Abundance and Distribution of the Three Species of Symbiotic Protozoa in the Hindgut of *Coptotermes formosanus* (Isoptera: Rhinotermitidae)[1,2]

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ABSTRACT

Workers, soldiers, nymphs and alates of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, all harbored the same 3 species of protozoa. Workers, nymphs, and alates had a larger number of protozoa than the soldiers. The relative abundance of the protozoa differed in the different castes. In the worker, *Holomastigotoides hartmanni* Koidzumi was the predominant species in numbers, followed by *Pseudotrichonympha grassii* Koidzumi and *Spirotrichonympha leidyi* Koidzumi while in the alate. *S. leidyi* was predominant. Each protozoan species apparently occupied a more or less specific location in the worker's hindgut. *P. grassii* was predominant in the first pouch, *H. hartmanni* in the second, and *H. hartmanni* and *S. leidyi* in the third pouch and excreta. This may indicate a difference in digestive roles of the protozoa and/or a difference in the oxygen requirements of the 3 species. There were no significant differences in the total number of protozoa found in the workers from 3 different colonies.

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki is the most important structural pest and is by far the most destructive among the four species of termites established in the Hawaiian archipelago (Bess 1970; Fujii 1975). The total damage caused by this termite in Hawaii exceeds 20 million dollars a year (Lai 1977).

C. formosanus feeds on anything containing cellulose, but, like other termites, cannot digest cellulose without the assistance of the symbiotic protozoa harbored in the hindgut (Hungate, 1938, 1939, 1944; Brown and Smith 1954; Mauldin et al. 1972).

There are three species of symbiotic flagellates in *C. formosanus* (Koidzumi 1921). These are *Pseudotrichonympha grassii* Koidzumi, *Holomastigotoides hartmanni* Koidzumi, and *Spirotrichonympha leidyi* Koidzumi. *P. grassii,* the largest of the three species, is spindle-shaped, ca. 200-300 μ long and 50-120 μ wide. *H. hartmanni* is oval or elliptical, 50-140 μ long and 30-80 μ wide. *S. leidyi* is cone-shaped and 15-50 μ long and 8-30 μ wide.

Since the symbiotic protozoa play such a major role in the digestion of cellulose in termites, it is important to establish their abundance and distribution in the termite. Such information may be valuable in elucidating the symbiotic relationship between termites and protozoa.

MATERIALS AND METHODS

A depression slide (Boerner) with 10 cells, was used for dissecting termites. The cells were filled with 0.2 ml of 10% formaldehyde solution. The formaldehyde solution was used to preserve the morphological and distributional integrity of the protozoa by killing the protozoa instantly. Workers collected from 3 isolated field

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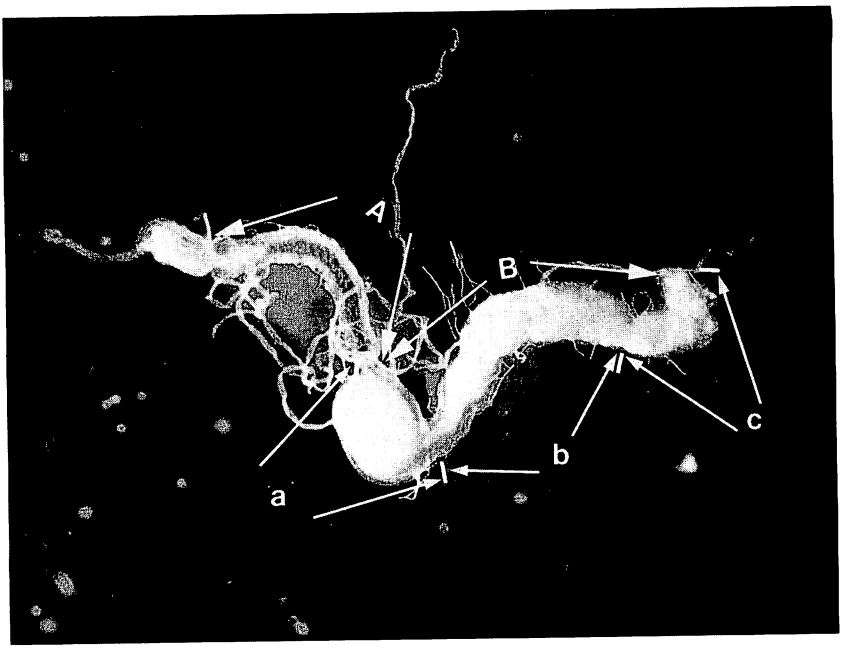

FIGURE 1. The digestive system of *Coptotermes formosanus* worker: A. Midgut; B. Hindgut; a. First pouch, b. Second pouch, c. Third pouch.

colonies, located on the campus of the University of Hawaii, Manoa, were used for this study.

A freshly collected worker from a field colony was held gently in an upright position with a pair of fine forceps in the area between the thorax and the first abdominal segment. The termite usually excreted a drop of fluid while it was held in this position. This first excretus was dropped into one of the cells containing 0.2 ml formaldehyde. Subsequent excreta were pooled together as the second excretus in the second cell. The termite was then placed in a clean slide in a drop of 0.2 ml of 10% formaldehyde solution and the anal segment was teased apart with a dissecting needle. The exposed intestine was gently pulled out from the posterior end and the cadaver was discarded.

The hindgut was then separated from the midgut at the pyloric region. The midgut and the Malpighian tubules were removed and discarded. The hindgut was then transferred to the third cell of the depression slide. An examination of the hindgut showed that it was divided into 3 pouches or enlargements which were delineated by constrictions (Fig. 1). The gut was cut into sections at these constrictions and each section was placed in a separate cell.

The separated pouches were gently macerated to expose the protozoa to the 10% formaldehyde solution. The protozoa in each cell was thoroughly mixed by blowing air through a disposable pipette on the surface of the suspension.

A 2 μl aliquot of the suspension was randomly drawn from each cell with a microsyringe (5 μl, Hamilton Co., Reno, Nevada) and placed on a clean slide. The aliquot was examined under a phase contrast microscope without using a coverslip. All the protozoa were counted and the number was recorded. This was repeated 3 times for each cell.

In addition, 10 termites of each caste were dissected and three 2 μl aliquots were drawn from each cell containing the different parts of the hindgut. The data were transformed using $\sqrt{x + 0.5}$ and subjected to analysis of variance of split plot design, 2 × 2 factorial tests, and the means were separated using Duncan's multiple range tests with the aide of a computer program (SAS User's Guide, 1979 Ed.).

Since the 2 μl sample counted was 1/100 of the total volume in each cell the population estimations of the protozoa were obtained by multiplying the average number of protozoa per 2 μl sample by 100. The populations of protozoa in the soldiers, nymphs and alates (male, female) were determined in a similar manner.

RESULTS AND DISCUSSION

Table 1 shows the average number of protozoa per 2 μl in *C. formosanus* workers from 3 different field colonies. The results of the analysis indicated that there were no significant differences in the total number of protozoa in workers from the 3 colonies (F = 1.16). In addition, there were no significant differences in the numbers of the individual species of protozoa in the workers from the three colonies. Workers from all 3 colonies had similar numbers of *P. grassii, H. hartmanni* and *S. leidyi.* Also, there were no significant differences in proportional distributions of the 3 species of protozoa in each location in the hindgut.

On the other hand, within each worker, *H. hartmanni* was significantly more abundant than *P. grassii* or *S. leidyi* (F = 31.3). *H. hartmanni* comprised 48.8% of the total population of protozoa followed by *P. grassii* with 27.4% and *S. leidyi* with 23.8%. The populations of *P. grassii* and *S. leidyi* were not significantly different from each other. The total number of protozoa per worker was ca. 3360 (Table 2).

The distribution of the 3 species of protozoa in the hindgut was not random (F = 38.8). There was a definite species distribution in different parts of the hindgut. Each species was more abundant in a particular location in the hindgut (Table 1). *P. grassii* was most abundant in the first pouch followed by *H. hartmanni* which was significantly more abundant than *S. leidyi.* In the second pouch the order of abundance was *H. hartmanni* > *P. grassii* > *S. leidyi.* In the third pouch and in the second excretus the order of abundance was *H. hartmanni* = *S. leidyi* > *P. grassii.*

Populations of *P. grassii* diminished in relative abundance as one moved towards the anus. Only 40 were found in the second excretus. *H. hartmanni,* on the other hand, seemed to be more uniformly distributed in the 3 pouches than either of the other 2 species. It was consistently present in relatively high numbers in all of the pouches, and was even most abundant in the second excretus. *H. hartmanni* was the most abundant of the 3 protozoa, almost equalling the total of the other 2 species combined.

The distribution of the third species, *S. leidyi,* was just the opposite of *P. grassii.* Its numbers increased as one moved towards the anus. In one of the colonies (Amphitheater), only 6 of the 30 samples had *S. leidyi* in the first pouch. The largest numbers of *S. leidyi* was found in the third pouch, but it was not the predominant species in any part of the hindgut.

The distribution of the protozoa indicated that there was a specific niche occupied by each species of protozoa. This niche type of distribution suggested that each species may have a specific function in the digestion of cellulose as indicated by Mauldin et al. (1972), Smythe and Mauldin (1972), and Mauldin and Smythe (1973). The distribution may also indicate that there were differences in oxygen tolerance by the 3 species. The anterior part of the hindgut was probably more anaerobic than the areas near the anus.

TABLE 1. Average number of protozoa from different locations in the hindgut of workers of *Coptotermes formosanus* from 3 field colonies. (n = 30)

		Average No. of protozoa per 2 μl sample					
Colony	Protozoa*	1st Pouch	2nd Pouch	3rd Pouch	2nd Excreta	Total**	Total protozoa per worker
Amphitheater	A	420	380	80	40	920[a]	
	B	290	760	400	190	1640[b]	3360[d]
	C	30	270	340	160	800[c]	
Miller Hall	A	410	240	70	60	780[a]	
	B	180	360	350	350	1240[b]	2760[d]
	C	10	80	360	290	740[c]	
Pope Lab	A	480	270	130	10	890[a]	
	B	230	400	470	100	1200[b]	2880[d]
	C	60	220	410	100	790[c]	

*A — *Pseudotrichonympha grassii*
B — *Holomastigotoides hartmanni*
C — *Spirotrichonympha leidyi*
**Numbers followed by the same letter are not significantly different from each other.

TABLE 2. The abundance and distribution of the 3 species of protozoa in the hindgut of *Coptotermes formosanus* workers. (n = 30)

	Estimated population in each location in the hindgut												
	1st Pouch			2nd Pouch			3rd Pouch			2nd Excreta			
Protozoa	A*	B**	C***	A	B	C	A	B	C	A	B	C	Sum
P. grassii	420	56.8	45.6	380	27.0	41.3	80	22.8	8.7	40	10.2	4.3	920
H. hartmanni	290	39.2	17.7	760	53.9	46.3	400	48.8	24.4	190	48.7	11.6	1640
S. leidyi	30	4.0	3.8	270	19.1	33.8	340	41.5	42.5	160	41.1	20.0	800
Sum	740			1410			820			390			3360

*A = no. of protozoa.
**B = Percentage of the species in the population of Protozoa of that location.
***C = Percentage of the total population of the given species found at that location.

TABLE 3. Population estimation of the symbiotic protozoa, *Pseudotrichonympha grassii, Holomastigotoides hartmanni,* and *Spirotrichonympha leidyi,* found in the hindgut of workers, soldiers, nymphs, and alates of *Coptotermes formosanus.**

	No. of *P. grassii*	Percent of Total	No. of *H. hartmanni*	Percent of Total	No. of *S. leidyi*	Percent of Total	Total** No. of Protozoa/ Termite
Worker	920	27.4	1640	48.8	800	23.8	3360[a]
Soldier	160	38.1	220	52.4	40	9.5	420[b]
Nymph	1660	41.8	1740	43.8	570	14.4	3970[c]
Alate (♂)	250	13.9	400	22.2	1150	63.9	1800[d]
Alate (♀)	530	22.8	710	30.6	1080	46.6	2320[d]

*Ten termites of each caste were dissected and 3 subsamples of 2 μl each were taken from each termite.
**Means followed by the same letter are not significantly different from each other at P = 0.01.

No protozoa were found in the first excretus. The lack of protozoa and the obvious difference in the consistency of the excreta indicated that the first excretus was primarily excrement. The first excretus mainly contained solid material.

The second excretus which was quite different from the first excretus resembled or was similar to the fluid excreted for proctodaeal feeding. This was an opaque, white, liquid which contained a number of protozoa. Obviously the termite either deposits the first excretus before initiating proctodaeal feeding or is somehow able to excrete the proctodaeal food without mixing it with excreta.

Although *H. hartmanni* was the predominant species in numbers, *P. grassii* was predominant in biomass since it was more than 3 times the size of *H. hartmanni* and more than 20 times the size of *S. leidyi.* The volume of protozoa almost completely filled the hindgut leaving what seemed to be very little space for food. The weight of an average hindgut was ca. 1.49 ± 0.22 mg which was 41% of the total weight of a worker which averaged 3.64 ± 0.01 mg.

In the other castes, i.e., soldiers, nymphs, and alates (males, females), the total numbers and relative abundances of protozoa differed from the workers (Table 3). The soldiers, as expected, harbored very few protozoa. Microscopic examination also proved that fragments of undigested food material were present in the hindgut of a worker. These undigested food material were probably passed on to the soldiers through trophallaxis. Therefore, not all the food obtained through trophallaxis was absorbed in the midgut.

The relative abundance of the protozoan species did not differ significantly from the workers except there were slightly more *P. grassii* and slightly less *S. leidyi. H. hartmanni* was still the predomiant species.

The nymphs had an unexpectedly large population of protozoa. The nymphs had the largest number of protozoa of all the castes. Since the abundance of protozoa, logically should be correlated with amount of feeding, the results were unexpected. The nymphs did not appear to feed as extensively or voraciously as the workers. In addition, the nymphs do not play a role in the feeding of soldiers and the immatures or in the maintanance of the colony.

Although the physical size of the nymph was larger than the worker, physical size alone could not account for the larger numbers of protozoa since the alates were also

larger than the worker and had less protozoa. The abundance of the protozoa, however, indicated that the nymphs probably did a significant amount of feeding.

That the nymphs do feed extensively was confirmed in a test where they were fed Sudan Red 7B, a dye that was used to mark *C. formosanus* for field studies. In this test only nymphs were held in the petri dishes so that there was no possibility of trophallaxis among nymphs and workers. The nymphs were all stained by the dye they ingested with the food.

The large amount of fat bodies present in this stage lends further evidence to support the hypothesis. Moreover, since they do not feed the immatures, or soldiers, all of the food consumed were utilized by the nymphs.

The composition of the protozoan fauna in the nymph also differed. There were relatively large numbers of *P. grassii* and a few *S. leidyi.* The relative abundance of *H. hartmanni* was similar to that of the workers.

The relatively small numbers of protozoa found in the alates were surprising, but even more surprising was the composition of the fauna. The predominant species in both the male and female was *S. leidyi,* and in the alate male there were more *S. leidyi* than the other two species combined. Apparently, there was a drastic change in the relative abundance of the protozoa after the last molt. There were significant differences in both the numbers of protozoa and in the species composition of the fauna between the nymphs and the adults.

Although the differences in the total numbers of protozoa among the castes were expected, the differences in the relative abundance of the protozoa among the castes were not. Whether this reflected differences in the nutritional requirements of each caste or whether it reflected physical differences in the hindgut was not determined. Each species of protozoa, however, was abundant or scarce in same general areas of the hindguts of all the castes.

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