

Biological changes in the Eastern subterranean termite, *Reticulitermes flavipes* (Isoptera, Rhinotermitidae) and its protozoa profile following starvation

X. P. Hu · D. Song · X. Gao

Received: 5 January 2010 / Revised: 26 April 2010 / Accepted: 28 July 2010 / Published online: 9 August 2010
© International Union for the Study of Social Insects (IUSSI) 2010

Abstract The effects of starvation on survival, body mass, movement, other behavior, and the symbiotic protozoan community in *Reticulitermes flavipes* (Kollar) were investigated in a 40-day assay. Groups of 100 termites in their natural worker/soldier ratio (98/2) were tested. Starvation resulted in significantly greater mortality and induced more cannibalism of workers than in the control. Worker survival rate gradually declined to 58% along with an increasing rate of cannibalism during the first 30 days, and then quickly decreased to 5% along with an increasing rate of necrophagia after 30 days. In contrast, starvation had no significant effect on the survival of soldiers (60%) as compared to the control (90%) and starved workers did not cannibalize soldiers. Ten protozoan species residing in the hindgut were identified. When compared with field termites, the 40-day starvation eliminated 5 species (*Trichonympha agilis*, *Pyrsonympha vertens*, and *P. major*, *D. gracilis*, *Holomastigotes elongatum*), significantly reduced the numbers of two species (*Dinenympha fimbriata*, *Spiro-nympha kofoidi*), had no effect on two species (*Trichomitus trypanoides* and *Spirotrichonympha flagellata*), and led to proliferation of one species (*Monocercomonas* sp.), whereas feeding on filter paper reduced the populations of five species

(*T. agilis*, *D. fimbriata*, *P. vertens*, *P. major*, and *S. flagellata*) to different degrees. Workers surviving starvation had similar body mass and short-range movement speed to workers fed on filter paper and workers freshly collected from natural setting. The energy-demanding survival and walking indicated that they might obtain sufficient nutrients from cannibalizing other workers. The observed behaviors are important in helping to understand termite survival strategies and the mechanisms by which termites maintain their social structure under stressful conditions.

Keywords Subterranean termites · Starvation effect · Symbiotic protozoa

Introduction

Termites are abundant in terrestrial ecosystems, and are ecologically and economically important because of their ability to decompose lignocelluloses (Ohkuma, 2008). In lower termites, the ability to digest lignocelluloses depends on a collaborative system involving the host (endogenous endoglucanases) and the intestinal microbiota (cellobiohydrolases inside protist cells) (Nakashima et al., 2002; Scharf and Boucias, 2010; Watanabe and Tokuda, 2010). Termites usually have plenty of food sources in nature, but could be expected to face periods of starvation or inadequate nutrition when a foraging group gets isolated from its food sources due to natural factors (temperature, flooding, etc.) and human activities (Esenther, 1969; Husby, 1980; Forschler and Henderson, 1995, Strack and Myles, 1997). Under such circumstances, like other organisms, termites might either lower metabolic rate to reduce energy expenditure (Marron et al., 2003) or modify their behavior (Su and La Fage, 1986; Miramontes and DeSouza, 1996).

X. P. Hu (✉)
Department of Entomology and Plant Pathology,
Auburn University, 203 Extension Hall,
Auburn, AL 36849, USA
e-mail: huxingp@auburn.edu

D. Song · X. Gao
Department of Entomology, China Agricultural University,
Beijing 100094, People's Republic of China
e-mail: songdl@cau.edu.cn

X. Gao
e-mail: gaoxiwu@263.net.cn

Two subterranean termites constitute >95% damage and control effort in Alabama, USA: the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Hu, unpubl.). Two early studies investigated starvation-induced behaviors in *C. formosanus* Shiraki (Su and La Fage, 1986; Song et al., 2006). Having a soldier proportion of 15–20% in natural colonies, the workers cannibalize soldiers before eating fellow workers when undergoing starvation. *R. flavipes* has a low soldier proportion of 1–2%. The effects of starvation on *R. flavipes* and the mechanisms it may use to survive starvation remain to be unknown.

In the present study, we investigated starvation-associated survival, cannibalism, body weight changes, and short-distance movement of *R. flavipes* in a 40-day laboratory experiment. We also examined the effects of 40-day starvation on hindgut protozoa profiles as compared to termites fed for 40 days and termites freshly collected from the field. The goal was to understand the survival strategies and this species may have evolved.

Materials and methods

Reticulitermes flavipes were collected on Auburn University campus at Auburn, Alabama, USA, in April from corrugated cardboard rolls (13 cm long, 11 cm diameter) installed inside open-bottomed, in-ground bucket traps (14 cm long, 12 cm diameter). The species were identified using soldier and alate morphology (Scheffrahn and Su, 1994; Austin et al., 2007). Termites were transferred into a clean plastic box to measure the worker/soldier ratio. Fully grown workers and soldiers were examined under a stereoscope individually for integrity. Intact termites were used in the study that was conducted on the day of field collection. Voucher specimens are preserved in 100% ethanol and stored in the insect collection of the Department of Entomology and Plant Pathology, Auburn University.

The experimental units were Petri dishes (1.5 cm high, 6.0 cm in diameter, Fisher Brand, Atlanta, GA) containing 10 g of sterilized play-sand moistened with 1.2 ml of distilled water. Control units were the same as the experimental units except for additional two pieces of 5.5-cm filter paper as food supply (Whatman #1). Groups of 100 workers and soldiers in their natural ratio (98:2) were introduced into the experimental units. The units were then sealed with parafilm (American National Can, Neenah, WI) and placed in a covered plastic box (27 × 15 × 9 cm) provisioned with two water containers to maintain $\geq 92 \pm 3\%$ RH. The plastic box was kept in an incubator at $22 \pm 1^\circ\text{C}$ and $85 \pm 3\%$ RH at constant darkness except during observations. In total, there were 80 experimental units (40 starved groups and 40 controls).

Effects on survival and cannibalism

Observations were made at 5-day intervals during a 40-day period. At each observation, five units from the treatment and control, respectively, were destructively examined under a stereoscope and not used again. The number of live intact, live cannibalized, dead intact, and dead cannibalized termites was recorded. The number of missing termites was counted as dead cannibalized. A termite was defined as dead if it did not move its appendage(s) when probed with a pen brush. A termite was classified as cannibalized if appendages or body parts were partially or entirely missing.

Body mass and vitality

Before the test commenced and on the day when the test was terminated, three groups of five live workers were weighed on an electronic analytical balance (M-220D, Denver Instrument, Arvada, CO) to compare the body mass among fresh field collected, starved, and paper-fed groups. Worker vitality was measured by their ability to move over a short distance. Ten workers from each of the three groups were tested individually to record the time needed to walk a 10 cm line drawn with a ballpoint pen (PAPERMATE, Sanford Bellwood, IL, USA) on a plain sheet of paper.

Hindgut protozoa profile

Fifteen live workers were randomly selected from each of the following three groups for examination of the hindgut protozoa profile: pre-test group collected from the field, 40-day starved, and 40-day filter-paper fed. The hindgut protozoa were prepared using the method developed by Lewis and Forschler (2004) and were identified using the non-dichotomous key (Lewis and Forschler, 2006). In brief, the hindgut of a termite was gently removed by pulling off the last two segments of the abdomen with a pair of forceps. The gut contents from five termites were pooled to form a sample, and were homogenized in 100 μl of 0.3% saline solution for 15 s using a disposable pestle in a 1.5 ml microcentrifuge tube. 10 μl of the gut suspension was loaded onto a hemacytometer (Arthur H. Thomas Company, Philadelphia, PA). Protozoa were identified and counted from a 0.5- μl sample under a microscope (MEIJI) to estimate the population of each protozoan species. The entire process for each sample was completed within 5 min. The estimated population of each protozoan species was calculated using the following formula:

$$\text{Number of cells counted} \times \text{volume saline solution} \\ \text{in original sample} / \text{volume on the hemacytometer.}$$

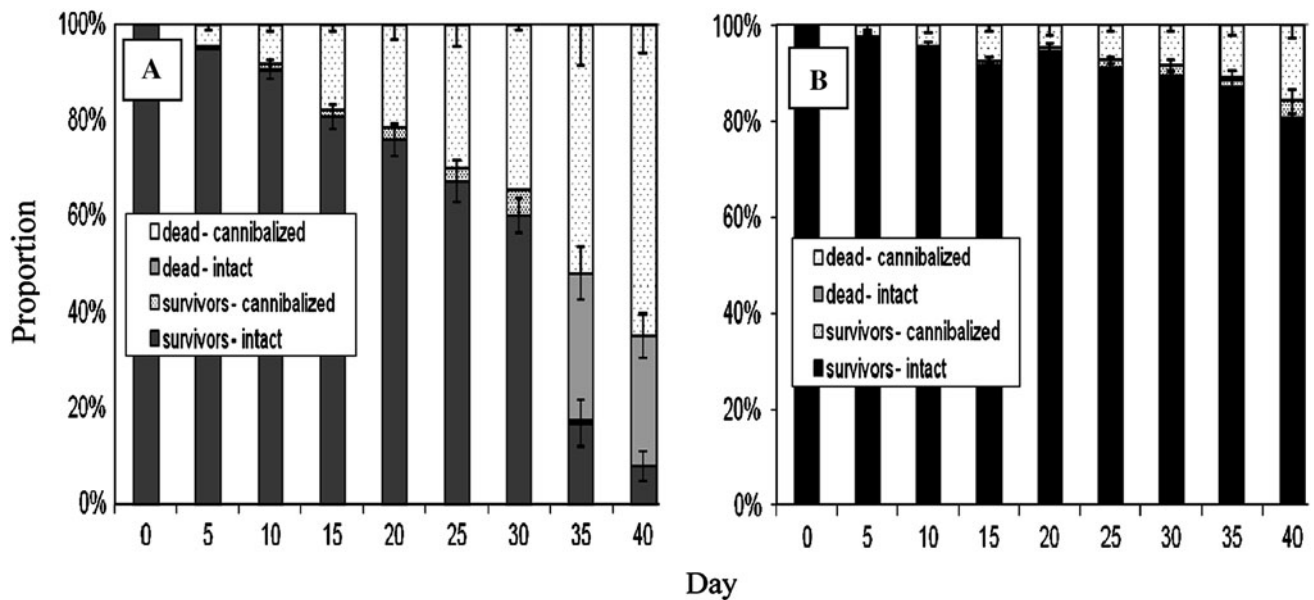


Fig. 1 Mean proportions of survived workers (intact or cannibalized) and dead workers (intact or cannibalized) during the 40-day experiment. **a** Starved, **b** paper-fed control

Table 1 Soldier survival (mean \pm SE) at 5-day intervals during the 40-day experiment

Treatment	Observation (days)								
	5	10	15	20	25	30	35	40	
Starved	100 \pm 0.0	100 \pm 0.0	100 \pm 0.0	100 \pm 0.0	100 \pm 0.0	80 \pm 12.2	70 \pm 12.2	60 \pm 10.0	
Fed	100 \pm 0.0	100 \pm 0.0	100 \pm 0.0	100 \pm 0.0	100 \pm 0.0	100 \pm 0.0	100 \pm 0.0	90 \pm 10.0	
$F_{1,9}$	–	–	–	–	–	2.67	6.0	4.5	
P	–	–	–	–	–	0.17	0.07	0.07	

Statistical analysis

Differences in the survival rate, body mass, short-range movement, and protozoan population between treatments were tested by analysis of variance ($P \leq 0.05$). Proportional data were arcsine transformed, and the estimated protozoan populations were square root transformed before data analysis. Significant differences between means were calculated by Tukey's comparison for survival, Dunnett methods for estimated protozoan populations, and LSD for body mass and short-range movement (Statistix 8.0 Analytical Software, Tallahassee, FL). Note that the untransformed data are provided in the tables and figures.

Results and discussion

Survival and cannibalism

An average of 5% workers and 60% soldiers, respectively, survived the 40-day starvation, whereas an average of 82 and 90%, respectively, survived on a filer paper diet

(Fig. 1; Table 1). The survival rates were calculated from the five replicates at each inspection date except for days 35 and 40 when two replicates undergoing starvation died from apparent bacterial infection, indicated by the soft, foul smelling, and decomposed carcasses. None of the groups were diseased during the first 30 days, and only 2 out of the 80 experiment units thereafter. A mortality of <20% was observed in the control groups, a range which is considered common in laboratory studies (Osbrink and Lax, 2002; Hu, 2005).

Except for the first 5-day inspection ($F = 19.1$, $P = 0.12$), worker mortality in starved groups was significantly greater than that in controls ($F > 56$, $P < 0.005$), whereas soldier mortality was not statistically different between the starved and the fed groups ($F < 4.5$, $P > 0.07$). During the first 30 days of starvation, worker mortality increased gradually and more workers were observed being eaten alive. Microscopic observations revealed black scars on wounds in the majority of cannibalized individuals, dead, or alive. After 30 days, the number of dead workers increased sharply and outnumbered live ones, but only a few of the carcasses had a black

scar on their wounds. Live insects are known to rely on a rapid coagulation of hemolymph and melanization to seal-off wounds (Grégoire, 1974). The black scar is probably the result of a general defensive response in the wound-healing process, i.e., scarring results from the activation of the prophenoloxidase cascade, blackening being due to chemical oxidation as part of the defense response (Bidochka et al., 1989; Scherfer et al., 2004). In immature insects, such as termite workers, the wound scars are supposed to stay until molting (Lai et al., 2002). Therefore, we believe that a black scar indicates that a worker was wounded/cannibalized while still alive or still fresh. It is less clear whether those without black scar were exclusively wounded/cannibalized after they had died.

Soldier mortality (Table 1) was not observed until 30 days of starvation. At 40 days, the soldier mortality did not statistically differ between the starved (60%) and the fed groups (90%) ($F = 4.5$, $P = 0.07$). Furthermore, cannibalism of soldiers was not observed during the entire test period. In termite colonies, the soldier proportion is regulated at a species-specific level (Haverty, 1977; Oster and Wilson, 1978; Haverty and Howard, 1981), but may vary in response to changes in environmental factors (season, temperature, availability, quality of food, etc.) and biotic factors (age, population density, physiological status, etc.) (Cleveland, 1925; Howard and Haverty, 1981; Su and LaFage, 1986; Roux and Korb, 2004). In addition to alarm and defense (Noirot, 1990), termite soldiers are known to have other roles that benefit their colony, including the regulation of other castes (Henderson, 1998; Roisin, 1999; Scharf et al. 2003; Roux and Korb, 2004; Park and Raina, 2005; Hrdy et al., 2006; Elliott and Stay, 2008). Soldiers also have a positive effect on colony survival in other social systems (e.g. ants: Hasegawa, 1993; aphids: Foster and Rhoden, 1998). Indeed, *C. formosanus* groups acquire nutrients to survive a 40-day starvation by eating more soldiers than workers during the first 10 days but cannibalizing both workers and soldiers after the soldier proportion falls from 20 to 2–5% (Su and LaFage, 1986). However, the current study showed *R. flavipes* groups survived the 40-day starvation through cannibalizing workers but not soldiers. This is very likely due to the considerable difference in soldier proportions between the two species. In *C. formosanus*, the benefit of reducing soldier proportion via cannibalism may outweigh the cost of maintaining the high proportion of soldiers. In contrast, as *R. flavipes* already has a low soldier proportion of 2%, keeping them may increase the benefit of group survival and reduce the cost of soldier differentiation, i.e. existing soldiers inhibit the formation of additional soldiers (Mao and Henderson, 2010), thus retaining the workers for food exploration and digestion.

Cannibalism, the consumption of conspecifics, is common in nature and has been observed in a wide variety of organisms (reviewed in Fox, 1975a). Many studies have shown that scarcity of food is the single most important factor promoting cannibalism in both herbivores and carnivores (Fox, 1975b, Elgar and Grespi, 1992). In termites, incidences of cannibalism have been observed when food sources were running low (Buchli, 1950), or were deficient in nutrients (Smythe and Williams, 1972; Mannesmann, 1973), or when termites starved (Su and La Fage, 1986). Raina et al. (2004) demonstrated that after defaunation reproductives cannibalized their progeny. The cues that lead to cannibalism in subterranean termites remain unknown. Korb and Lenz (2004) pointed out that in diploid termites, no relatedness asymmetries per se exist that would favor altruistic helping. Fox (1975b) proposed that cannibalism works as another form of “social” behavior in surviving food shortage. In a starvation situation, many of the “sub-organismal signals” may be overridden and less influential; it is all about getting something to eat and staying fit/alive. Cannibalism in termites has been described as to provide a supply of nitrogen (Cook and Scott, 1933) or to provide cellulolytic symbionts so that workers can maintain their cellulose digesting ability when food becomes available (Seifert and Becker, 1965). We assume that termites could tell a fit individual from a weak one and that the fitter could run faster or defend itself better than a weaker one. Hence, we hypothesize that the workers that survived were the fittest.

Body mass and vitality

Starvation for 40 days did not affect body mass and short-range movement ability of the survivors (Fig. 2). Workers that were starved or paper fed for 40 days or freshly from natural setting had approximately the same body mass

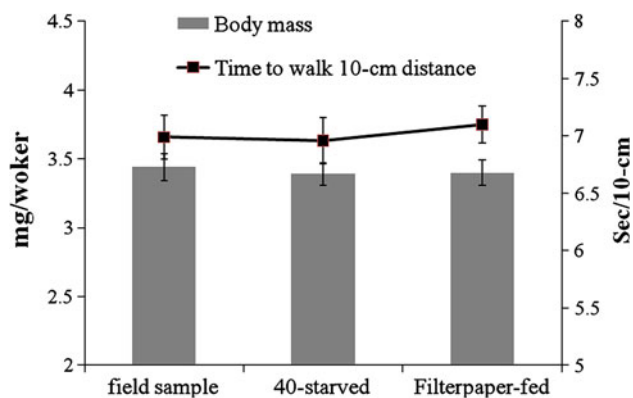


Fig. 2 Body mass and moving ability of *R. flavipes* workers (mean \pm SD)

Table 2 Protozoa population estimate (mean \pm SD) in the hindgut of *R. flavipes* workers ($n = 15$) from different groups

Species (family)	Field collected	40-day paper fed	40-day starved
<i>Trichonympha agilis</i> Leidy (Trichonymphidae)	3,600 \pm 1,486c	2,020 \pm 555b	0a
<i>Dinenympha fimbriata</i> Kirby (Pyrsonymphidae)	7,050 \pm 1,350c	5,222 \pm 1,200b	150 \pm 95a
<i>Pyrsonympha vertens</i> Leidy (Pyrsonymphidae)	4,000 \pm 400c	2,666 \pm 600b	0a
<i>Spirotrichonympha flagellate</i> (Grassi) (Spirotrichonymphidae)	5,111 \pm 890c	2,222 \pm 1,070b	0a
<i>P. major</i> Powell (Pyrsonymphidae)	2,800 \pm 200c	777 \pm 85b	0a
<i>D. gracilis</i> Leidy (Pyrsonymphidae)	21,100 \pm 3,016b	11,333 \pm 1,440b	0a
<i>Holomastigotes elongatum</i> Grassi (Holomastigotidae)	3,222 \pm 977b	2,050 \pm 700b	0a
<i>Spirotrichonympha kofoidi</i> Koidzumi (Spirotrichonymphidae)	2,020 \pm 528b	2,005 \pm 833b	755 \pm 50a
<i>Trichomitus trypanoides</i> (Dobosq and Grasse) (Trichomondadiae)	1,444 \pm 1,030a	1,000 \pm 950a	950 \pm 178a
<i>Monocercomonas</i> sp. Grassi (Monocercomonadidae)	1,222 \pm 1,230a	1,444 \pm 555a	2,160 \pm 205b

Means (\pm SD) with the same letter in a row do not differ significantly ($df = 44$, $P < 0.05$, Dunnett's method)

($F = 3.09$, $P = 0.08$) and needed the same time to walk a 10-cm distance ($F = 0.16$, $P = 0.85$). The energy-demanding survival and walking indicate they might obtain sufficient nutrients from cannibalizing other workers (Reinhold, 1999; Lorenz and Gäde, 2009). In addition to the two studies on *C. formosanus*, here we provide more evidence corroborating the observation made by Cook and Scott (1933), i.e. cannibalism can provide a food supply enabling termites to extend their life span. However, there may still exist many physiological differences between the tree types of termites, investigated here, i.e. termites either starved, fed on filter paper or freshly collected from the field, with regard to e.g. energy and nitrogen reserves, general metabolism, and water content.

Intestinal protozoa profiles between starved, paper-fed, and field workers

Starvation had a significant effect on both the community structure and the total number of the intestinal protozoa (Table 2). Of the 11 species reported from the neighboring state of Georgia (Lewis and Forschler, 2004), we identified 10 protozoan species in our samples. The total protozoan population estimates and the relative protozoan species abundance were within the ranges reported previously (Cleveland, 1925; Lewis and Forschler, 2004). The one we did not find is *Microfoenia fallax* (Dubosq and Grassé). This should not be a surprise because variation in species composition and population of termite species-specific protozoan fauna can occur between sites, among castes, and even individual termites (Smythe and Williams, 1972; Carter et al., 1981; Cook and Gold, 1998).

In the starved groups, six species were eliminated (*Trichonympha agilis*, *Pyrsonympha vertens*, *P. major*, *Dinenympha gracilis*, *Spirotrichonympha flagellata*, and *Holomastigotes elongatum*), two species were significantly reduced in number (*D. fimbriata* and *Spirotrichonympha kofoidi*),

one species was not affected (*Trichomitus trypanoides*), and one species proliferated (*Monocercomonas* sp.). Of the six species eliminated, *T. agilis* is considered the most important cellulolytic species and is critical in termite nutrition physiology. Cleveland (1925) and Mauldin et al. (1981) reported that termites (*Reticulitermes* spp.) would die soon after losing it. Haverty and Howard (1979) showed that all it took was 13 days of starvation to reduce its population to zero. In the current study, the mortality at the 15th day was only 20%, indicating that either *T. agilis* had persisted longer or it was not as critical as previously thought. The other five species (*P. vertens*, *P. major*, *D. gracilis*, *S. flagellata*, and *H. elongatum*) have been assigned different contributions to the nutritional physiology of lower termites (Grosovsky and Margulis, 1982; Yoshimura, 1995; Belitz and Waller, 1998). Of particular interest are the two species (*D. fimbriata* and *S. kofoidi*) that were reduced significantly in numbers, but persisted through the 40-day starvation. Their roles in termite food metabolism have been in dispute. Some researchers (Cleveland, 1925; Grosovsky and Margulis, 1982) consider them as non-cellulolytic and others (Kirby, 1924; Belitz and Waller, 1998) believe they are cellulolytic. Dissecting termites fed on wood and red paper particles revealed wood and paper particles in the cells of *D. fimbriata*, and particles are metabolized at a rate similar to that of *T. agilis* and *P. vertens* (Belitz and Waller, 1998). The high populations of *T. trypanoides* and *Monocercomonas* sp. in the starved survivors corroborate early reports that they are not associated with wood ingestion or cellulose degradation. The significant increase in *Monocercomonas* sp. population may indicate that the elimination and reduction of other species encourage its proliferation.

When compared with termites from natural settings, filter paper-fed groups showed population reductions in five species (*T. agilis*, *D. fimbriata*, *P. vertens*, *P. major*, and *S. flagellata*). Filter paper is composed of >95 cellulose and

is xylan-free, and nutritionally different from the food termites most likely encounter in the field (Cook and Scott, 1933; Smith and Koehler, 2007). Each of the symbiotic protozoa is known to have its specific role(s) in food digestion (Mauldin et al., 1981; Yoshimura, 1995; Cook and Gold, 2000; Watanabe and Tokuda, 2010). Therefore, the population reductions may also indicate their specialization in nutrient metabolism, and that their requirements are no longer met on an impoverished diet of filter paper (Manesmann, 1973; Breznak and Brune, 1994; Yoshimura, 1995; Cook and Gold 2000; Smith and Koehler 2007).

Still to date, many aspects of the role of protozoa in termite metabolism remain to be unknown because most of the protozoa cannot be cultivated (Radek, 1999). Cleveland (1924) and Honigsberg (1970) reported that termites died within 2 weeks, despite continued feeding if the protozoa were killed. Later studies have shown a dual cellulose system: termites produce their own endogenous endoglucanases that act in the midgut and symbiotic protozoa work in the hindgut (Breznak and Brune, 1994; Nakashima et al., 2002). Recent genetic information on “digestome”, a pool of enzyme genes of termites and gut symbionts, has suggested a more or less collaborative system, in which host and symbiont enzyme systems complement each other to achieve efficient lignocelluloses digestion (Scharf and Tartar, 2008; Watanabe and Tokuda, 2010).

Acknowledgments We thank Michael Lenz, Matthew Roehrig, and Janette Lenz for constructive comments and editing various drafts of this manuscript. We thank John Vandenberg, David Grzywacz, Kenneth Wilson, Nannan Liu and Arthur Appel for helpful discussions. Two anonymous referees provided useful criticisms and helpful suggestions. Our special thanks go to Franklin Quarcoo and Lavanya Polana for technical assistance. This work was supported by the Multistate Hatch program of Auburn University.

References

- Austin J.W., Bagnères A.-G., Szalanski A.L., Scheffrahn R.H., Heintschel B.P., Messenger M.T., Clément J.-L. and Gold R.E. 2007. *Reticulitermes mallei* (Isoptera: Rhinotermitidae): a valid Nearctic subterranean termite from Eastern North America. *Zootaxa* **1554**: 1–26
- Belitz L.A. and Waller D.A. 1998. Effect of temperature and termite starvation on phagocytosis by protozoan symbionts of the eastern subterranean termite *Reticulitermes flavipes* Kollar. *Microb. Ecol.* **36**: 175–180
- Bidochka M.J., Gillespie J.P. and Khachatourians G.G. 1989. Phenoxidase activity of acridid grasshoppers from the subfamilies Melanoplinae and Oedipodinae. *Comp. Biochem. Physiol. B: Biochem. Molec. Biol.* **94**: 117–124
- Breznak J.A. and Brune A. 1994. Role of microorganisms in the digestion of lignocellulose by termites. *Annu. Rev. Entomol.* **39**: 453–487
- Buchli H.R. 1950. Recherche sur la fondation et le développement des nouvelles colonies chez le termite lucifuge (*Reticulitermes lucifugus* (Rossi)). *Physiol. Comp. Oecol.* **2**: 145–160
- Carter F.L., Mauldin J.K. and Rich N.M. 1981. Protozoan populations of *Coptotermes formosanus* Shiraki exposed to heartwood samples of 21 species. *Mater. Org.* **16**: 29–38
- Cleveland L.R. 1924. The physiological and symbiotic relationships between the intestinal protozoa of termites and their host, with special reference to *Reticulitermes flavipes* (Kollar). *Biol. Bull.* **46**: 203–223
- Cleveland L.R. 1925. The feeding habit of termite castes and its relation to their intestinal flagellates. *Biol. Bull.* **48**: 295–306
- Cook S.F. and Scott K.G. 1933. The nutritional requirements of *Zootermopsis (Termopsis) angusticollis*. *J. Cell. Comp. Phys.* **4**: 95–110
- Cook T.J. and Gold R.E. 1998. Organization of the symbiotic flagellate community in three castes of the Eastern Subterranean Termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiology* **31**: 25–40
- Cook T.J. and Gold R.E. 2000. Effects of different cellulose sources on the structure of the hindgut flagellate communities in *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). *Sociobiology* **35**: 119–130
- Elgar M.A. and Crespi B.J. 1992. Ecology and evolution of cannibalism. In: *Ecology and Evolution among Diverse Taxa* (Elgar M.A., and Crespi B.J., Eds). Oxford University Press, NY. pp 1–12
- Elliott K.L. and Stay B. 2008. Changes in juvenile hormone synthesis in the termite *Reticulitermes flavipes* during development of soldiers and neotenic reproductives from groups of isolated workers. *J. Insect Physiol.* **54**:492–500
- Esenher G.R. 1969. Termites in Wisconsin. *Ann. Entomol. Soc. Am.* **62**: 1274–1284
- Forschler B.T. and Henderson G. 1995. Subterranean termite behavioral reaction to water and survival of inundation: implications for field populations. *Environ. Entomol.* **24**: 1592–1597
- Foster W.A. and Rhoden P.K. 1998. Soldiers effectively defend aphid colonies against predators in the field. *Anim. Behav.* **55**: 761–765
- Fox L.R. 1975a. Cannibalism in natural populations. *Annu. Rev. Ecol. Syst.* **6**: 87–106
- Fox L.R. 1975b. Factors influencing cannibalism, a mechanism of population limitation in the predator *Notonecta hoffmanni*. *Ecology* **56**: 933–941
- Grégoire C. 1974. Hemolymph coagulation. In: *The Physiology of Insecta* vol. 5 (Rockstein M., Ed). Academic Press, Boca Raton, FL. pp 309–360
- Grosovsky B.D. and Margulis L. (1982). Termite microbial communities. In: *Experimental Microbial Ecology* (Burns R.G. and Slater J.H., Eds) Blackwell Scientific, Oxford, U.K. pp 519–532
- Hasegawa E. 1993. Nest defense and early production of the major workers in the dimorphic ant *Colobopsis nipponicus* (Wheeler) (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **33**: 73–77
- Haverty M.I. 1977. The proportion of soldiers in termite colonies: A list and bibliography. *Sociobiology* **2**: 199–216
- Haverty M.I. and Howard R.W. 1979. Effects of insect regulators on subterranean termites: induction of differentiation, defaunation, and starvation. *Ann. Entomol. Soc. Am.* **72**: 503–508
- Haverty, M.I. and Howard, R.W. 1981. Production of soldiers and maintenance of soldier proportions in laboratory experimental groups of *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks). *Insect. Soc.* **28**: 32–39
- Henderson G. 1998. Primer pheromones and possible soldier caste influence on the evolution of sociality in lower termites. In: *Pheromone Communication in Social Insects* (Vander Meer R.K., Breed M.D., Espelie, K.E. and Winston M.L., Eds). Westview Press, Boulder, CO. pp 314–330
- Honigsberg B.M. 1970. Protozoa associated with termites and their role in digestion. In: *Biology of Termites*, Vol. 2 (Krishna K. and Weesner F.M., Eds). Academic Press, N.Y. pp 1–36

- Howard R.W. and Haverty M. 1981. Seasonal variations of caste proportion of field colonies of *Reticulitermes flavipes* (Kollar). *Environ. Entomol.* **10**: 456–459
- Hrdy I., Kuldova J., Hanus R. and Wimmer Z. 2006. Juvenile hormone III, hydroptene and a juvenogen as soldier caste differentiation regulators in three *Reticulitermes* species: potential of juvenile hormone analogues in termite control. *Pest Manage. Sci.* **62**: 848–854
- Hu X.P. 2005. Evaluation of efficacy and nonrepellency of indoxacarb and fipronil treated soil at various concentrations and thicknesses against two subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* **98**: 509–517
- Husby W.D. 1980. Biological studies on *Reticulitermes flavipes* (Kollar) (Dictyoptera, Rhinotermitidae) in southern Ontario. M.S. thesis, University of Guelph, Guelph
- Kirby H. 1924. Morphology and mitosis of *Denenympa fimbriata* sp. nov. *Univ. Calif. Publ. Zool.* **26**: 199–221
- Korb J. and Lenz M. 2004. Reproductive decision-making in the termite, *Cryptotermes secundus* (Kalotermitidae), under variable food conditions. *Behav. Ecol.* **15**: 390–395
- Lai S., Chen C. and Hou R.F. 2002. Immunolocalization of prophenoloxidase in the process of wound healing in the mosquito *Armigeres subalbatus* (Diptera: Culicidae). *J. Med. Entomol.* **39**: 266–274
- Lewis J.L. and Forschler B.T. 2004. Protist communities from four castes and three species of *Reticulitermes* (Isoptera: Rhinotermitidae). *Ann. Entomol. Soc. Am.* **97**: 1242–1251
- Lewis J.L. and Forschler B.T. 2006. A nondichotomous key to protist species identification of *Reticulitermes* (Isoptera, Rhinotermitidae). *Ann. Entomol. Soc. Am.* **99**: 1028–1033
- Lorenz MW and Gäde G. 2009. Hormonal regulation of energy metabolism in insects as a driving force for performance. *Integr. Comp. Biol.* **49**: 380–392
- Mannesmann R. 1973. Comparison of twenty-one commercial wood species from North America in relation to feeding rates of the Formosan termite, *Coptotermes formosanus* Shiraki. *Mater. Org.* **8**: 107–120
- Mao L. and Henderson G. 2010. Group size effect on worker juvenile hormone titers and soldier differentiation in Formosan subterranean termite. *J. Insect Physiol.* (in press)
- Marron M.T., Markow T.A., Kain K.J. and Gibbs A.G. 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. *J. Insect Physiol.* **49**: 261–270
- Mauldin I.K., Carter F.L. and Rich N.M. 1981. Protozoan populations of *Reticulitermes flavipes* (Kollar) exposed to heartwood blocks of 21 American species. *Mater. Org.* **16**: 15–28
- Miramontes O. and DeSouza O. 1996. The nonlinear dynamics of survival and social facilitation in termites. *J. Theor. Biol.* **181**: 373–380
- Nakashima K., Watanabe H., Saitoh H., Tokuda G. and Azuma J.-I. 2002. Dual cellulose-digesting system of the wood-feeding termites, *Coptotermes formosanus* Shiraki. *Insect Biochem. Mol. Biol.* **32**: 777–784
- Noirot C. 1990. La caste des soldats chez les termites: originalité, évolution. *Actes Coll. Insect. Soc.* **6**: 21–26
- Ohkuma M. 2008. Symbioses of flagellates and prokaryotes in the gut of lower termites. *Trends Microbiol.* **16**: 345–352
- Osbrink W.L.A. and Lax A. 2002. Effect of tolerance to insecticides on substrate penetration by Formosan subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* **95**: 989–1000
- Oster G.F. and Wilson E.O. 1978. *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, N.J. 352 pp
- Park Y.I. and Raina A.K. 2005. Regulation of juvenile hormone titers by soldiers in Formosan subterranean termite, *Coptotermes formosanus*. *J. Insect Physiol.* **51**: 385–391
- Radek R. 1999. Flagellates, bacteria, and fungi associated with termites: diversity and function in nutrition - a review. *Ecotropica* **5**: 183–196
- Raina A. Park Y.I. and Lax A. 2004. Defaunation leads to cannibalism in primary reproductives of the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Ann. Entomol. Soc. Am.* **97**: 753–756
- Reinhold K. 1999. Energetically costly behavior and the evolution of resting metabolic rate in insects. *Funct. Ecol.* **13**: 217–224
- Roisin Y. 1999. Philopatric reproduction, a prime mover in the evolution of termite sociality? *Insect. Soc.* **46**: 297–305
- Roux E.A. and Korb J. 2004. Evolution of eusociality and the soldier caste in termites: a validation of the intrinsic benefit hypothesis. *J. Evol. Biol.* **17**: 869–875
- Scharf M.E. and Boucias D.G. 2010. Potential of termite-based biomass pre-treatment strategies for use in bioethanol production. *Insect Sci.* **17**: 1–9
- Scharf M.E. and Tartar A. 2008. Termite digestomes as sources for novel lignocellulases. *Biofuels Bioprod. Bioref.* **2**: 540–552
- Scharf M.E., Ratliff C.R., J Hoteling T., Pittendrigh B.R. and Bennett G.W. 2003. Caste differentiation responses of two sympatric *Reticulitermes* termite species to juvenile hormone homologs and synthetic juvenoids in two laboratory assays. *Insect. Soc.* **50**: 346–354
- Scheffrahn R.H. and Su N.-Y. 1994. Keys to soldier and winged adult termites (Isoptera) of Florida. *Fla. Entomol.* **77**: 460–474
- Scherfer C., Karlsson C., Loseva O., Bidla G., Goto A., Havemann J., Dushay M. and Theopold U. 2004. Isolation and characterization of hemolymph clotting factors in *Drosophila melanogaster* by a pullout method. *Curr. Biol.* **14**: 625–629
- Seifert K and Becker G. 1965. Der chemische Abbau von Laub- und Nadelholzarten durch verschiedene Termiten. *Holzforschung* **19**: 105–111
- Smith J.A. and Koehler P.G. 2007. Changes in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) gut xylanolytic activities in response to dietary xylan content. *Ann. Entomol. Soc. Am.* **100**: 568–573
- Smythe R.V. and Williams L.H. 1972. Feeding and survival of two subterranean termite species at constant temperatures. *Ann. Entomol. Soc. Am.* **65**: 226–229
- Song D., Hu X.P. and Su N.-Y. 2006. Survivorship, cannibalism, body weight loss, necrophagia, and entombment in laboratory groups of the Formosan subterranean termite, *Coptotermes formosanus* under starvation (Isoptera: Rhinotermitidae). *Sociobiology* **47**: 27–39
- Strack B.H. and Myles T.G. 1997. Behavioral responses of the eastern subterranean termite to falling temperatures (Isoptera: Rhinotermitidae). *Proc. Entomol. Soc. Ont.* **128**: 13–17
- Su N.-Y. and LaFage J.P. 1986. Effects of starvation on survival and maintenance of soldier proportion in laboratory groups of the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Ann. Entomol. Soc. Am.* **79**: 312–316
- Watanabe H. and Tokuda G. 2010. Cellulolytic systems in insects. *Annu. Rev. Entomol.* **55**: 609–632
- Yoshimura T. 1995. Contribution of the protozoan fauna to nutritional physiology of the low termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). *Wood Res.* **82**: 68–129