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Life cycles and host-parasite relationships of Microsporidia in culicine mosquitoes

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Abstract. Heterosporous (polymorphic) microsporidia in mosquitoes are characterized by intricate life cycles involving multiple spore types responsible for horizontal (*per os*) and vertical (transovarial) transmission. They affect two generations of the mosquito and some involve an obligate intermediate host. Heterosporous microsporidia are generally very host and tissue specific with complex developmental sequences comprised of unique stages and events. Full details on the intricate relationships between heterosporous microsporidia and their mosquito hosts have only recently been elucidated. *Edhazardia aedis* (Kudo, 1930) and *Culicospora magna* (Kudo, 1920) have developmental sequences in larvae that involve gametogony followed by plasmogamy and nuclear association to form diplokarya. These diplokaryotic stages then undergo karyogamy and form binucleate spores responsible for transovarial transmission. In the filial generation, haplosis occurs as a result of nuclear dissociation to produce uninucleate spores infectious to larval mosquitoes. *Amblyospora californica* (Kellen et Lipa, 1960) has similar sequences except that haplosis is by meiosis to produce spores infectious for a copepod intermediate host. A third spore type is formed in the intermediate host responsible for infection in a new generation of the mosquito host.

Microsporidia are fascinating organisms. They are an extremely large group that has invaded nearly all animal phyla. Perhaps best known to invertebrate and fish pathologists, they have also drawn the attention of human pathologists. While considerable knowledge has accumulated on morphological aspects of sporulation, relatively little information is available on early development and details of individual host-parasite relationships. One reason for this lack of knowledge is the incredible complexity of some groups of microsporidia which makes them much more difficult to work with than other parasite groups. Another hinderance is the difficulty associated with the study of a complex parasite within a host that can not be easily manipulated or colonized in the laboratory. Some of these obstacles can be overcome by the study of microsporidia in mosquitoes. First, most mosquitoes have relatively short life cycles (2–3 weeks) are readily manipulated in the laboratory and can be easily colonized. In addition, microsporidia are common to mosquitoes and include diverse groups with both simple and complex life cycles. For these reasons, the study of microsporidia parasitic in mosquitoes has provided many insights into the relationships between microsporidia and their hosts.

Microsporidia – general features

The microsporidia are a large and ubiquitous group of obligate, intracellular parasites that produce unicellular spores containing a polar filament (tube). They are primitive eukaryotes without mitochondria that have small (70S) ribosomes containing RNA's of prokaryotic size (Vossbrinck et al. 1987). They infect nearly all major animal groups from protozoa to man but are especially common in arthropods. Microsporidia have been found worldwide in mosquitoes with descriptions of over 100 species in more than 15 different mosquito genera (Hazard and Oldacre 1975, Hazard and Chapman 1977, Castillo 1980, Andreadis 1990, Sweeney and Becnel 1991).

The spore is the infective stage and transmission typically takes place in one of two ways. Horizontal (*per os*) transmission occurs when spores are ingested by a suitable host. Germination takes place within the gut and the infective germ (sporoplasm) is inoculated into a host cell via the polar tube. Vertical (transovarial) transmission occurs when spores germinate in or near the ovaries of the adult female and the sporoplasm is presumably inoculated into the developing egg. Some species are only known to be transmitted orally while others can be transmitted both orally and transovarially.

Microsporidia in mosquitoes

Heterosporous microsporidian parasites of mosquitoes are a diverse group of organisms with complex life cycles. Hesse (1904) was probably the first researcher to document a true microsporidian parasite of a mosquito when he described *Thelohania (=Parathelohania) legeri* (Hesse, 1904) Codreano, 1966 from *Anopheles maculipennis* Meigen. Later, the most comprehensive study of these mosquito parasites was a series of eight publications by Kudo (1921, 1922, 1924a,b, 1925a,b, 1929, 1930) under the general title „Microsporidia Parasitic in Mosquitoes“. This series was notable in that extensive details and drawings on the developmental stages of various microsporidia provided much new information which has proven to be remarkably accurate. But given the minute size and complexity of these parasites, along with the limitations of the instruments at that time, many details were misinterpreted or overlooked. For these reasons, certain ideas concerning sexuality and conclusions about the transmission, development and relationships of microsporidian parasites of mosquitoes were accepted and perpetuated by subsequent researchers. A primary obstacle to obtaining accurate information was the inability to orally transmit these parasites to the mosquito host. It was not until this obstacle was overcome that significant advancements were made in understanding the complex life cycles.

Kellen and co-workers published a notable series of studies on host-parasite relationships. They described and classified microsporidia in mosquitoes according to tissue specificity and host sex in which the sporogonic cycle occurred in transovarially infected progeny (Kellen and Lipa 1960, Kellen and Willis 1962a,b, Kellen et al. 1966a,b, 1967). This led to the important discovery of spore dimorphism and the role of transovarial transmission in some species of *Thelohania* (=*Parathelohania*) found parasitizing *Anopheles* mosquitoes; an important finding for the taxonomy of the Microsporidia. Hazard and Weiser (1968) reported that a binucleate spore formed in the adult female was responsible for transmitting the pathogen to progeny. Their studies revealed that in infected male larval progeny, uninucleate spores (meiospores) were produced. In infected female progeny, however, spore development was delayed until pupation and adult emergence. In these females, binucleate spores of the original type were produced to repeat the cycle. This represented the first clear documentation of a link between infections in adults and progeny and proved that the two morphologically distinctive spores found in larvae and adult hosts (formerly believed to belong to two genera) represented a single species.

While the role of binucleate spores in transovarial transmission continued to be documented, the means by which these microsporidia were transmitted horizontally remained a mystery until the discovery that meiospores formed in larvae were infectious to a copepod intermediate host (Sweeney et al. 1985). When ingested by mosquito larvae, the spores from the copepod intermediate host initiate a sequence of development that ends with binucleate spores in the adult female mosquito. The developmental cycles of some of these microsporidian parasites of mosquitoes have therefore been shown to involve both vertical and horizontal transmission affecting two generations of the mosquito host with the involvement of an intermediate host.

Recent investigations have used this group of parasites in mosquitoes to establish patterns of nuclear cycles for the microsporidia (Sprague et al. 1992). Possession of this new information, together with established knowledge, provides the opportunity to discuss the host-parasite relationships of microsporidian parasites of mosquitoes.

Table 1. Nuclear phases in the life cycle of heterosporous microsporidia in mosquitoes.

PARENTAL GENERATION OF MOSQUITO HOST

HAPLOPHASE. A sequence of haploid stages that starts with the products of haplosis and ends with plasmogamy and nuclear association to produce meronts (diplokaryotic cells).

Schizogony. Division of individuals with unpaired nuclei.

Gametogony. Production of gametes by proliferation of cells in the haplophase. Used in distinction from gametogenesis, often applied to gamete production accompanied by meiosis.

Plasmogamy. Cytoplasmic fusion of 2 gametes without karyogamy.

DIHAPLOPHASE. That part of a life cycle in which the nuclei occur in pairs known as diplokarya.

Nuclear association. The pairing of 2 haploid nuclei at the end of the haplophase to form a diplokaryon.
Diplosis in microsporidia.

Karyogamy. Fusion of nuclei.

DIPLOPHASE. That part of the chromosome cycle in which the nuclei are diploid. Limited to zygote in microsporidia.

DIHAPLOPHASE. That part of a life cycle in which the nuclei occur in pairs known as diplokarya.

First Merogony. Defined here as proliferation of meronts (diplokaryotic cells).

Binucleate spore formation.
Transovarial transmission.

FILIAL GENERATION OF MOSQUITO HOST

DIHAPLOPHASE. That part of a life cycle in which the nuclei occur in pairs known as diplokarya.

Second Merogony – Defined here as proliferation of meronts (diplokaryotic cells).

HAPLOPHASE

Haplosis. Reduction of the chromosome number from diploid to haploid.

Nuclear dissociation. The separating of the members of a diplokaryon to form 2 independent haploid nuclei. One of 2 types of haplosis in microsporidia.

Meiosis. Haplosis accompanied by synapsis and disjunction of homologous chromosomes.

Haploid spore formation.
Spore from dissociation. Directly infectious to the mosquito.
Meiospore. Infectious for a copepod intermediate host.

INTERMEDIATE HOST

HAPLOPHASE

Schizogony. Division of individuals with unpaired nuclei. Asexual sequence.

Uninucleate spore. Directly infectious to the mosquito host.

Life cycles and host-parasite relationships

Microsporidia in mosquitoes are commonly divided into two categories based on their life cycles and host-parasite relationships. Some species of microsporidia exhibit simple life cycles with one spore type responsible for oral transmission. They affect only one generation

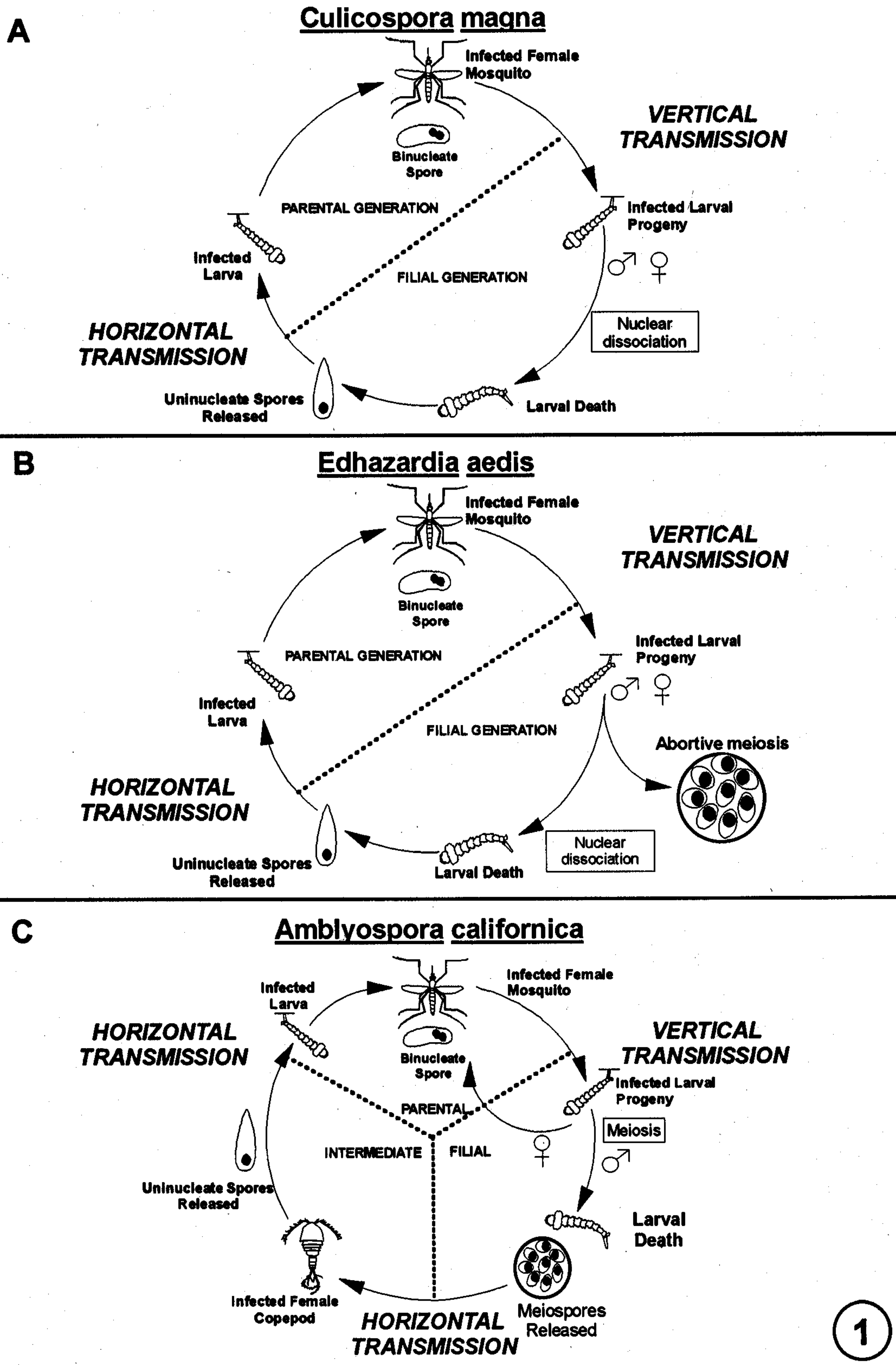
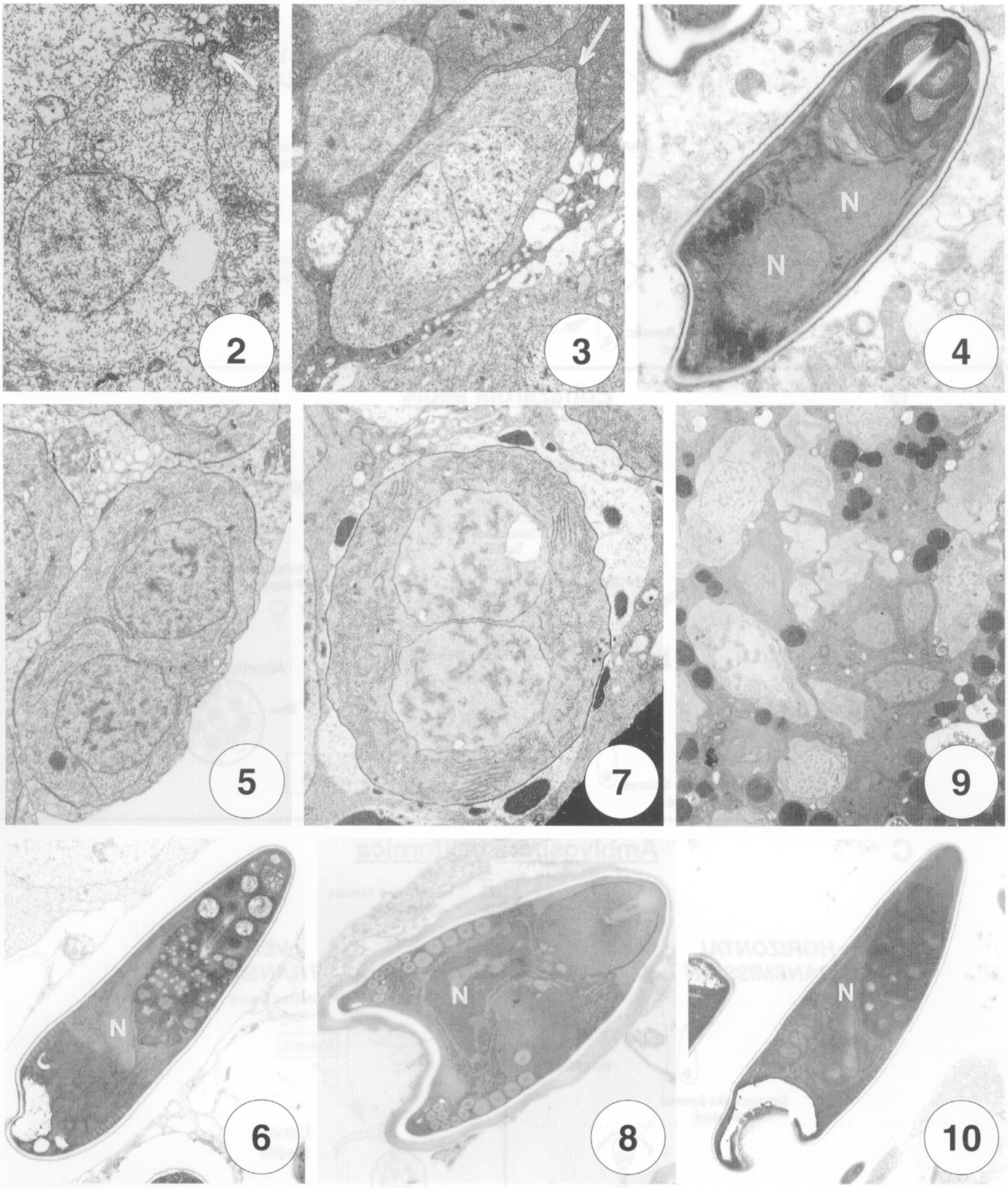


Fig. 1. Schematic representations of the life cycles of heterosporous microsporidia. **A.** *Culicospora magna* in the mosquito *Culex restuans*. **B.** *Edhazardia aedis* in the mosquito *Aedes aegypti*. **C.** *Amblyospora californica* in the mosquito *Culex tarsalis* and the copepod intermediate host *Macrocyclops albidus*.



Figs. 2–10. Electron micrographs of selected developmental stages involved in the life cycles of heterosporous microsporidia. **Fig. 2.** Gamete of *Culicospora magna* with a papilla at the anterior end (arrow) x 5000. **Fig. 3.** Newly formed diplokaryotic meront of *Edhazardia aedis* with a papilla persisting at one pole (arrow) x 5000. **Fig. 4.** Binucleate spore of *Edhazardia aedis*. (N=nucleus). x 10000. **Fig. 5.** Haplosis by nuclear dissociation in *Culicospora magna*. x5000. **Fig. 6.** Uninucleate spore of *Culicospora magna*, the end product of nuclear dissociation. x 5000. **Fig. 7.** Diplokaryotic sporont, haplosis by meiosis in *Amblyospora californica*. x 5000. **Fig. 8.** Meiospore of *Amblyospora californica*. x11,400. **Fig. 9.** Uninucleate schizonts of *Amblyospora californica* in the ovaries of the copepod intermediate host. x 2500. **Fig. 10.** Asexually formed uninucleate spore in the copepod intermediate host. x 5000.

of the mosquito and are not usually host or tissue specific. *Nosema algerae* Vávra et Undeen, 1970 and *Vavraia culicis* (Weiser, 1947) are examples of species with one spore type and a simple host-parasite relationship.

Heterosporous (polymorphic) microsporidia are characterized by intricate life cycles involving multiple spore types responsible for horizontal and vertical transmission. They affect two generations of the mosquito and some involve an obligate intermediate host. Heterosporous microsporidia are generally very host and tissue specific with complex developmental sequences comprised of unique stages and events. Full details on the intricate relationships between heterosporous microsporidia and their mosquito hosts have only recently been elucidated (Becnel et al. 1987, 1989, Andreadis 1988, Sweeney et al.1988, Becnel 1992). The life cycles of some heterosporous microsporidia in mosquitoes have been viewed as an alternation of generations associated with haploidy and diploidy in the nuclei (Becnel et al. 1987). The change from the diploid cell state (the dihaplophase) to the haploid cell state (the haplophase) is by haplosis of two types, meiosis or nuclear dissociation. The change from the haplophase to the dihaplophase is accomplished by nuclear association without nuclear fusion (Table 1).

Culicospora magna (Kudo, 1920) and *Edhazardia aedis* (Kudo, 1930) have similar life cycles (Becnel et al. 1987, 1989) that require two mosquito generations to complete (Figs. 1A and B). In the parental generation, uninucleate sporoplasms are inoculated into the gastric caeca of larvae following the ingestion of spores. Gametogony results in the formation of gametes identified by the presence of a papilla at the anterior pole of the cell (Fig. 2). They come together in pairs followed by cytoplasmic fusion (plasmogamy) and nuclear association to form diplokaryotic meronts (Fig. 3). In some cases, these diplokaryotic stages undergo karyogamy (Becnel et al. 1989). Subsequently, oenocytes become infected where binucleate spores in adults are responsible for transovarial transmission (Fig. 4). Infections in larval progeny occur in fat body cells. Haplosis of diplokaryotic sporonts is as a result of nuclear dissociation (Fig. 5) producing uninucleate spores (Fig. 6) that are infectious *per os* to a new generation of larval mosquitoes. *Edhazardia aedis* has an additional sporulation sequence involving meiosis that aborts in the latter stages, rarely forming meiospores (Fig. 1B).

Amblyospora californica (Kellen et Lipa, 1960) has developmental sequences similar to those of *C. magna* and *E. aedis* (Fig. 1C) but with some fundamental differences (Becnel 1992). Female progeny from infected adults have benign larval infections that are restricted to oenocytes. In the adults, binucleate spores are formed after a blood meal and these are responsible for transovarial transmission to progeny. Male progeny from infected adults develop fat body infections with haplosis of diplokaryotic sporonts by meiosis (Fig. 7) producing meiospores (Fig. 8). Meiospores are infectious *per os* to the copepod intermediate host *Macrocyclops albidus* (Jurine). Asexual development of uninucleate schizonts begins in the ovaries of the intermediate host (Fig. 9) and ends with the production of uninucleate spores (Fig. 10) that are infectious *per os* to a new generation of the mosquito host. In larval mosquitoes, gametogony is followed by plasmogamy and nuclear association to form diplokarya and eventually binucleate spores in adults to complete the cycle.

These three microsporidia in mosquitoes exhibit common life cycle themes of vertical and horizontal transmission involving two successive generations of the mosquito host. Tissue specificity is another common feature with the initiation of infection in the gastric caeca and subsequent spread to the oenocytes, egg yolk and fat body of the mosquito hosts, and the ovaries of the intermediate copepod host. The mechanisms involved in the movement between specific tissues are unknown, but they may be related to specific biochemical and biophysical conditions found in the hosts. This hypothesis is supported by recent host specificity studies involving species of *Amblyospora* and *Hazardia* wherein the microsporidia are able to initially infect alternate mosquito host larvae but are unable to infect the ovaries of adult hosts and complete their respective life cycles via transovarial transmission (Andreadis 1989, Becnel and Johnson 1993).

These microsporidia also share common nuclear cycles expressed as an alternation between haploid and diploid cell states with haplosis occurring in one of two ways. In *C. magna* and *E. aedis*, functional haplosis is by nuclear dissociation followed by the production of uninucleate spores infectious for larval mosquitoes. *E. aedis* undergoes an additional sporulation sequence that involves meiosis but this process aborts and does not form functional meiospores. Haplosis in *A. californica* is by meiosis with meiospores infectious for an obligate intermediate host. This apparent shift in the mechanism of haplosis by nuclear dissociation, with *E. aedis* an intermediate form, may represent an example of regressive evolution wherein microsporidia in multivoltine mosquitoes with overlapping generations may be evolving away from meiosis and two host systems to simpler, more efficient one host systems.

CONCLUSIONS

Major advances have been made in elucidating the complex developmental cycles of heterosporous microsporidia in mosquitoes, some of which involve an intermediate host. The taxonomic implications of these findings for the Microspora as a group are presently being evaluated but the impact will without doubt be far reaching. This detailed information on the host-parasite relationships of microsporidia in mosquitoes can hopefully serve as a "template" for other groups of microsporidia and, together with some of the new molecular techniques, solve many of the unanswered questions about this fascinating group of organisms.

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