

MICROSPORIDIA AND LARVAL CONTROL¹

WILLIAM R. KELLEN

Bureau of Vector Control, State Department of Public Health, Fresno, California

Microsporidia are a highly specialized and poorly understood group of Protozoa, and although they are common parasites of mosquitoes, they are not very well known to most individuals engaged in mosquito control operations; therefore, a few introductory remarks on the general relationships of the microorganisms will be presented.

Microsporidia are single-celled animals belonging to the Class Sporozoa and are closely related to the more widely known group of Protozoa responsible for malaria. Unlike parasites of malaria, however, Microsporidia require only a single host to complete their life cycle and they do not infect warm-blooded animals. About 90 percent of the known species of Microsporidia parasitize invertebrates, while the remainder have been reported infecting cold-blooded vertebrates. Like all members of the Sporozoa, Microsporidia have two distinct phases in their life cycle: an asexual or schizogonic and a sexual or sporogonic phase. The significance of the two phases will become evident when mosquito-parasite relationships of some Microsporidia are presented below.

The typical life cycle of a microsporidian may be exemplified by members of the genus *Thelohania*. The resting stage of the life cycle is spent as a resistant spore which is deposited in the soil or water frequented by the host. Members of the genus *Thelohania* are characterized by the formation of groups of eight spores (Figures 1, 2, 3); however, such groups of spores may dissociate as they mature and infected hosts succumb to the disease and decompose. Spores may accumulate in

the habitat and subsequently may be ingested by suitable hosts.

The process of initiating new infections via ingestion of resting spores is poorly understood; however, it is usually suggested that spores normally germinate in the intestinal tract of a host, releasing mononucleate amoeboid "planonts" which penetrate the gut wall and migrate to suitable tissues. During the process of germination, spores of all Microsporidia characteristically extrude a hollow polar filament. The infective "planont" escapes the spore by passing through the filament. Once a "planont" arrives at a suitable tissue, it transforms into a mononucleate schizont, which is immotile, and thus enters the schizogonic phase of its life cycle.

Schizonts multiply rapidly and serve to increase the number of parasites attacking a host. After a given period of multiplication, however, some schizonts may undergo nuclear reorganization and transform into sporonts. The sporogonic phase of the life cycle is a dead-end process and leads only to the formation of spores, which, when ingested, germinate in a new host and repeat the cycle.

The life cycle of Microsporidia apparently is relatively simple; however, the simplicity of both the life cycle and the morphology of the microorganisms has made it difficult for specialists to identify and classify the parasites. Protozoologists have, however, divided the Microsporidia into several major families based on the morphology of spores. The largest family, and the one to which most of the known parasites of mosquitoes belong, is the Nosematidae; it is characterized as having spores which are more or less oval.

The seven genera belonging to the family Nosematidae are characterized by the number of spores formed during the sporogonic phase of the life cycle: the genus

¹This research was supported in part by Research Grant No. E-3496 from the National Institute of Allergy and Infectious Diseases of the National Institute of Health, Public Health Service.

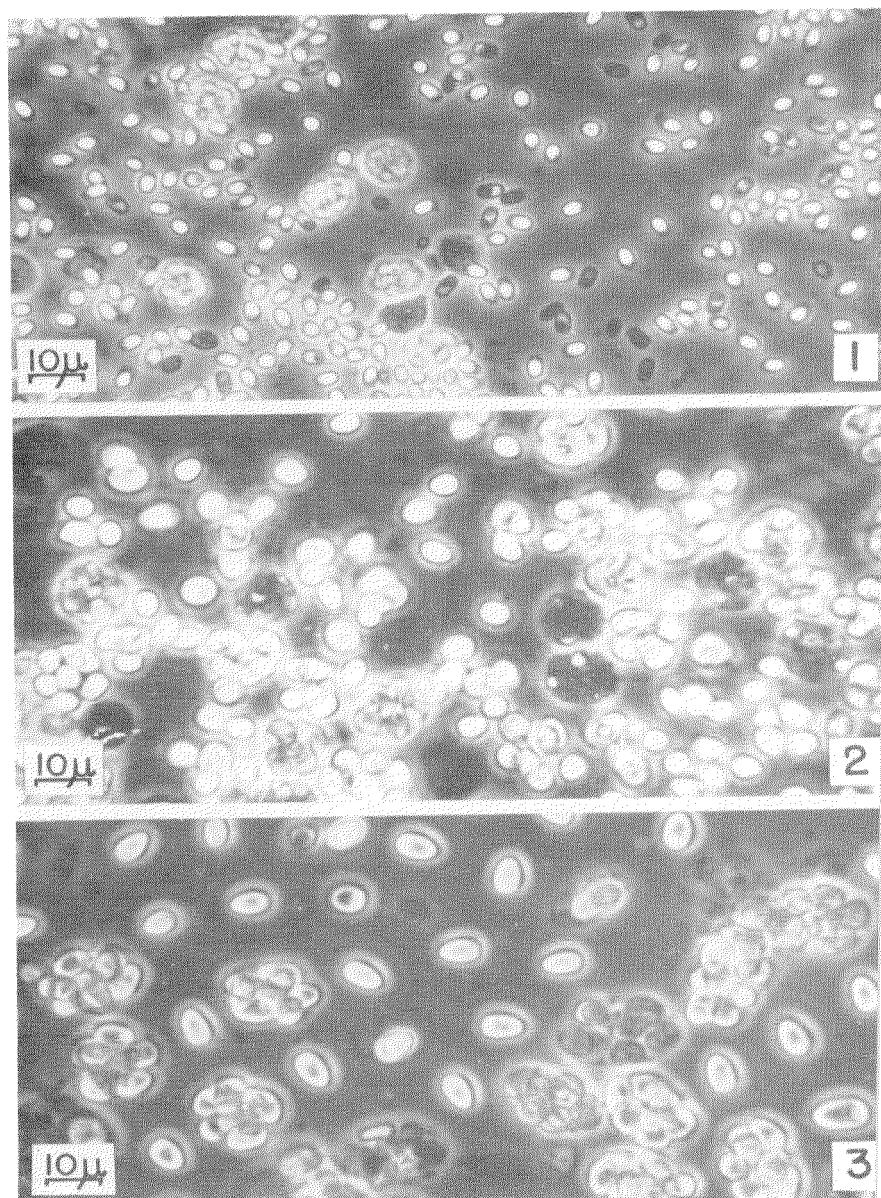


FIG. 1.—Living spores of *Thelohania periculosa* from *Anopheles p. franciscanus*. FIG. 2.—*T. melanimon* from *Aedes melanimon*. FIG. 3.—*T. californica* from *Culex tarsalis*.

Nosema has sporonts which give rise to only a single spore; the genus *Glugea* forms two spores, *Gurleya* four spores, *Thelohania* eight spores, *Duboscqia* sixteen spores, and *Plistophora* more than sixteen spores. Finally, members of the genus *Stempellia* may form a variable number of spores, but never more than eight.

Of the nosematids known to parasitize mosquitoes, members of the genus *Thelohania* are by far the most common. Nineteen species of *Thelohania* have been reported from 29 different species of mosquitoes from various parts of the world. In contrast to this, there have been only five species of *Nosema* described from mosquitoes, one species of *Stempellia* and three species of *Plistophora*. Although his fairly large number of nosematids has been described parasitizing mosquitoes, very little is known regarding geographical distribution, seasonal incidence, or host-parasite relationships.

When studies of mosquito pathogens were initiated in California about three years ago there were no described species of Microsporidia from mosquitoes known to occur in the state. It is now recognized, however, that such parasites occur commonly in Californian mosquitoes, and it has been further concluded that probably all species of mosquitoes are hosts to one or more species of Microsporidia.

Table 1 lists all of the *Thelohania* which are now recognized from Californian mosquitoes; two new species of the genus *Nosema* are also indicated. These new nosematids were originally isolated from larvae, but information is now available which suggests that they probably occur in adult hosts as well. Some data are also presented in Table 1 which indicate in broad terms the levels of infection which were observed in natural populations of larvae. These levels of infection were estimated from collections made at irregular intervals and are not intended to present seasonal averages.

Data concerning population studies and seasonal incidences of infection for certain species of mosquitoes are still very incom-

TABLE 1.—Synopsis of data on Microsporidia reported from mosquitoes in California

Parasite	Host
<i>Thelohania benigna</i> K. and W.	<i>Culex apicalis</i> Adams
	In hemocoel; causes minute localized areas of infection; 1-3% apparent larval infections.
<i>T. bolinasae</i> K. and W.	<i>Aedes squamiger</i> (Coq.)
	In hemocoel; causes mottled appearance; 1-5% apparent larval infections.
<i>T. californica</i> K. and L.	<i>Culex tarsalis</i> Coq.
	In hemocoel; larvae become swollen and white; about 5-25% apparent infections.
<i>T. campbelli</i> K. and W.	<i>Culiseta incidens</i> (Thomson)
	In hemocoel; causes swollen grayish-white appearance; usually less than 1%, but up to 80% apparent larval infections observed.
<i>T. gigantea</i> K. and W.	<i>Culex erythrorhoxus</i> Dyar
	In hemocoel; larvae appear opaque, white and swollen; 1-5% apparent infections.
<i>T. inimica</i> K. and W.	<i>Culiseta inornata</i> (Will.)
	In hemocoel; causes swollen grayish-white appearance; most larval populations with 1-5% apparent infections.
<i>T. noxia</i> K. and W.	<i>Culex thriambus</i> Dyar
	In hemocoel; causes dull gray coloration ventrally; 1-3% apparent larval infections observed.
<i>T. periculosa</i> K. and W.	<i>Anopheles pseudopunctipennis franciscanus</i> McCracken
	In hemocoel; causes white ventral coloration of infected areas; usually 1-10% apparent natural infections.
<i>T. unica</i> K. and W.	<i>Aedes melanimon</i> Dyar
	In hemocoel; causes grayish-white ventral surface; less than 1% apparent infection observed.
<i>T. sp.</i>	<i>Culex apicalis</i> Adams
	In hemocoel; causes enlarged massive infections; 5-10% apparent infections in some populations.
<i>T. sp.</i>	<i>Culex tarsalis</i> Coq.
	Similar to <i>T. californica</i> , but forms smaller spores; levels of natural infections unknown.
<i>T. sp.</i>	<i>Culex peus</i> Speiser
	In hemocoel; causes grayish-white appearance; most larvae populations with low levels of apparent infection.
<i>T. sp.</i>	<i>C. peus</i> Speiser
	In hemocoel; causes localized infections and enlargement of single abdominal segments; levels of natural infections unknown.
<i>Nosema sp.</i>	<i>Culex tarsalis</i> Coq.
	In hemocoel and oenocytes of larvae and adults; percentage of infections unknown.
<i>Nosema sp.</i>	<i>Anopheles pseudopunctipennis franciscanus</i> McCracken
	In hemocoel and oenocytes (?) of larvae and adults; percentage of infections unknown.

plete. Indications are, however, that levels of infection may vary considerably in different localities even for the same species of host and parasite. Furthermore, some species of mosquitoes evidently experience much higher levels of infection than other species, or at least manifest their infections to a larger degree. For example, natural larval populations of *Culiseta incidens* now under study in California have been observed with levels of infection with *Thelohania campbelli* of over 80 percent; however, such populations were not typical. Studies are in progress to discover factors which influence such high levels of infection.

In contrast with *C. incidens*, larval populations of *Culex tarsalis* observed in the San Joaquin Valley of California usually had levels of infection of *Thelohania californica* ranging from about 5 to 15 percent. Most other mosquito species observed had relatively low levels of infection ranging from about 1 to 5 percent.

The data on levels of infection given in Table 1 were obtained by sampling larval populations and counting the number of individuals with apparent infections. Larvae with patent infections of *Thelohania* could usually be recognized in the laboratory without much difficulty and frequently could be recognized in the field with the naked eye. Typical fourth-instar larvae of *C. tarsalis* with patent infections of *T. californica* usually exhibited white masses which were very evident in the thorax and along the lateral margins of the abdomen; such white areas in diseased larvae were produced by accumulations of many thousands of sporonts and spores deposited in the body cavity. Larvae with advanced infections became greatly swollen and usually died during the fourth larval stadium.

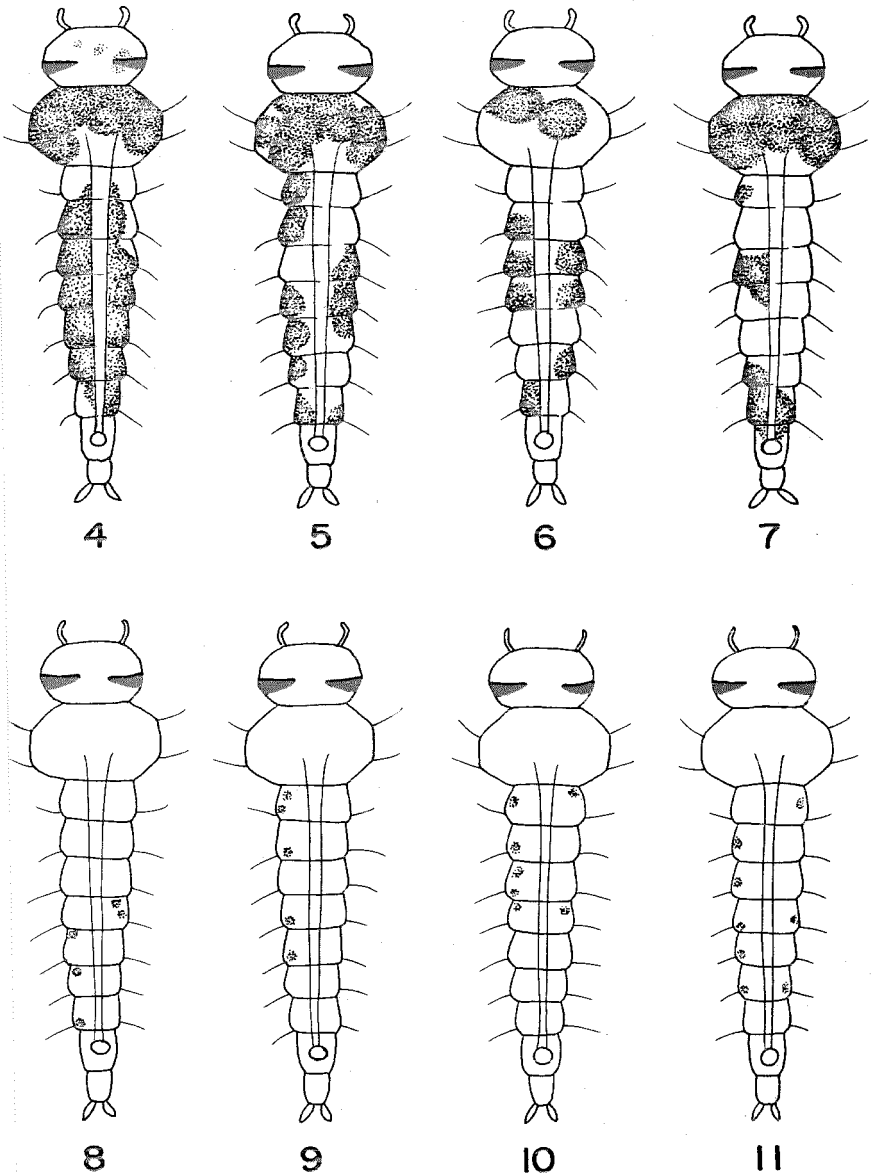
Thelohania parasites of mosquitoes, and perhaps other nosematid parasites as well, are apparently highly host specific; however, host-specificity has not been adequately demonstrated in the laboratory and such conclusions have been largely based on field observations. Although several different species of mosquitoes were

frequently observed sharing common habitats, different species of parasites were not shared by the hosts. Apparently each mosquito is only susceptible to its own particular species of parasite for cross-infections were never observed.

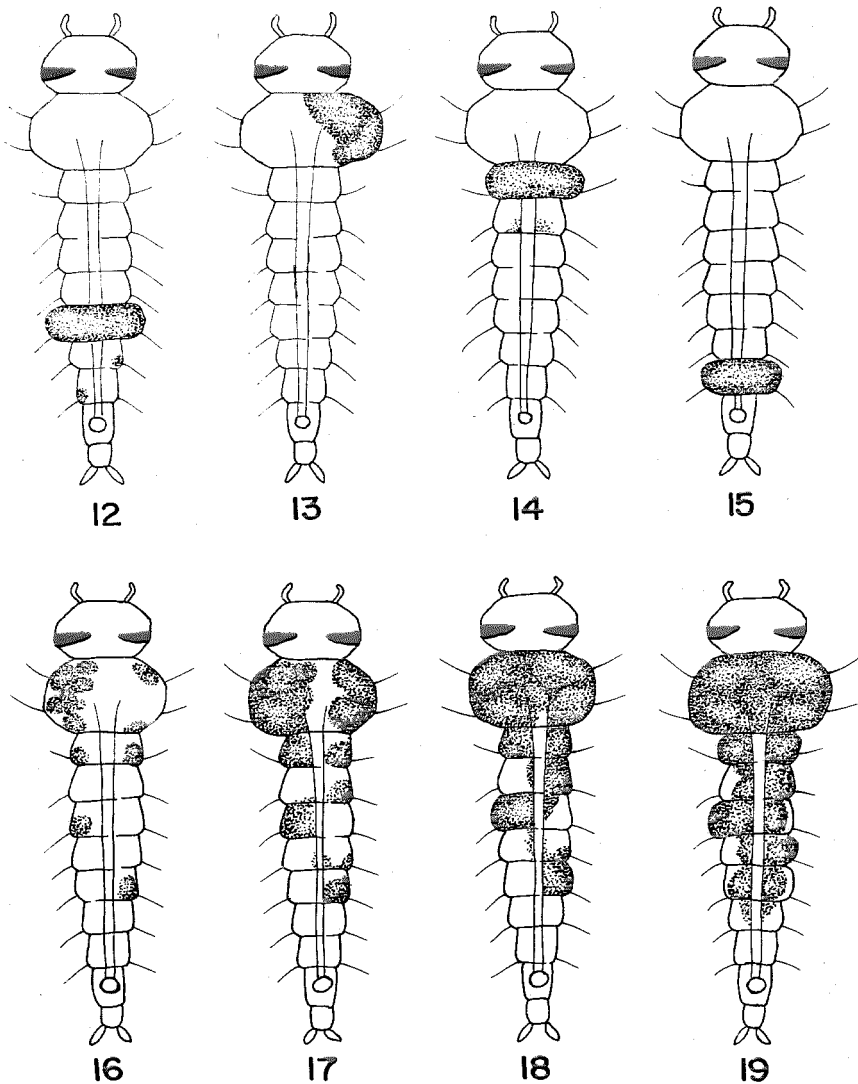
Each of the *Thelohania* now known to occur in Californian mosquitoes is easily recognizable on the basis of characteristic spore shape and size. Figures 1-3, for example, illustrate living spores from larvae of *Anopheles p. franciscanus*, *Aedes melanimon*, and *Culex tarsalis* which had recently succumbed to their infections. Not only are different species of thelohanian parasites distinctive on the basis of their morphology, but larval hosts may also exhibit characteristic manifestation of their specific infections which are distinctive.

Larvae of *C. tarsalis* with apparent infections have enlarged areas along the abdomen and thorax (Figures 4-7). Fourth-instar larvae of *C. apicalis* infected with *T. benigna*, however, rarely develop more than very small localized sites of spore deposition (Figures 8-11). Such infected areas usually appear as very light spots which are usually barely apparent to the naked eye. In contrast, fourth-instar larvae of *C. peus* with infections of an undescribed *Thelohania* were observed with areas of enlargement localized in a single abdominal segment or part of the thorax (Figures 12-15).

Larvae of most mosquitoes observed with apparent thelohanian infections developed massive infections as illustrated by *C. tarsalis* infected with *T. californica*. *C. apicalis* was the only species observed with small localized and benign infections. Larvae of all species of mosquitoes, however, usually did not show signs of the infections until late in the third larval stadium when small opaque spots usually became evident. As hosts matured, the areas of apparent infection enlarged, indicative of increased spore deposition. Laboratory observations suggest that such infected larvae frequently develop normally until the fourth larval stadium. Matu-



FIGS. 4-7.—Fourth-instar larvae of *Culex tarsalis* infected with *Thelohania californica*. FIGS. 8-11. Fourth-instar larvae of *C. apicalis* infected with *T. benigna*.



FIGS. 12-15.—Fourth-instar larvae of *Culex peus* infected with *Thelohania* sp. FIGS. 16-19.—Progressive development of *T. californica* infection in a fourth-instar larva of *C. tarsalis*. Period development = 14 days.

larvae have areas of infection which are usually fairly large. Diseased individuals are unable to pupate normally, and may continue to survive for an abnormally long time during the fourth larval stadium as development of spore material within the tissues continues to increase. Some infected larvae of *C. tarsalis* observed in our laboratory survived for as long as two weeks in the fourth larval stadium at 25° C. (Figures 16-19).

Our present data indicate that host-parasite relationships between *Thelohania* and mosquitoes are not simple, nor do the same or even similar relationships exist for each species of parasite and mosquito. The most complete data available on host-parasite relationships are for *C. tarsalis* and *T. californica*. These data suggest that transovarian transmission of parasites occurs commonly. Evidence of transovarian passage of *T. californica* was first obtained from larvae reared from 28 egg rafts collected from an irrigated pasture near Fresno, California. The rafts were placed in clean water in individual containers for rearing. Larvae from three of the 28 rafts developed infections of *T. californica* which were grossly evident in fourth-instar individuals. Additional collections subsequently were made at the same locality. From a total of 201 *C. tarsalis* egg rafts, 19 (9%) gave rise to larvae having apparent infections.

Adult gravid females of *C. tarsalis* were so collected from the study area and were maintained in small cages in our laboratory. The field-collected females subsequently laid 84 egg rafts which were reared in separate containers; 14 rafts (17%) gave rise to larvae with apparent infections.

A further attempt was made to rear complete progenies from infected females, and of 2353 larvae reared to the fourth-instar, 1106 larvae (47%) developed apparent infections. The median proportion of all progenies exhibiting apparent infections was 50 percent.

Larvae with apparent infections usually died in these studies. The surviving larvae, i.e., larvae from infected rafts but

which appeared not to be infected, were reared to the adult stage and appeared normal in every respect. Of the 291 adults which resulted, 97 percent were females and only 3 percent were males.

Eggs laid by females reared from infected rafts were examined for parasites, and almost all such eggs were found to contain from one to about 50 mono- and binucleate schizonts of *T. californica* (Figures 21-22). Larvae with apparent infections had massive accumulations of spores and sporonts; such larvae were males (Figure 20). Surviving sister larvae were also examined microscopically and were found to contain only mono- and binucleate schizonts. Sporozonic stages of *T. californica* have not been observed in female hosts. To summarize: *T. californica* is a host-specific parasite of *C. tarsalis*. Infected females lay infected egg rafts and schizogonic stages have been demonstrated in both. Infected male larvae contain both schizogonic and sporogonic stages of the parasite, and develop massive accumulations of spores; such male larvae die before pupation. Infected female larvae, however, have only schizogonic stages and survive to transmit the infection to their progeny.

The transovarian transmission of *T. californica* in *C. tarsalis* is qualitatively different from any previously reported microsporidian life-cycle in that the female host suppresses the sporogonic cycle of the parasite and survives with little apparent effect of the infection. The male larva, in contrast, experiences a malignant infection and usually dies during the fourth larval stadium. An adequate supply of healthy males may, therefore, become a critical factor in the continued existence of this parasite within wild *C. tarsalis* populations.

Several mosquitoes known to be hosts of the thelohanian parasites have been colonized in our laboratory, and preliminary studies of their host-parasite relationships have suggested that they are different from that observed for *C. tarsalis*. Data from a number of mosquitoes, notably *C. peus*, *C. apicalis*, *C. erythrorhox*, *Culiseta incidens* and *C. inornata* indicate that these

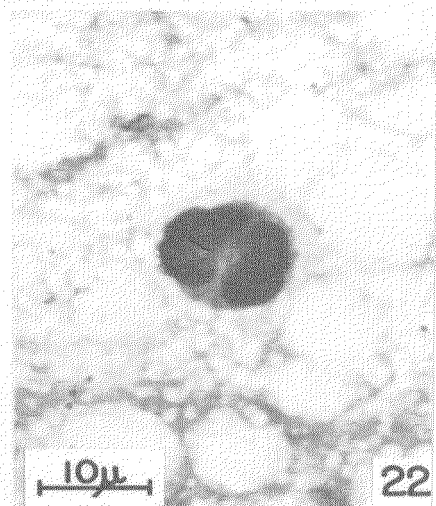
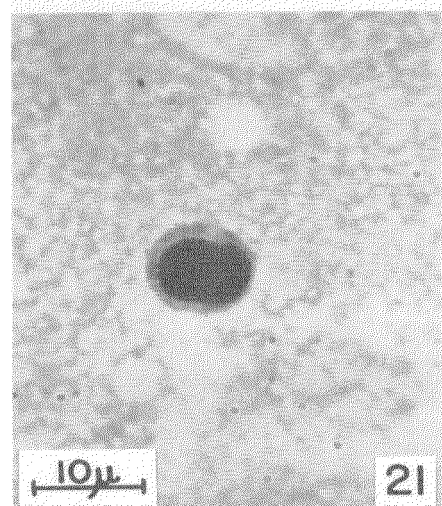
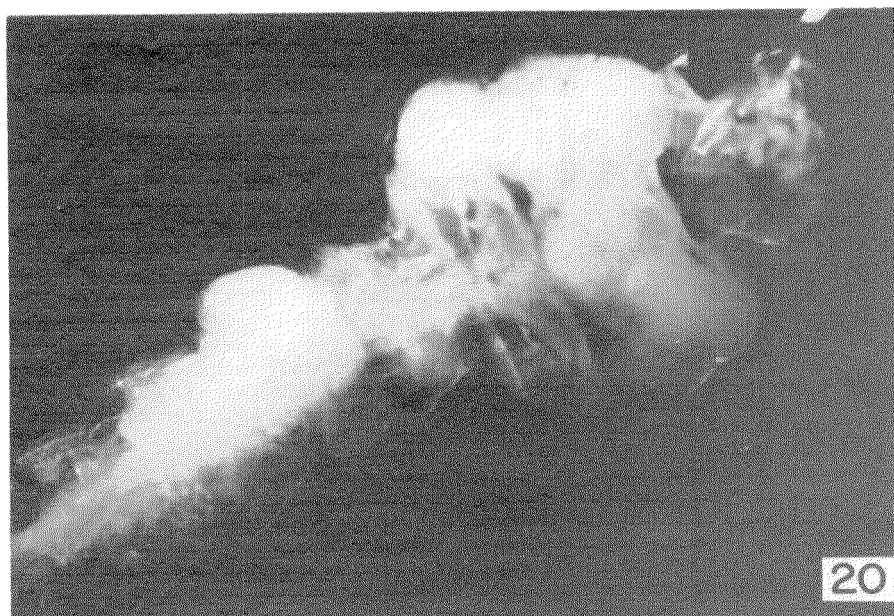


FIG. 20.—Fourth-instar male larva of *Culex tarsalis* with apparent infection of *Thelohania californica*. FIGS. 21 and 22.—Mono- and binucleate schizonts of *T. californica* from eggs of *C. tarsalis*.

species also are capable of transmitting their infections transovarially.

It has been concluded that probably all mosquitoes are capable of transmitting thelohanian parasites via the egg route. However, attempts to transmit *Thelohania* parasites in our laboratory by exposing larvae to spores have been uniformly unsuccessful. It seems highly unlikely that such thelohanian spores are incapable of initiating new infections, as transmission by spores via the alimentary canal has been demonstrated for Microsporidia known to parasitize honey bees, silk worms, and many other insect hosts. It is possible, however, that some mosquito parasites require a special conditioning period to render spores infective.

Another microsporidian parasite of *C. tarsalis* under study in our laboratory is a member of the genus *Nosema*. The life cycle of this parasite is incompletely understood; however, it is known that transovarian transmission occurs, and both male and female hosts experience sporogony. Furthermore, massive apparent infections do not develop.

The *Nosema* parasites of *C. tarsalis* undergo schizogony while floating free in the hemolymph of the larval host. The sporogonic phase of activity, however, is highly tissue-specific and is initiated in larval oenocytes occurring as isolated small groups of cells along the lateral margins of each abdominal segment. If the infected host survives to the adult stage, parasitic development is stimulated and the oenocytes become greatly enlarged and filled with sporonts and spores. Hypertrophied oenocytes lose their normal attachments and may be carried by the hem-

olymph to various other sites within the body. Such hypertrophied cells may ultimately become attached to the walls of the alimentary canal, malpighian tubules, and ovaries of the adult host.

Since the function of oenocytes in larvae is to secrete materials required for normal development and metamorphosis, total destruction of the cells by parasites prevents the host from developing beyond the larval stage. Infected populations of *C. tarsalis* observed in our laboratory usually experienced about 20 to 50 percent mortality, while surviving females transmitted their infections transovarially.

Field data are available which suggest that other *Nosema* or similar microsporidian parasites may attack many species of mosquitoes, and that the formation of hypertrophied cells is characteristic of such infections. Studies are underway to determine the levels of *Nosema* infections existing in natural populations of mosquitoes. This is done by sampling adult populations and examining individuals for the presence of spores or hypertrophied cells in their body cavities.

The more we understand about microsporidian infections of mosquitoes, the more it becomes evident that such parasites are capable of exerting a strong influence toward limiting natural populations. Whether it will be feasible to apply Microsporidia in a biological control program, however, will largely depend upon our ability to manipulate such parasites. If it were possible to induce infections in healthy populations of larvae by exposing them to infective microsporidian spores, then such a biological control program may be feasible.