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Studies on Microsporidia Parasitic in Mosquitoes.

III. On *Thelohania legeri* HESSE (= *Th. illinoisensis* KUDO).¹⁾

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(With 1 textfigure and Plate 7)

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Introduction.

In former papers, the writer (1921a, 1922) showed that Anopheline mosquito larvae of North America were subject to an infection by a microsporidian. At the time of the brief description of this species, the material on hand was scant for a thorough study

¹⁾ Contributions from the Zoological Laboratory of the University of Illinois. No. 241.

and the writer failed to carry out any satisfactory observations upon its phases of development. It manifested a close relationship to *Thelohania legeri* which had been found and described by HESSE (1904, 1904a). A mere comparison of the preparations with HESSE's description of his form, led the writer to name the American species, *Th. illinoisensis* and to state that „except for the irregularity and slight difference in the dimensions of the spores and the difference of the host species, the two species would be distinguished from each other only with difficulty“.

In the summer of 1922, the writer collected some more material in New York from the vicinity of the place where 1920 collection had been made, which proved to be well fit for the present study. In addition a number of infected Anopheline larvae from Southern United States were added in 1922 and 1923 to the collection through the kindness of Doctor M. B. BARBER, Special Expert, United States Public Health Service.

During the course of the study, a careful comparison of *Th. legeri* and *Th. illinoisensis*, both in permanent smears, has been made, which leads the writer to conclude that the difference between them, aside from that of the host species, is too small to maintain them as two distinct species.

The study revealed that a peculiar fusion of two nuclei occurs at the end of schizogony, that the nucleus of the sporont is formed by this union, that a mitotic division takes place in the stages of sporogony and that the microsporidian is specific to the genus of host larvae which relation is similar to that which exists between adult Anopheline mosquitoes and the malarial organisms. The present paper is intended to bring forth these findings of interesting character and further to discuss the general situation with respect to the microsporidian infection in Anopheline mosquitoes.

Material and methods.

The material used in the present study are as follows:

1. Fresh and stained smears of the two larvae of *Anopheles punctipennis* which were collected in Illinois, 1919 (September).
2. Fresh and stained smears of two larvae and sections of one larva of *A. quadrimaculatus* which were collected in New York, 1920 (August).
3. Smears and sections of five larvae of *A. crucians* which were collected in Georgia, 1921 and Alabama, 1922 (February).

4. Smears and sections of nine larvae of *A. quadrimaculatus* which were collected in New York, 1922 (August, September).

5. Smears and sections of six larvae of *A. punctipennis* (?) which were collected in Louisiana, 1923 (February)¹).

6. Smears and sections of numerous larvae, pupae and adults of *A. quadrimaculatus* which were collected from the place where materials listed under 2 and 4 were collected.

7. HESSE's smears²):

a) One smear of a larva of *A. maculipennis*, 1902.

b) Four smears of larvae of *A. bifurcatus*, 1918 (April, October).

c) One smear of a larva of *A. bifurcatus*, 1919 (September).

The methods of fixation and staining were same as the writer had used in his previous studies. SCHAUDINN'S and BOUIN'S fluids were employed exclusively with excellent results, while HEIDENHAIN'S iron haematoxylin and GIEMSA'S stain were used as before with satisfactory results. The polar filament was easily studied by the method which the writer described elsewhere (KUDO, 1921).

Schizogony.

The youngest stage was found in a lightly infected adipose tissue cell. It is an oval or rounded body with compact chromatic granules in it. At present the changes that take place between liberated amoebula stage and this stage are unknown to the writer. The compact chromatic grains may be composed of one large and four or five minute granules scattered in the cytoplasm (Fig. 2) or five or six equally large granules clustered together to form a mass (Fig. 3).

These compact grains transform into a vesicular nucleus which possesses now a distinct membrane with apparently large amount of nuclear sap through which achromatic network with attached chromatin grains penetrates (Figs. 4, 5). With the growth of the cytoplasmic body, the nucleus undergoes a division. Although the writer has seen stages shown in Figs. 6 and 7 which one may interpret as prophase in the division, no other succeeding stages

¹) The writer is greatly indebted to Dr. BARBER who not only supplied the author excellently preserved material on several occasions, but also informed him his personal experience and view as to the effect of the parasite upon the host larvae.

²) The writer is under obligation to Dr. EDMOND HESSE of the University of Grenoble, France, for six beautifully prepared smears of *Thelohania legeri*, one of which (1902) was taken as the type specimen by the writer.

suggestive of possible promitotic division were found. Occasionally one finds a small deeply staining grain or two located near the dividing nuclei, but its nature is not known. It seems most probable that the entire achromatic substance assumes an irregularly coiled thread with attached chromatin granules (Figs. 6 and 7). The cytoplasmic body becomes elongated in the direction of the nuclear division, while the thread becomes separated into two groups or parts which at first are connected with each other by a rather thick strand, but which later become independent. The schizont now divides into two daughter cells, each containing a single nucleus. These young schizonts grow and repeat the divisions, thus increasing their number in the host cell. This binary fission seems to continue while there is a large space left in the host cell which is usually the case at the beginning of the infection.

Some of the schizonts which contain two daughter nuclei remain without being followed by a complete cytoplasmic division and grow in size. In the mean time, each of the two nuclei undergoes a division (Figs. 12 to 18). The division of these nuclei takes place almost always simultaneously so that when it is completed, a tetranucleated large and elongated schizont is produced, the two daughter nuclei in the opposite portions remaining in pairs (Fig. 12 to 18). These stages are found chiefly in the periphery of an adipose tissue cell of the host larva, the central portion of which is filled with the stages of sporogony. This body with four nuclei arranged in two pairs, divides into two schizonts, each containing the paired nuclei (Figs. 19 to 21). These binucleate schizonts are in reality what one may term sporont mother-cells, since they seem to undergo a peculiar division once and form binucleate cells. Through the fusion of the nuclei, each cell transforms into a sporont. The nuclei of such a cell lose their vesicular appearance and assume a compact form in which one may occasionally distinguish chromatin and achromatic portions (Figs. 21). At the beginning of this peculiar nuclear division there may be seen two deeply stained granules on the opposite ends (Fig. 22). The nucleus divides into two parts which remain connected with a single strand even after they are separated widely from each other. The division of the two nuclei is always simultaneous so that one sees stages such as shown in Figs. 24 to 28, typical figures in these stages. The connecting strands of the nuclei cross each other in many cases. Occasionally there are seen two minute chromatic granules present

near the crossing point of the strands. Finally the strands become absorbed in the cytoplasmic network (Figs. 27) and disappear. The body now divides into two portions by a constriction through the center, each half possessing two nuclei, derived from two different nuclei and not two daughter nuclei (Figs. 22 to 28 and textfigure). These nuclei are characterized by the possession of a conspicuous oval chromatin grain usually located excentrically in the achromatic network.

Throughout the various stages of schizogonic divisions, the cytoplasm of the schizonts is free from any inclosures and is coarsely vacuolated. In this respect it differs greatly from that of sporogonic stages which will be described below.

The schizogony described above differs considerably from that which was observed by HESSE (1904a) who described it as follows: Les mérontes sont des corps arrondis 3 à 4 μ de diamètre à cytoplasme fortement colorable, à noyau assez petit, formé d'un amas de grains chromatiques entouré par une zone claire. La méronte grossit et peut atteindre jusqu'à 6 μ de diamètre (fig. 1). Comme chez *Th. mülleri* L. PFEIFFER d'après STEMPELL, les mérontes se divisent par un étranglement transversal du cytoplasme précédé de la division directe du noyau (fig. 2). On observe parfois des chaînes de trois mérontes qui se séparent ensuite. La division du noyau n'entraîne pas toujours immédiatement celle du cytoplasme." In view of HESSE's statement that „on trouve surtout des spores mûres, on rencontre néanmoins d'autres stades qui permettent de reconstituer les traits principaux du développement du parasite“, apparently late stages in schizogony where binucleate forms appear were not present in his material. In fact in HESSE smears which the writer examined, he failed to make out clearly the stages just mentioned.

The schizogony under consideration is greatly different from those of other *Thelohantias* which the writer studied up to the present. For instance in *Th. magna*, besides a binary fission, a schizont seemed to divide into eight daughter cells by repeated nuclear divisions. A multinucleate elongated (sausage) forms have been reported to occur in *Th. mülleri* (STEMPELL, 1902), *Th. chaetogastris* (SCHRÖDER, 1909) etc. Repeated binary fission and the peculiar divisions of binucleate schizonts are characteristics of the present species. Somewhat similar changes of binucleate schizonts prior to the production of sporonts seemed to have been observed by DEBAISIEUX and GASTALDI (1919) in *Plistophora simulii*.

As to the nuclear divisions during schizogony of different species of Microsporidia, a controversy still prevails. Due to the extreme minuteness of the nucleus of most of the schizonts, authors maintain that the nuclear division is amitotic. PÉREZ (1905), however, observed in the dividing nucleus of the schizont of *Th. maenadis*, the appearance of eight Y-shaped masses of chromatic substance which after passing through spireme stage, became divided into two groups of chromosomes. In *Th. giardi*, MÉRCIER (1909) saw what one may interpret as an anaphase in the nuclear division of the corresponding stage.

In the present species, the writer's observations failed to determine its nature clearly, but the nuclear division is not of as simple a type as was observed by him in *Nosema bombycis* (1916) or *N. baetis* (1921 a). The entire nuclear substances seem to undergo a sort of thread formation (Figs. 6, 7) which results in division into two groups (Figs. 8 to 10). No achromatic spindle which is plainly visible in the nuclear divisions of the sporonts, was distinctly recognizable in schizogony. The nuclear changes stated above resemble closely to those of *Th. magna* (KUDO, 1921 a).

Sporogony.

As was stated before, at the end of schizogony the final stage is an enlarged binucleate form. Each nucleus shows a conspicuous chromatic body, compact in its appearance and located usually eccentrically (Fig. 29). The cytoplasm is vacuolated, but free from any inclusions.

Following this stage, the nuclei exhibit a remarkable change and activity. They become large by a considerable increase in the nuclear sap, and come to lie side by side (Fig. 30). In the meantime the chromatin substances become strikingly conspicuous and spread over the achromatic network (Fig. 30), and the wall between the two nuclei disappears. The nuclear substances of the nuclei then fuse completely into one mass (Figs. 31, 32), while a large number of chromatin granules of variable size appear in the cytoplasm (Figs. 31, 32). These extranuclear granules apparently are thrown out by the nuclei during the process of fusion. The resulting form has a large nucleus with chromatin grains attached to the achromatin network and to the nuclear membrane and a cytoplasm in which chromatin granules of variable size and number are imbedded. This is the sporont, from which by later nuclear

and cytoplasmic divisions eight sporoblasts and further eight spores are formed.

HESSE (1904 a) described the sporont of the present species as follows: „Les sporontes au début sont des corps ovalaires, sans paroi différenciée, mesurant de 9 à 10 μ de grand axe sur 4 à 6 de petit axe. Ils possèdent un cytoplasme beaucoup plus clair que celui des mérontes, un gros noyau à membrane colorable contenant, outre de petits grains de chromatine disposés sur un réseau, un karyosome complexe, formé de quatre amas chromatiques (fig. 3).“ Thus he has not noticed the changes described above.

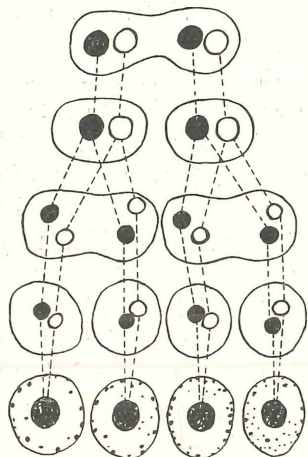
Whether or not a karyogamy as in the case of *Th. giardi* (MERCIER, 1909) or an autogamy as in the cases of Simulid Microsporidia (DEBAISEUX, 1919 and DEBAISEUX and GASTALDI, 1919) and of *Glugea mülleri* and *G. danilewskyi* (DEBAISEUX, 1919 a) exists in other Microsporidia has not been thoroughly studied. Up to the present time, the writer has never observed any stages which may justly be reasoned as belonging to such sexual unions between two elements in the various species of Microsporidia which were brought under his observation. In *Th. magna* studied in 1919, the writer (1921 a) saw binucleate forms among the schizonts, but was led to “consider them as stages of division instead of fusion of two forms“, because of the conditions under which they existed.

In the present species, the writer found by tracing the schizogonic divisions that apparently the last stage in the division is a binucleate body with clear cytoplasm such as shown in Fig. 29. That this stage is not formed by the union of two isogametes as is the case in *Th. giardi* according to MERCIER (1909), seemed to be clear by the existence of the stages shown in Figs. 19 to 28 and further because of the absence of uninucleate bodies in that part of the host cell where stages shown in Figs. 19 to 51 were present. In fact the uninucleate schizonts (Figs. 3 to 6) were only found in host cells where schizogonic and sporogonic divisions had not yet commenced. Hence the writer is inclined to believe that this stage (Fig. 29) is not formed by a union of two uninucleate cells.

Similar binucleate bodies observed in *Th. varians*, *Th. multispora*, *Th. fibrata* and *Th. bracteata* and named by DEBAISEUX (1919) as „diplocarya autogamique“ contain „deux noyaux accolés intimement et provenus de la division d'un même parent“.

In *Th. legeri*, the two nuclei which undergo fusion to form the nucleus of the sporont are not two daughter nuclei resulting from a division of a nucleus, but the „cousin“ nuclei as will readily be

understood by glancing over the textfigure. In *Plistophora similii*, DEBAISEUX and GASTALDI (1919) show stages (their figures 73 to 78) in the formation of the binucleate forms which transform into sporonts, but the nuclei of which are „cousin“ nuclei and not the two daughter nuclei. In this connection, the authors state, without emphasising the nature of the nuclei, that „il faut considérer comme telles les divisions d'aspect très particulier, Figs 66 to 77, dans lesquelles on observe deux divisions bien distinctes évoluent parallèlement“.



A

Textfigure.

Diagram showing the nuclear changes in late schizogony and early sporogony.

B

A, tetranucleate stage. Compare with Figs. 18, 19.

C

B, Binucleate form, sporont mother cells. Compare with Figs. 20, 21.

D

C, Dividing forms. Compare with Figs. 22—28.

D, The final form with two „cousin“ nuclei. Compare with Fig. 29.

E

E, Sporonts where the fusion of the cousin nuclei has taken place. Compare with Figs. 31—33.

Therefore one sees the nuclear union of the present species as described above is a peculiar one and not an ordinary autogamy in its strict sense, because the fusing nuclei are not daughter nuclei, but cousin nuclei.

DEBAISEUX (1919) observed the expulsion of small chromatin granules from the nuclei prior to the fusion and stated as follows: „Ils montrent souvent à l'état d'ébauche une division suivant le grand axe de l'appareil nucléaire double; l'un des granules est expulsé dans le protoplasme et disparaît complètement... Les noyaux du diplocaryon sont encore le siège d'un second phénomène; la substance chromatique de chaque noyau, réduite maintenant à une seule masse centrale, s'étire dans le sens de la largeur du noyau, c'est-à-dire parallèlement à la surface d'accolement des deux noyaux. Finalement la masse chromatique allongée se scinde et on trouve dans chaque noyau deux granules, dont l'un plus petit. Ce petit granule est expulsé du noyau principal, entraînant avec lui une portion du réseau achromatique. Les phénomène se passe simultanément dans les deux noyaux, et symétriquement; aussi à certain moment trouve-t-

on deux petits noyaux complets enclavés dans l'angle d'accolement des deux grands. Les deux petits noyaux dégénèrent, ils se réduisent de plus en plus, jusqu'à ne plus entre finalement que deux granules chromatiques, qui eux-mêmes disparaissent."

In *Th. legeri*, no such regular expulsion of chromatin granules was noticed by the writer, although as was stated above, one sees often small grains during the nuclear division of the sporont mother-cells (Figs. 25, 27).

The appearance of chromatin granules in the sporogonic stages similar to the present species seems to have been noted by MERCIER (1909) in *Th. giardi*. In this *Thelohania*, MERCIER observed: „Des granulations chromatiques, véritables chromidies, se détachent du syncarion, se répartissent dans le cytoplasme et finalement viennent se rassembler sous la membrane d'enveloppe du sporonte. Lorsque l'émission des chromidies est terminée, l'appareil nucléaire prend un aspect tout à fait caractéristique; les grains chromatiques, après s'être fusionnées, se disposent de façon à former une étoile dont les branches rappellent assez des chromosomes."

DEBAISIEUX (1919) also observed a similar phenomenon in *Th. varians* and stated that „au moment où la fusion des deux noyaux s'achève, parfois même plus tôt, on voit apparaître dans le protoplasme d'abondants granules chromatiques“.

The nucleus of the sporont after passing through a short resting stage, undergoes a division. The chromatin forms a spireme (Figs. 34 to 36) which at first is rather fine and closely convoluted (Figs. 35, 36), but later thickens and shortens (Figs. 37 to 39). At this latter stage one can see clearly that the thread is single. The nuclear membrane becomes less conspicuous and entire nucleus assumes an elongated shape, the axis coinciding with the general direction of the spireme (Figs. 37, 38). The spireme breaks to form rounded or oblong chromatic granules which may be called chromosomes (Figs. 40, 41). Whether these chromosomes are transversely broken threads or not, cannot be determined due to the minuteness of the object, but it is possibly formed by the former process.

The achromatic network becomes stretched in the direction of the nuclear division and forms a sort of a spindle (Figs. 38 to 41), at the equatorial part of which the chromosomes become located. The number of chromosomes seemed to vary: in one stage, six were seen (Fig. 42), in another over ten were counted (Fig. 43), but in the anaphase, the writer thought that he saw fairly regularly eight chromosomes — two groups of four moving toward the opposite ends

(Figs. 44, 45). The writer is unable to determine whether a typical metaphase exists here or not. The two groups of the chromosomes finally reach the opposite ends (Figs. 46, 49 to 51) and the two daughter nuclei are reconstructed (Figs. 52 to 53). In some instances, one sees a deeply staining centrosome-like body at each end of the dividing nucleus (Fig. 40, 41, 43, 47).

A similar nuclear division of the sporont was described by MERCIER (1909) for *Th. giardi*, who stated that „Cette division nucléaire s'effectue suivant un de ces modes spéciaux aux Protozoaires et intermédiaires entre la division directe et la division indirecte. La figure 18, en particulier, rappelle assez le mécanisme d'une mitose typique“. But the figures given by this author do not lead one to agree with MERCIER to maintain that the nuclear division is a typical mitosis.

The nuclei thus formed have an appearance as shown in Fig. 54. Each possesses a large chromatin mass, usually located excentrically, from which radiating achromatic threads reach the nuclear membrane, to which smaller chromatin granules are attached. The resting period of these nuclei is very short, and soon the second division takes place.

This division is somewhat different from the first one in the following respects: the achromatic substance forms a longitudinal axis, a small deeply staining granule is located at each end of the axis (Figs. 55 to 57), the chromatin substance becomes condensed into two compact masses which lie on the sides of the axis at its middle part (Figs. 55 to 58). These chromatin masses are drawn to the opposite ends quickly (Figs. 59 to 61). The connecting strand of the two daughter nuclei seems to be single in most cases, but in some instances two (Figs. 59, 60). The division of the nuclei is synchronous so that when it is completed, stages such as shown in Fig. 62, are reached.

The third division which is in the main similar to the last follows quickly and the sporont now reaches a stage which contains eight compact nuclei (Figs. 63 to 66). Each of these nuclei becomes surrounded by an island of cytoplasm and transforms into a sporoblast.

During these three successive nuclear divisions, the extra-nuclear chromatin granules seem to remain without any visible changes, although in a few cases union of some of the granules seemed to take place (Fig. 56).

As to the development of the sporoblast into a spore, the writer

failed to make satisfactory observations due mainly to the size of the object and also to the crowded conditions under which the sporulating individuals were present in the host cells. The changes, however, seemed to take place in a manner similar to those observed in *Th. magna*, a much larger form (KUDO, 1921 a).

The writer maintains that the significance of the extranuclear chromatin granules is two-fold. In the first place, these granules are extruded from the nuclei at the time of fusion in order possibly to get rid of excess amount of chromatin substance, and secondly these granules probably change into substances that compose the spore membrane, since they gradually disappear as the spore formation progresses, and since the writer has recently noticed an analogous change in a new myxosporidian, *Myxosoma catostomi*.

The identity of *Thelohania legeri* and *Th. illinoisensis*.

As was stated before, when the writer first described *Th. illinoisensis*, he emphasised a close similarity between it and *Th. legeri* (KUDO, 1921a). The study of the scant material which he had at hand, brought out a slight difference in the dimensions of the spores of the two forms. The difference of host species leads frequently to a specific difference between apparently closely related Microsporidia. Therefore, on the basis of these two characters, I gave the American form, the name, *Th. illinoisensis*.

Further collection of infected host larvae in 1920, 1922 and 1923, revealed the fact that the microsporidian is not specific to the species of the host mosquito, but to the genus, since the parasite was found in the larvae of *Anopheles punctipennis*, *A. quadrimaculatus* and *A. crucians* from the different parts of North America and further according to Dr. HESSE's smears, of *A. maculipennis* and *A. bifurcatus* of France.

A series of careful comparison of a large number of spores of HESSE's and the writer's forms in smears, showed that the dimensions of the spores of these two forms vary within the same range. The fact that the developmental stages described in the preceding chapters were absent in HESSE's preparations, is of little importance in this respect, because the later stages of sporogony of the two forms are identical with each other.

Therefore, the writer considers that *Thelohania illinoisensis* KUDO 1921 is identical with *Thelohania legeri* HESSE 1904 and holds it as a synonym to the latter name.

The relation between the parasite and the host larvae.

Concerning the occurrence of *Thelohania legeri* in the larvae of *Anopheles maculipennis* and the relation between the microsporidian and the host larvae, HESSE (1904) states as follows: „Le parasite est rare; je l'ai rencontré seulement deux fois sur quarante larves déjà grosses recueillies dans les marais de la région littorale entre Cavalière et Saint-Tropez. Il envahit le corps gras, l'intestin restant indemne. Je n'ai pas recherché cette Microsporidie chez les *Anopheles* adultes, mais il ne me paraît pas douteux qu'elle y parvienne, car les larves infestées ne semblaient nullement souffrir de sa présence.“

The writer has not seen any paper by HESSE regarding his findings of some larvae of *A. bifurcatus* infected by the present microsporidian in 1918 and 1919.

With respect to some diseased larvae of *A. crucians* which were found in Georgia and majority of which seemed to have been parasitised by this protozoon, Dr. BARBER informed the writer in one of his letters as follows: „I do not think there was a month from January to November when we did not find this parasite on Anopheline larvae. ‚Doughbelly‘ was our nickname for it; you readily understand the applicability of the name. None of the several sets of large larvae — one possible exception (?) — would pupate, but the life of the infected larvae was usually fairly long in the laboratory But our chief reason for thinking that this disease is of very limited value as anti-Anopheline measure was the fact that our breeding places which were well infected with the parasite, continued to produce Anophelines abundantly, month after month.“ From these observations it would appear that *Thelohania* infection among the larvae of *Anopheles maculipennis* of France and *A. crucians* of Southern United States does not bring about any serious effects among the host larvae.

On the other hand, the writer's observations upon the larvae of *A. quadrimaculatus* are entirely different from those of others quoted above. One of the three larvae infected by the parasite which he observed in 1920 „was so strikingly opaque in color and inactive that its diseased condition could be diagnosed as due to some microsporidian parasites while it was still in the water. It died in about thirty minutes after capture“ (KUDO, 1922). Further findings in 1922 tend to confirm this observation. In that year nine infected larvae of the same host species were obtained and

studied from August 23 to September 1. All showed the typical symptoms of the infection and none pupated. The data at hand are still too meager to allow the writer to make any generalized statement as to the results of the infection, but lead him to believe that the infection is detrimental to the host larvae.

The fact that although the writer have studied in both fresh and stained smears and sections several hundreds of pupae and adults of both sexes of this particular *Anopheles* species which either metamorphosed in the rearing jars or were collected from the naturally infected place, yet so far he has failed to find any unmistakable cases of microsporidian infection in them, and further that GRASSI'S and ROSS' cases¹⁾ seem to be the only records where adult mosquitoes were found to harbor what these authors maintained the microsporidian parasites, seems to indicate that the infection has fatal effect upon the host larvae in general.²⁾

How does the infection of a new host larva take place? At present moment, the writer presumes that the larva becomes infected by devouring fresh spores of the microsporidian per os at certain stages of its development.

It is well known fact that Anopheline mosquito larvae feed ordinarily on the surface of the water in which they live. The food consists in the main of minute organisms which are held by the surface film. NUTTALL and SHIPLEY (1901) state that they „have on several occasions observed larvae devouring their dead fellows“, and according to their quotation, GRAY (1900) noted that Anopheline larvae fed „on the dead bodies of drowned mosquitoes“. The writer's observations also confirm that Anopheline larvae feed upon the vegetable as well as animal matters that float on the surface of the water.

As was noted in many species of Microsporidia, the present form is specific in its habitat, invading only the adipose tissue cells. When heavily infected, the fatty tissue cell may rupture and the parasites become liberated in the general body cavity of the host; but all other tissues remain free from the infection. The muscle cells while not infected become degenerated so that the infected larvae are exceedingly weak. The extended fatty tissue cells which contain hypertrophied nuclei, give rise to the opacity and deformity

¹⁾ The statements of these authors were quoted elsewhere (KUDO, 1922).

²⁾ STRICKLAND'S (1913) observations regarding the relation between the microsporidian parasites and *Simulium* larvae agree with the above.

of the host body. And a slight external pressure frequently results in the disruption of the body wall through which the parasitic masses emerge into the surrounding water. The liberated mature spores will undoubtedly gain easy entrance to the alimentary canal of the larvae present and feeding nearby.

The fact that the Anopheline larvae live in the running water and are surface feeders appear to explain the less frequent occurrence of the infection among them by *Thelohania legeri* than that in *Culex* larvae by *Th. magna* or *Th. opacita*¹⁾ which live ordinarily in still and stagnant water and feed mostly at the bottom.

In this connection, it is interesting to note that the microsporidian parasites of mosquito larvae are specific to the genus of the host. *Th. legeri* is an exclusive parasite of the genus *Anopheles*, having been found in the larvae of *A. maculipennis*, *A. bifurcatus* (France), *A. punctipennis*, *A. quadrimaculatus* and *A. crucians* (U. S. A.). On the other hand, *Thelohania magna* and *Th. opacita* seem to confine themselves to the mosquito larvae of the genus *Culex*. Thus the former was seen in the larvae of *C. pipiens* and *C. territans* (U. S. A.), while the latter was found to parasitise larvae of *C. testaceus* and *C. territans* (U. S. A.).²⁾

Thus here one sees a generic specificity between the microsporidian parasites and the host mosquito larvae somewhat analogous to that which exists and has been known for many years without satisfactory solution of the phenomenon, between malarial parasites of man and birds and the adult mosquitoes. The causes — whether physiological or anatomical — which bring forth this specificity remain to be determined in the future.

Summary.

1. *Thelohania illinoisensis* KUDO 1921 is identical with *Th. legeri* HESSE 1904.

2. The schizogony is repeated binary fission. At the end of the fission, binucleate schizonts are produced from which tetranucleate forms result by the nuclear division. The tetranucleate form breaks into two sporont mother cells. These further divide into

¹⁾ These Microsporidia will be the subjects of separate papers.

²⁾ NÖLLER (1920) saw sporogonic stages of a *Thelohania* in a larva of *Aedes nemorosus* from Wohldorf bei Hamburg and stated that „wahrscheinlich aber handelt es sich um *Thelohania legeri*“ which view does not appear to be well founded.

two as the nuclei undergo division. Thus formed binucleate cell contains two „cousin“ nuclei.

3. These two nuclei undergo fusion, forming the nucleus of the sporont. At the same time, fine chromatic granules appear in the cytoplasm. They are probably extruded from the nuclei, in order to get rid of excess amount of chromatin material and later seem to take part in the formation of spore membrane.

4. The first nuclear division of the sporont is mitotic. At the anaphase, eight chromosomes are observed.

5. In the second nuclear division of the sporont, distinctly visible, deeply staining centrosome-like bodies are noticed at the opposite ends of the dividing nucleus.

6. After the third division, eight sporoblasts are formed inside a sporont, each of which transforms into a spore.

7. As far as the observation up to date is concerned, *Thelohania legeri* seems to be an exclusive parasite of Anopheline mosquito larvae which relation is analogous to that which exists between adult Anopheline mosquitoes and the malarial parasites of man.

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Explanation of Plate 7.

All the figures relate to *Thelohania legeri*. Zeiß compensation oculars $\times 15$ and $\times 20$ and homogeneous oil immersion objective 90 were used. Figs. 2—5, 7—11, 13—17, 19—66 and 71—74 were observed in section preparations stained with HEIDENHAIN'S iron haematoxylin, while Figs. 6, 12, 18 and 67—79 were seen in smears, stained or fresh. Magnification: Fig. 1, $\times 15$; Figs. 67—70, $\times 2200$; and the rest $\times 3200$.

Fig. 1. A moderately infected larva of *Anopheles crucians*, showing several parasitic masses contained in the adipose tissue.

Figs. 2—4. Young schizonts from lightly infected adipose cells.

Figs. 5—20. Early stages in schizogony.

Figs. 21—29. Further schizogonic stages leading to the formation of sporonts.

Figs. 30—33. Formation of sporont through the fusion of the nuclei.

Figs. 34—54. Stages in the first nuclear division of the sporont.

Figs. 34—38, prophase; Figs. 39—42, metaphase; Figs. 43—51, anaphase;

Figs. 52—54, telophase.

Figs. 55—62. Stages in the second nuclear division of the sporont.

Figs. 63—66. Stages in the last nuclear division of sporont which now contains eight sporoblast nuclei.

Figs. 67—70. Preserved unstained spores studied in water.

Figs. 71—74. Spores stained with HEIDENHAIN'S method and differentiated to different degrees.





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