

STARVATION-ASSOCIATED MORTALITY, CANNIBALISM, BODY WEIGHT, AND INTESTINAL SYMBIOTIC PROTIST PROFILE OF *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE)

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Abstract This study investigated the effects of food deprivation on survival, cannibalism, body weight, and hindgut symbiotic protist communities in Eastern subterranean termites, *Reticulitermes flavipes* (Kollar). Termite groups in natural worker and soldier ratio were tested in Petri dishes that were half filled with sand, with or without a food source, under laboratory conditions for 40 days. Starvation resulted in significantly higher mortality than groups with access to food. Compared to controls, the surviving workers showed little weight loss and exhibited little or no reduction in aliveness. Close examination indicated that the surviving workers primarily lived by cannibalizing nest-mates. When the experiment was terminated at 40 days, the surviving workers were dissected to count the symbiotic protists inhabiting their hindguts. Among the 10 identified protists, starvation eliminated 3 species (*Trichonympha agilis*, *Pyronympha vertens*, and *P. major*), significantly reduced the population of 4 species (*Dinenympha fimbriata*, *D. gracilis*, *Holomastigotes elongatum*, and *Spirotrichonympha kofoidi*), but had little effect or even encouraged population growth in 3 species (*Monocercomonas* sp., *Trichomitius trypanoides*, and *Spirotrichonympha flagellata*).

Key Words food deprivation, physiological behavior, ecological behavior, intestinal microbes

INTRODUCTION

The symbiotic protists residing in their hindguts play a critical role in cellulose digestion of subterranean termites (Honigsbeg 1970; Carter et al., 1981). Starvation or inadequate nutrition may cause physiological, morphological, and behavioral changes, affect development and reproduction, and ultimately result in the death of the termite colony (Grosobsky and Margulis, 1982; Su and La Fage, 1986; Lenz, 1994; Song et al., 2006). This study investigated the effects of food deprivation on the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar). The objectives were to elucidate starvation-induced survival and cannibalism, the impact on survivors' body mass, aliveness, and changes in hindgut symbiotic protist communities.

MATERIALS AND METHODS

Reticulitermes flavipes were collected from cardboard rolls (13 cm length, 11 cm diameter) which were placed inside open-bottomed buckets (14 cm length x 12 cm diameter) installed in-ground on Auburn University's campus in April of 2006. Termites were transferred into a clean plastic box (27 x 19 x 10 cm) using a fine brush to count the ratio of workers and soldiers. Termites were examined under a stereoscope within 1 day of the collection. Active, intact, and mature individuals were used for this study.

Petri dishes (6.0 cm in dia by 1.5 cm high) containing 10 g of sterilized sand moistened with 1.2 ml distilled water were used as experimental units. Control units had an additional piece of moistened filter paper (Fisher Brand, Pittsburgh, PA) as a food source. Termites were tested in groups of 100 workers and soldiers in natural ratio (98:2). After introduction of termites, the units were sealed with Parafilm (American National Can, Neenah, WI) to retain moisture, and then were placed in an incubator (23 ± 1°C) with constant darkness except during observations. In total, there were 104 experimental units (48 starved groups and 56 controls).

Survival and Cannibalism

Observations were made at 5-day intervals during a 40-day period. On each of the 8 observation days, 6 replications from starvation treatment and control, respectively, were taken out of the incubator for

examination and not used again. The units were examined under a stereoscope to record the number of live intact, live cannibalized, dead intact, and dead cannibalized termites. A termite was defined as dead if it did not move appendage(s) when probed. A termite was classified as cannibalized if appendage(s) or body parts were either partially or entirely missing. Data were normalized before the analysis of two-sample t-test at $\alpha = 0.05$ (Statistix 8.0 Analytical Software, Tallahassee, FL).

Body Mass And Aliveness

Twenty workers from the following 3 groups were weighed to determine changes in body mass: fresh field-collected, survivors of 40-day starvation, and survivors of 40-day filter paper feeding. These workers were randomly selected and weighed on an electronic analytical balance. ANOVA was used to determine the difference between the 3 groups. The same groups of workers were then tested individually for aliveness by measuring the time needed to walk a 10 cm distance on a plain paper.

Intestinal Protist Profile

After aliveness measurement, 5 workers from each group were randomly selected and dissected for examination using the method developed by Lewis and Forschler (2004). Hindguts were gently removed from the last two abdominal segments using fine-tipped forceps. The gut content was homogenized in 100 μ l 0.3% saline solution for 10 seconds using a disposable pestle in a 1.5-ml microcentrifuge tube. Ten μ l of the gut suspension was then loaded into a hemacytometer (Arthur H. Thomas Company, Philadelphia, PA). Protists were identified and counted from 5 large squares at 400 \times magnification under a microscope (MEIJI). The entire process for each individual worker was completed within 5 minutes. The estimated population of each protist species was calculated using the following formula: number of cells counted \times volume saline solution in original sample / volume on the hemacytometer.

RESULTS AND DISCUSSION

Survival and Cannibalism

The actual proportions of surviving (intact or cannibalized) and dead workers (intact or cannibalized) during the 40-day test are presented in Figure 1. Starvation resulted in significantly greater termite mortality compared to the control ($P < 0.005$). During the first 30 days, mortality in starved units increased gradually, corresponding to the increased number of cannibalism as evidenced on nearly all of the dead bodies as well as an increased number of living workers that either had appendage(s) dislodged or missed body parts. Close examination showed that a majority of the dead termites had black scars on the wound formed by the clotting of hemolymph, indicating they were eaten while alive. Thirty days after starvation, mortality rate increased rapidly, but cannibalism (on dead or living termites) was barely evidenced. This suggests that starvation was the direct cause of death, and that the few remaining survivors no longer sacrificed living members. This result is consistent with our previous starvation study on Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Song et al., 2006). However, unlike *C. formosanus*, which utilized soldiers as a food source after 10 days of starvation, starved *R. flavipes* workers did not sacrifice soldiers as an emergency ration during the entire 40-day starvation. When the study was terminated at 40 days, soldiers only suffered a mortality of 11% (10 died out of the 96 soldiers in the 48 starved groups), a lever lower than the overall mortality of 15% from control. A possible explanation is that *R. flavipes* had a very low soldier ratio (2%) and had to keep them alive for defense, while *C. formosanus* had a high soldier ratio (20%), and consuming some soldiers mitigated the trophic burden under starvation conditions. Mortality of laboratory groups with access to filter paper is common and may not associated with any treatment (Osbrink and Lax 2002). The speculation is that termites eat their own members to gain protein because the nitrogen content of filter-paper used as food source is lower than the nitrogen content in wood (La Fage, 1976). At the last day of this 40-day test, about 90% and 15% had died in starved and control groups, respectively.

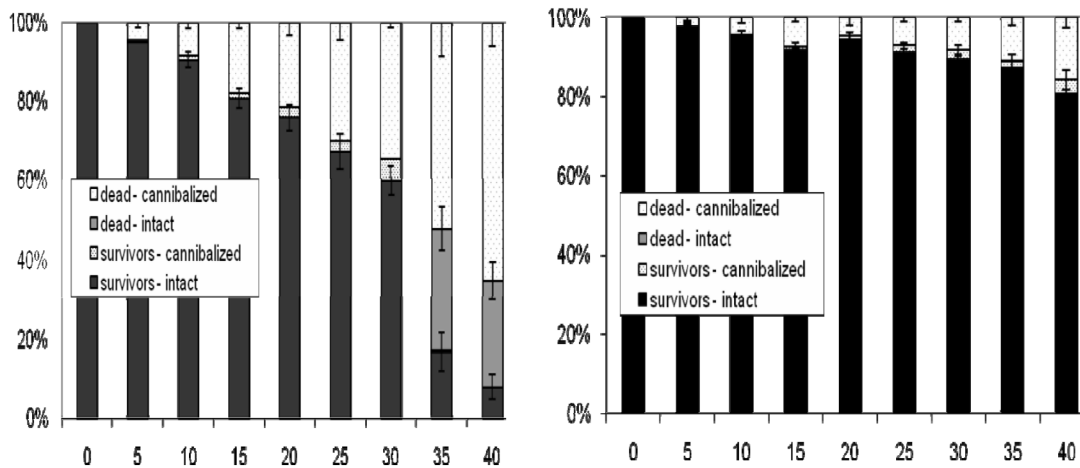


Figure 1. Proportions of surviving (intact or cannibalized) and dead workers (intact or cannibalized) during the 40-day test (starvation vs. control)

Body Mass and Aliveness

Table 1 shows worker body mass and aliveness expressed by travel ability. Workers starved or fed on filter paper for 40 days showed little or no reduction in body weight in comparison with fresh field collected termites ($F_{(2)} = 3.09, P = 0.08$). Workers from the 3 groups also exhibited a similar level of aliveness ($F_{(2)} = 0.16, P = 0.85$). These results indicate the ability of the starving *R. flavipes* workers to maintain body weight and aliveness by eating nest-mates in the absence of cellulosic food supply.

Table 1. Body mass and aliveness of *R. flavipes* worker (Mean \pm SD).

	Body mass (mg/worker)	Time to travel 10 cm distance (second)
Fresh field-collected	3.436 \pm 0.041	6.992 \pm 0.860
40-day feeding on filter paper	3.400 \pm 0.042	7.106 \pm 0.905
40-day starvation	3.390 \pm 0.049	6.962 \pm 0.714

Intestinal Protist Profile

We identified 10 out of the 11 protist species described from *R. flavipes* by Lewis and Forschler (2004) (Table 2). Compared to fresh field-collected workers, workers feeding on filter paper caused selective population reductions of 6 species (*D. fimbriata*, *H. elongatum*, *P. major*, *S. kofoidi*, and *T. agilis*). The reduction may be a result of the inadequate nutrition in filter paper (Carter et al., 1981, Breznak and Brune 1994). Starvation resulted in the total elimination of 3 species (*P. major*, *P. vertens*, and *T. agilis*), significant population reduction of 4 species (*D. fimbriata*, *D. gracilis*, *H. elongatum*, and *S. kofoidi*), while the other 3 species (*Monocercomonas* sp., *Trichomitus trypanoides*, and *Spirotrichonympha flagellata*) were either not affected or increased in number. Early studies proposed *T. agilis* and *T. fimbriata* as cellulolytic because they were the first to be eliminated in starvation experiment (Celveland, 1925; Grosovsky and Margulis, 1982). *P. vertens* were proposed to be facultatively cellulolytic because it disappeared after elimination of cellulolytic species (Haverty and Howard 1979). The other 7 species were thought to be less sensitive to starvation and did not ingest wood fragments but might be involved in the later stages of cellulose degradation. Our study confirmed the cellulolytic status of *T. agilis* (the most dominant species in fresh field-collected workers) and the facultative status of *P. vertens*, but challenged the cellulolytic role of *T. fimbriata*. The more or less increase in *M. sp.*, *S. flagellate* and *T. trypanoides* populations suggests that elimination of other species might have encouraged their growth and development.

Table 2. Estimated protist population (Mean \pm SD) in *R. flavipes* worker's hindgut content.

Species	Fresh field-collected	40-day feeding on filter paper	40-day starvation
<i>Dinenympha fimbriata</i>	2,050 \pm 428	1300 \pm 200	100 \pm 120
<i>D. gracilis</i>	1,100 \pm 216	1,000 \pm 140	200 \pm 50
<i>Holomastigotes elongatum</i>	1,700 \pm 277	600 \pm 100	100 \pm 50
<i>Monocercomonas</i> sp.	900 \pm 130	1,000 \pm 600	2,160 \pm 250
<i>Pyrsonympha major</i>	1,800 \pm 200	700 \pm 80	0
<i>P. vertens</i>	1,000 \pm 400	900 \pm 600	0
<i>Spironympha flagellate</i>	200 \pm 70	300 \pm 80	200 \pm 60
<i>S. kofoidi</i>	2,200 \pm 328	900 \pm 100	100 \pm 50
<i>Trichomitus trypanoides</i>	400 \pm 130	600 \pm 150	400 \pm 80
<i>Trichonympha agilis</i>	3,660 \pm 186	1,200 \pm 555	0

CONCLUSIONS

This study showed that food-deprivation resulted in significant mortality attributing primarily to cannibalism of other living members during the first 30 days but starvation between 30 to 40 days. The first 30 days of starvation, *R. flavipes* workers exhibited cannibalism of other workers, both alive and dead, and also their own metabolic stores as additional carbon and energy sources to sustain body weight, aliveness, and probably protists as well. However, they no longer sacrificed living members after 30-days when the dead bodies outnumbered survivors. This may be an evolved strategy in *R. flavipes* to remain biologically active and to conserve colony survival under starvation conditions.

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