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Studies on the Intestinal Protozoa of Termites

I. Starvation Experiments on the Commonest Japanese Termite, *Leucotermes speratus*

By

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Having started in April 1928 a physiological study on the intestinal protozoa of Japanese termites as a graduation thesis, I had first to examine the intestinal protozoa of *Leucotermes speratus* which is the commonest termite in Japan proper, and carried out some starvation experiments on that insect. The object of the present paper is to report briefly on the time-relation of the protozoal-disappearance in the starvation experiments I carried out from September to December 1929.

Here, I wish to express my warmest thanks to Prof. TAMIJI KAWAMURA for his kind direction and help.

PROTOZOA

The intestinal protozoa of Japanese termites were precisely described for the first time by MAKOTO KOIDZUMI (1921) who reported that *Leucotermes speratus* in Japan proper harbored the following protozoa: 1. *Trichonympha agilis* var. *japonica*, 2. *Teratonympha mirabilis*, 3. *Holomastigotoides elongatum*, 4. *Pyrsonympha grandis*, 5. *P. modesta*, 6 *Dinenympha exilis*, 7. *D. rugosa*, 8. *D. nobilis*, 9. *D. leidyi*, 10. *D. parva*, and 11. *D. porteri*. Collecting many colonies of *Leucotermes speratus* in the vicinity of Kyôto and carefully observing the intestinal protozoa, the writer also found in all the colonies all the forms of protozoa that KOIDZUMI had mentioned, except that in three colonies, which were collected in the summer of 1929, no *Teratonympha mirabilis* was to be found.

EXPERIMENTS

Workers from seven colonies (A—G) of *Leucotermes speratus* were brought from several pine-forests near Kyoto into the laboratory, removed from the wood in which they had lived, and prepared for the experiments in the following manner :—immediately or after they had been fed with common or commercial absorbent cotton for two weeks, the workers were made free from all food particles; 200–300 individuals from a colony were placed in a Petri dish, which was kept in a dark place at the room temperature. The moisture in the vessel was kept by means of inserting a sheet of wet gauze between the Petri dish and its cover. This dish was replaced every day by a clean one to keep the termites from eating debris or from being infected by bacteria. In this manner almost all the individuals could be kept active and apparently normal throughout the experiment. The intestinal protozoa in the living materials were examined under the microscope every 24 hours.

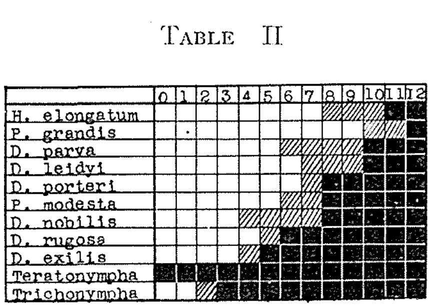
TABLE I

Starvation experiments

Experiment	Period during which the experiment was carried out	The colony that was used in the experiment	Whether the animals had been fed with cotton before they were starved, or not	Table in which the results of experiment are shown
1 and 2	7th to 19th Sept.	A and B	Not	II
3 and 4	19th Sept. to 6th Oct.	A and B	Fed	III
5, 6, and 7	29th Oct. to 22nd Nov.	C, D, and E	Not	IV
8	21st Nov. to 24th Dec.	F	Not	V
9			Fed	VI
10	28th Nov. to 29th Dec.	G	Not	VII

Ten experiments were carried out as five successive series at five periods from September to December as shown in Table I. The first and the second of the series each consisted of a similar experiment with the termites from two different colonies, the third series was three identical experiments with animals from three different colonies, the fourth consisted of two experiments with animals from the same source but fed with different diets, the fifth was a single experiment. Similar results were obtained from the experiments which were carried out parallel under the same conditions. Hence, summarising all these results into one, six tables (II.—VII.) were made as shown in the right hand column of Table I. In these six tables the following rules are observed: (a) the names of the protozoa are arranged in the left hand column, (b) the date of the experiment is given in the uppermost row, (c) the blank area of each row represents the period during which the presence of that protozoon was confirmed in any individuals of the host, (d) the hatched area indicates the period during which that protozoon was still maintained by some termites but already lost in the other members of the same colonies, and (e) the black area indicates the period during which that protozoon was entirely absent in all the individual termites. For example, in experiments 1 and 2 (Table II.) every termite harbored *H. elongatum* up to the end of the 7th day of the starvation and then some were found to be harboring this protozoon but the others were free from it from the 8th till the 10th day, and at last all the individual termites lost *H. elongatum* completely on the 11th day.

TABLE II



Each of the ten experiments mentioned above ended with more or less divergent results. As for the conditions which are concerned with this diversity, I am at present inclined to appreciate two factors as important, namely, the nutriment of the termites and the temperature to which the animals were exposed during the experiments. As can be seen by comparing Tables II. and III., when the termites were starved after they had been fed with cotton for two weeks (Experiments 3 and 4.), every form of the protozoa lived some days longer than when the termites were starved at once (Experiments 1 and 2.). Since there are no great changes of temperature during these two series of experiments (cf. Table VIII.), this factor may safely be neglected. It seems very probable, therefore, that the prolongation of the times of disappearance of protozoa is due chiefly to the difference in the quantity of nourishment (cellulose) available for the protozoa in the intestine of the host, as Cleveland (1925) remarked. The colonies A and B harbored no *Teratonympha* (cf. Table II, and III.) so that data concerning this protozoon are lacking in these experiments.

TABLE III

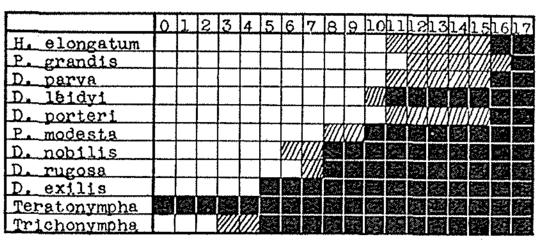
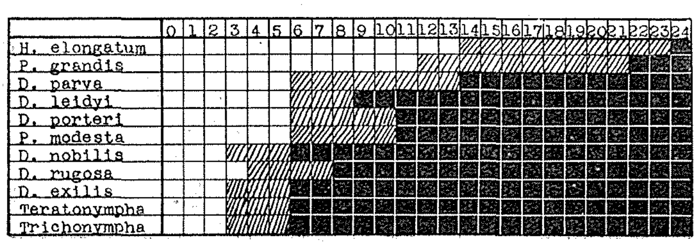


TABLE IV



In experiments 5, 6, and 7 (cf. Table IV.) *H. elongatum* and *P. grandis* lived considerably longer than in each of the previous experiments, while the other protozoa showed similar periods of disappearance to those in experiments 1 and 2. The longevity of the protozoa is perhaps related to the fall of temperature, which was more distinct in this case than in the first two experiments. This effect of the temperature diminution is more striking in the next three cases (cf. Tables V. VI. and VII.), the life of the protozoa being from two to five times as long as in experiments 1–7. While in experiments 5, 6, and 7 we saw that the lives of two species of protozoa, *H. elongatum* and *P. grandis,* were prolonged most distinctly, in experiments 8, 9, and 10 nine other forms of protozoa were equally affected by that condition. Indeed, in these experiments every form of protozoon disappeared very late. Furthermore, in these cases every protozoon, especially *Trichonympha* and *Teratonympha,* showed very high variations in the time of disappearance according to the individual host, the hatched area being much extended as is to be seen in the tables. In experiment 9 (Table VI.) the termites were starved after they had been fed with cotton for two weeks, while in experiment 8 (Table V.) which was performed at the same time as it the termites were starved immediately. Neverthless, in both cases there is no such difference in protozoal life as was found in experiments 1, 2 and 3, 4. This fact may well be explained by supposing that the temperature was too low for the termites to take the diet, though plenty of it was given before they were starved.

TABLE V

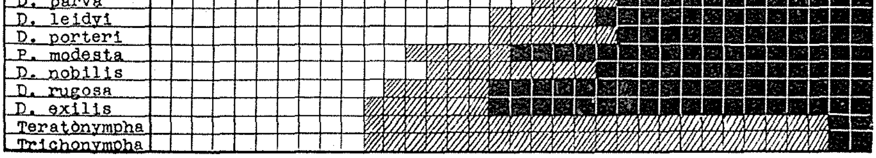


TABLE VI

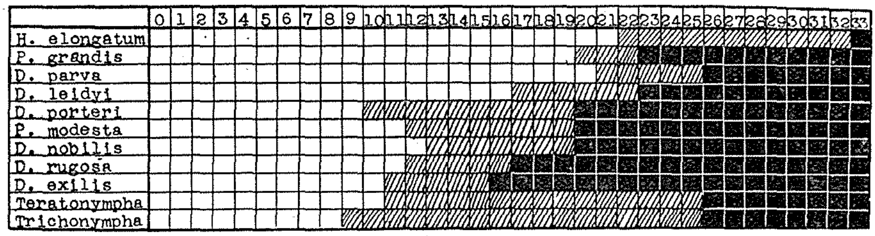
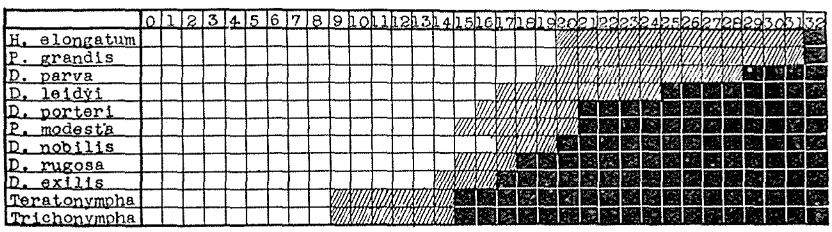


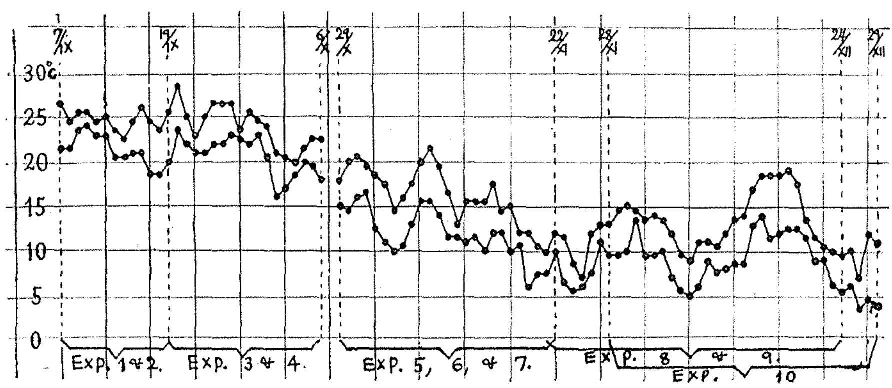
TABLE VII



In 1924 and 1925 L. R. Cleveland found in his excellent studies on the symbiotic relationships between the termites and their intestinal protozoa, that incubation, starvation, and oxygenation affected the termites so much that the hosts lost their intestinal protozoa, species by species, in a definite order in each experiment. Although such a definite order in the strict sense of disappearance of the protozoa was

TABLE VIII

The maximum and minimum room temperature of the laboratory during the experiments



not confirmed in my starvation experiments, it was found that eleven forms of the protozoa in question may be grouped into four with different period of disappearance: first, *Trichonympha* and *Teratonympha;* second, *D. exilis, D. rugosa, D. nobilis,* and *P. modesta;* third, *D. porteri, D. leidyi,* and *D. parva;* and fourth and last, *P. grandis* and *H. elongatum.*

GENERAL CONSIDERATIONS

CLEVELAND (1925) has stated that the termites lose their protozoa more slowly when they are fed first with pure cellulose, instead of wood, for several months before being starved and then are cellulose-starved, and it is true that in my experiments 3 and 4 the protozoa lived some days longer than in my experiments 1 and 2. From experiment 9, although it was not so distinct in this case as in experiments 3 and 4, it might be supposed that the temperature was too low for the termites to devour in two weeks the necessary amount of food from the cotton diet supplied.

Furthermore, if we assume as CLEVELAND (1925) did, that in the starvation experiments the protozoa die of actual cellulose starvation, the quantity of food available for the protozoa in the intestine of the host must play an important rôle in shortening or prolonging the period of disappearance of the protozoa. From this, the diversity in the results obtained with different colonies of the termites is naturally to be expected, since the different nests of the termites may retain different conditions in the nutrition of the termites, and consequently different times of longevity may be enjoyed by the intestinal protozoa in various colonies of termites when starved.

It seems probable from these experiments that the temperature is much more effective in modifying the time of death of the protozoa. For instance, in experiments 5, 6, and 7 *H. elongatum* and *P. grandis* lived rather longer than in the four previous experiments. In experiments 8, 9, and 10, when the temperature falled still more, every species of protozoon lived considerably longer, as mentioned above.

As far as these facts appeal to us, the duration of life in the parasitic protozoa in starved termites seems to be a function of temperature. The lower the temperature falls the longer the protozoa may live, in accordance with the slowness of the metabolic process.

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