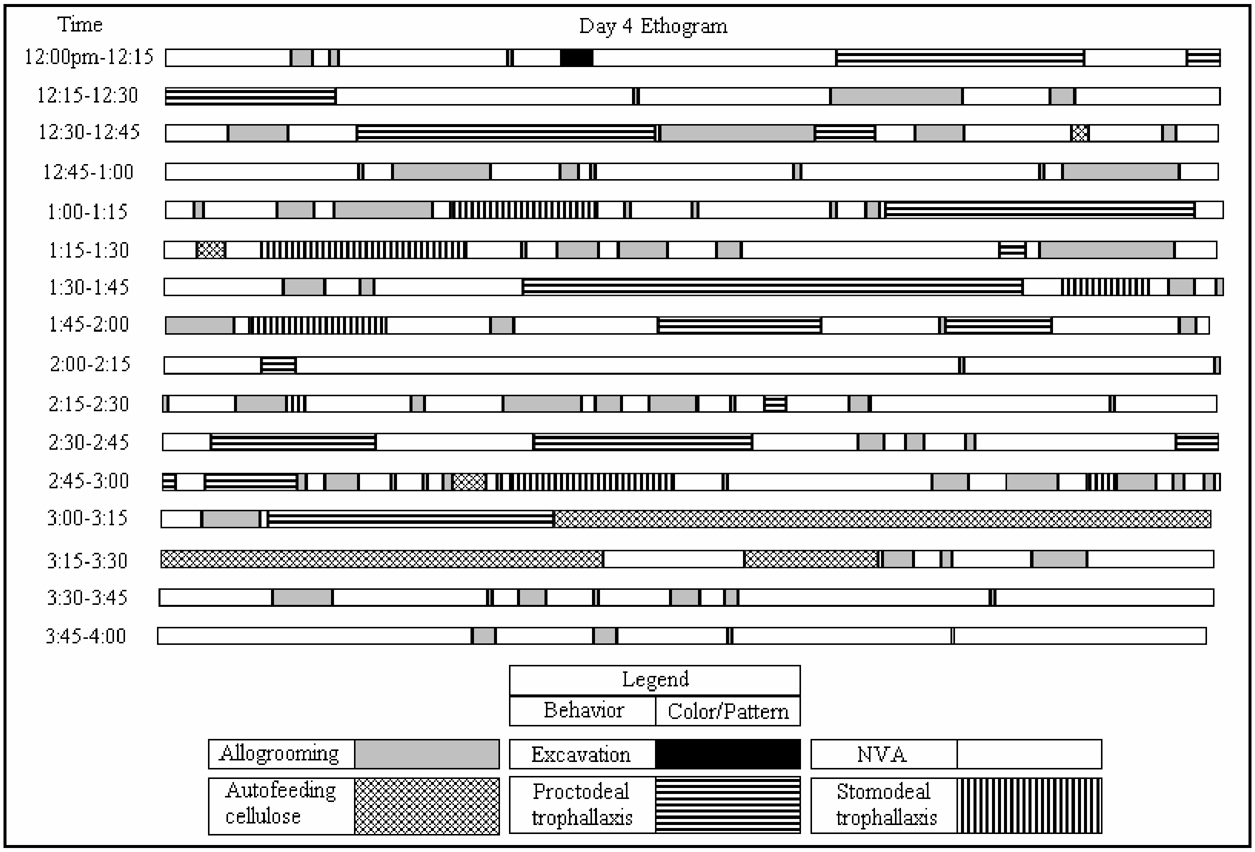

Figure 4.4. Ethogram of a single termite scored for 4 consecutive hours on Day 4 of filming, each bar representing a contiguous 15-min interval

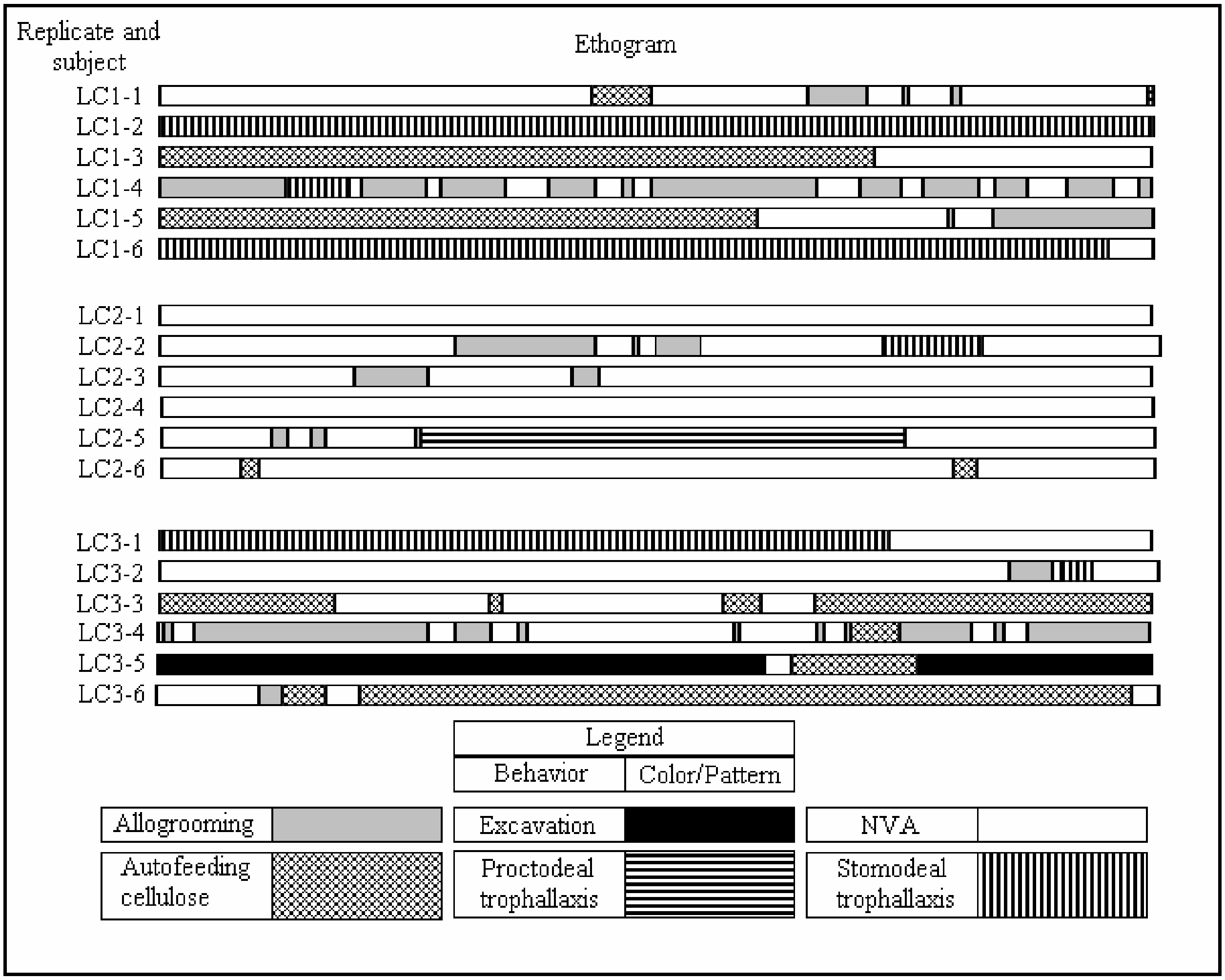

Figure 4.5. Ethograms of laboratory-reared (LC) termites scored for a single simultaneous 15-min interval on Day 1, by replicate

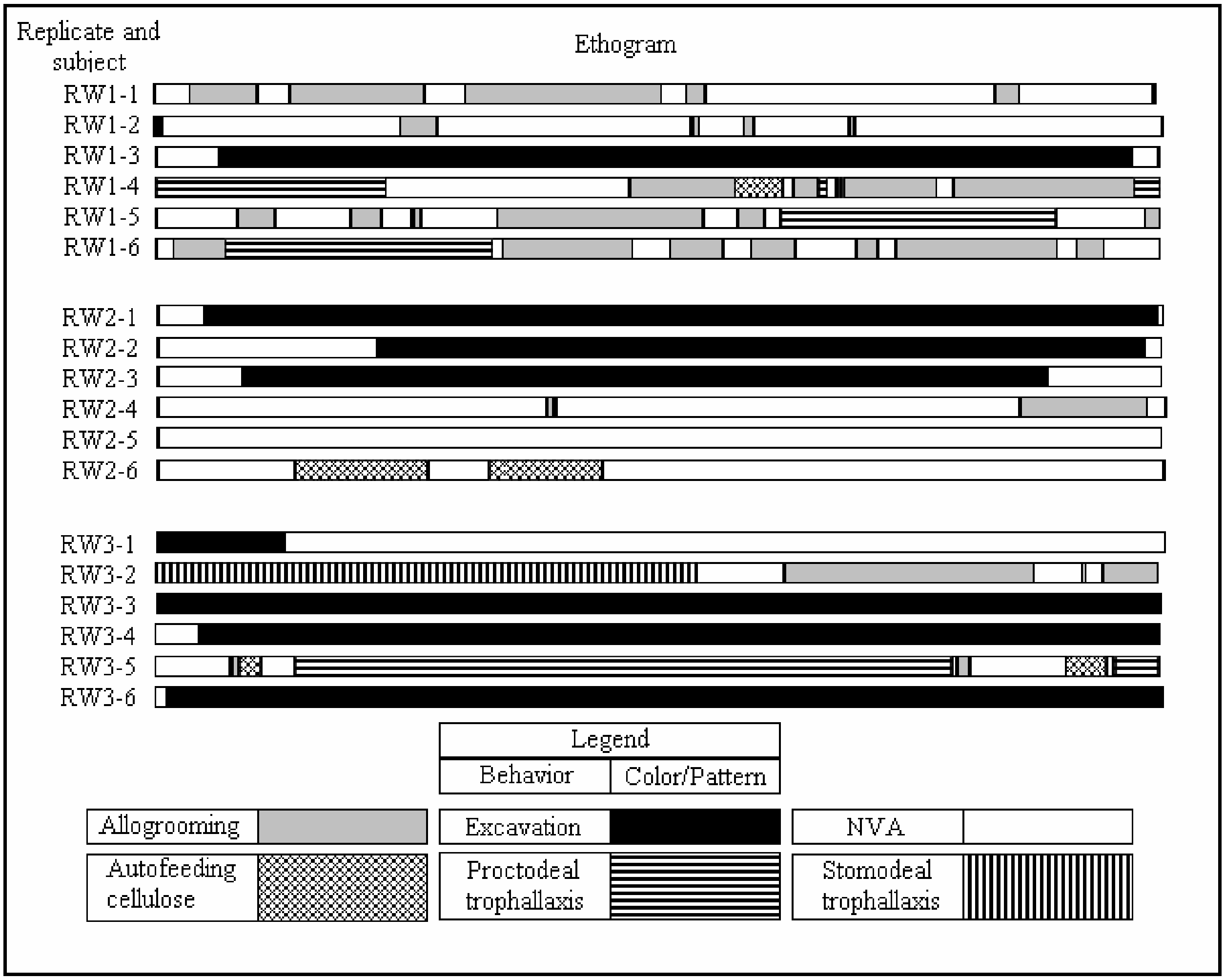

Figure 4.6. Ethograms of randomly-selected (RW) termites scored for a single simultaneous 15-min interval on Day 1, by replicate

CHAPTER 5

SUMMARY

We described, in detail, several behaviors of *R. flavipes* – mating events, ecdysis, the use of substrate 'pills' in tunnel formation and gallery excavation, oscillatory movements, and trophallaxis. The frequency of the mating events we observed agrees with previous predictions (Raina et al. 2003, Costa and Patricio 2005). Ecdysis as an event that involves multiple nest mates has implications for the evolution of eusociality in this insect (Thorne 1997) as well as colony-level immune responses (Rosengaus et al. 1999b, Traniello et al. 2002) and toxicant transfer (Su and Scheffrahn 1993, Sheets et al. 2000, Valles and Woodson 2002). The role of substrate 'pills' in termite construction could affect studies involving termite foraging, feeding site selection, and movement of soil toxicants. We were able to link Type 4 OMs with defecation. That regurgitated material was shared only rarely by trophallaxis clearly shows the model of stomodeal trophallaxis as a means of chemical transfer requires further examination.

Our data showed that randomly-selected workers can be used to model the behaviors of intact colonies, as both of our treatments displayed the same repertoire of behaviors. However, there was a great deal of variation at the individual level in both the frequency and duration of all behaviors. The behavioral differences found between the treatments in allogrooming and trophallaxis have been linked to social communication and, we believe, represent the re-establishment of a social hierarchy on the part of the randomly-selected workers (Wilson 1971, Bagneres et al. 1991, Nalepa 1994, Thorne 1998, Suarez and Thorne 2000). We saw an initial burst of activity which recommended the use of a brief period of acclimatization before beginning an experimental regime. However, we also observed a gradual overall slowing down of activities and this should be taken into account when interpreting data.

The termites we observed spent the majority of their time inactive and had comparable feeding rates to other lower termites (Rosengaus and Traniello 1993, Maistrello and Sbrenna

1996). The behavioral patterns we saw in feeding indicate that while it is difficult to interpret the behavior of termites on an individual level, the fact that only one-third of a termite's food intake involves unprocessed cellulose is important to predicting bait efficacy and feeding variability. Finally, our data clearly show that excavation of tunnels and galleries is not performed by all *R. flavipes* workers and therefore seems liable to fall under some form of division of labor.

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