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REPRODUCTIVE STRATEGIES AND ADAPTATIONS FOR SURVIVAL AMONG OBLIGATORY MICROSPORIDIAN AND FUNGAL PARASITES OF MOSQUITOES: A COMPARATIVE ANALYSIS OF *AMBLYOSPORA* AND *COELOMOMYCES*

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ABSTRACT. *Amblyospora* (Microsporida: Amblyosporidae) and *Coelomomyces* (Chytridiomycetes: Blastocladiales) have independently evolved a diverse array of unique and highly specialized mechanisms that have allowed them to more fully exploit their mosquito hosts and the aquatic environment that their hosts inhabit. *Amblyospora* and *Coelomomyces* both have complex life cycles that include obligatory development in an intermediate microcrustacean host and 2 mosquito generations for completion. *Amblyospora* is polymorphic with 3 separate and distinct developmental sequences, asexual and sexual reproduction, and aspects of both vertical (transovarial) and horizontal transmission. Infective stages of *Coelomomyces* are motile, a temporal gating mechanism coordinates gamete release, and, even though there is no transovarial transmission, infection of primary host ovaries is important in dissemination of the fungus to new habitats. The intent of this review is to examine how these and other strategies and adaptations facilitate parasite reproduction within the host(s) and enhance transmission and survival between hosts.

INTRODUCTION

Microsporidia belonging to the genus *Amblyospora* Hazard and Oldacre (Microsporida: Amblyosporidae) are a highly successful group of obligate intracellular parasites that are among the most common and widespread parasites associated with mosquito populations in nature. To date, at least 31 described and 49 undescribed species or forms have been reported from 71 different host mosquitoes representing 8 genera worldwide (see Andreadis 1994, for host list), and this probably represents a small fraction of the total number that exist. They have also been reported from blackflies, caddisflies, and amphipods (Hazard and Oldacre 1975).

Coelomomyces Keilin (Chytridiomycetes: Blastocladiales) consists of more than 50 described species from at least 135 species, in 22 genera, of mosquitoes and 2 species of chironomids (Couch and Bland 1985). Both *Amblyospora* and *Coelomomyces* are particularly common among *Aedes* and *Culex* mosquitoes. *Coelomomyces* is also frequently found in *Anopheles* species.

Amblyospora and *Coelomomyces* have independently evolved a number of unique and highly specialized mechanisms that have allowed them to more fully exploit their mosquito hosts and the aquatic environment they inhabit. The purpose of this review is to examine how these adaptations facilitate parasite reproduction, transmission, and survival.

LIFE CYCLES

Amblyospora: All species of *Amblyospora*, in so far as we know, are transovarially transmitted by adult female mosquitoes and undergo obligatory development in an intermediate copepod host as a prerequisite to horizontal transmission. A representative life cycle of one species, *Amblyospora connecticus* Andreadis, as it occurs in the brown saltmarsh mosquito, *Aedes cantator* (Coquillett) and the copepod, *Acanthocyclops vernalis* (Fischer) is shown in Fig. 1 and is described below (Andreadis 1983, 1985a, 1988a, 1990).

The microsporidium overwinters as an active infection in copepodid stages of *A. vernalis*. Infections are transmitted horizontally to larval mosquitoes via oral ingestion of extracellular haploid spores that are released into the water with the death of infected copepods in the spring. Spores germinate within the lumen of the larval gut and invade epithelial cells of the midgut and gastric caecum by injection of the sporoplasm through the evaginating polar tube. After a brief period of multiplication by binary fission, the microsporidium spreads to muscle tissue and oenocytes where it undergoes a sexual phase of development involving gametogenesis and plasmogamy, thereby restoring itself to the diploid condition. There is no apparent pathology associated with infection and larval hosts develop normally and emerge as healthy adults. The microsporidium undergoes limited multiplication in the adult female and sporulates when the female mosquito acquires a blood meal. Sporula-

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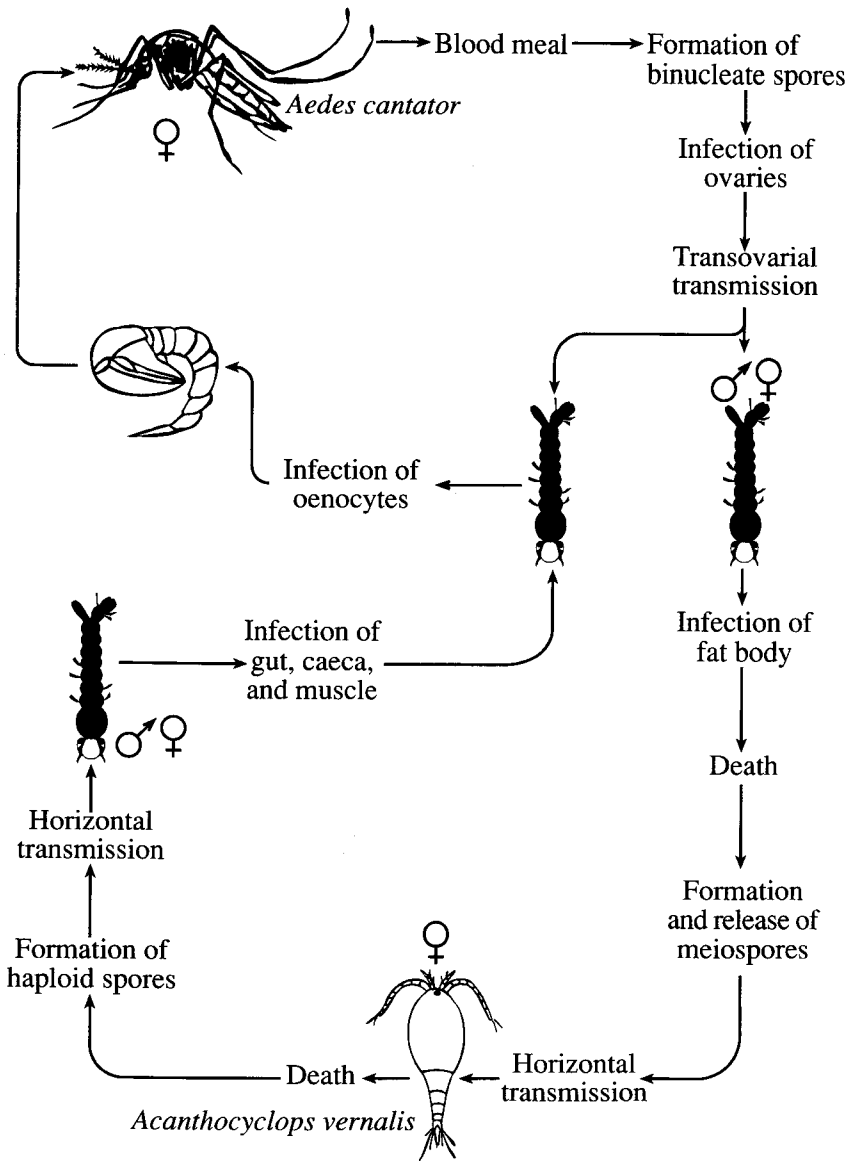

Fig. 1. Life cycle of *Amblyospora connecticus.*

tion coincides with the maturation of the ovaries and is stimulated by host reproductive events, probably hormonal (Lord and Hall 1983). This results in the formation of a binucleate spore that infects the ovaries and is responsible for transovarial transmission of infection to the F_1 generation.

Parasite development in larval progeny is typically dimorphic and dependent to some degree on the host sex. In certain female mosquitoes, the microsporidium will infect the oenocytes and undergo a simple developmental sequence wherein it sustains limited multiplication by binary and multiple fission. These larvae show no adverse effects and develop normally to adulthood. When mated with healthy males and following a blood meal, these females transovarially transmit infections as in the previous generation. In other females and usually all male progeny that hatch from infected eggs, the microsporidium invades fat body tissue and exhibits an entirely different developmental sequence. It undergoes meiosis and a prolonged sporulation sequence that results in the production of tens

of thousands of haploid meiospores. These infections typically kill the larval host during the 4th stadium by destroying normal fat body function and depleting larvae of essential reserves. Meiospores produced in these mosquito larvae are orally infectious to female stages of *A. vernalis*. Following ingestion and subsequent spore germination, the microsporidium infects copepod ovarian tissue and undergoes repeated merogony followed by polysporoblastic sporogony and the formation of thousands of haploid spores. This ultimately kills the copepod, permitting the release of spores into the water where they can be eaten by mosquito larvae to complete the cycle.

Coelomomyces: Coelomomyces alternates between mosquitoes and copepods or ostracods (Whisler et al. 1974, Whisler 1985). As representative, the life cycle of *Coelomomyces stegomyiae* Keilin in the yellow fever mosquito, *Aedes aegypti* (Linn.), and the harpacticoid copepod *Phyllognathopus viguieri* (Maupas) is shown in Fig. 2 and is described below. The fungus overwinters as resting sporangia (RS) that develop from diploid hyphae in infected mosquito larvae. Normally, the larvae die in the 4th stadium and RS are released as the cadavers decompose. Meiosis occurs in the RS (Whisler et al. 1983) and the posteriorly uniflagellate meiozoospores that emerge from the RS infect the appropriate microcrustacean host and establish the haploid, heterothallic gametophytic stage, which develops in the hemocoel. Coincident with the death of the copepod, motile isogametes (Lucarotti and Federici 1984b) (+ and − mating types from respective gametophytes) emerge from the bodies of the host copepods and pair to form zygotes that reestablish the diplophase in the primary mosquito host. Larvae of mosquitoes infected by certain species of *Coelomomyces* can pupate and eclose to produce infected adults. In adult female *Ae. aegypti,* the infection is mostly localized in the ovaries (Lucarotti 1987). During the first 72 h following eclosion, as the ovaries enlarge under the influence of juvenile hormone (Gwadz and Spielman 1973, Hagedorn et al. 1977), the hyphae in the hemocoel are transferred to the interstitial spaces of the ovaries. This transfer is effected, at least in part, by hyphae infecting epithelial cells, which penetrate the ovaries to produce tracheoles to aid respiration in the maturing ovaries (Wigglesworth 1991, Lucarotti 1992). Infected adult males and females will mate (Lucarotti 1987), but the hyphae in the ovaries will only mature to RS following a blood meal (Lucarotti and Klein 1988). The factor that initiates this maturation of the hyphae to RS is the increase in 20-hydroxyecdysone that follows a blood meal (Lucarotti 1992). Eggs do not develop in the ovaries of *C. stegomyiae*-infected individuals; the females do, however, attempt to oviposit, but RS are laid in the place of eggs (Lucarotti 1987). Meiozoospores from these RS infect the copepod host, *P. viguieri,* thereby completing the life cycle (Padua et al. 1986, Lucarotti 1987).

VERTICAL TRANSMISSION

The evolution of a separate and distinct developmental cycle within the female mosquito that results in transovarial transmission of *Amblyospora* from one host generation to the next probably represents the single most important adaptation for survival that has evolved within this group of microsporidia. This phenomenon, which has evolved many times in a range of microbial parasites (Smith and Dunn 1991), provides a highly efficient and specialized mechanism wherein parasites can be temporally maintained within a mosquito population when mosquito densities are low and/or when no intermediate host is present. Transovarial transmission also helps to stabilize parasite populations where mosquito and copepod densities oscillate from year to year and when one or both hosts possesses distinct nonoverlapping generations.

The degree to which maternal-mediated transovarial transmission contributes to the continual maintenance of *Amblyospora* within a mosquito population varies widely among species and their respective mosquito hosts (Kellen et al. 1965, 1966). In some species such as *A. connecticus* (Andreadis 1983) and *Amblyospora dyxenoides* Sweeney, Graham and Hazard (host = *Culex annulirostris* Skuse) (Sweeney et al. 1988), where most infected female progeny develop benign infections and survive to transmit infections from one successive generation to the next, transovarial transmission can sustain the parasite for several generations. In other species, such as *Amblyospora polykarya* Lord, Hall and Ellis (host = *Aedes taeniorhynchus* (Weidemann)) (Lord et al. 1981) and *Amblyospora indicola* Vavra, Bai and Panicker (host = *Culex sitiens* Weidemann) (Sweeney et al. 1990), where both male and female mosquito larvae succumb to meiospore infections during the 4th stadium, the parasite is transovarially transmitted for one generation only. It is important to recognize that even in those species of *Amblyospora* where transovarial transmission is continuous, some degree of horizontal transmission must eventually take place. This is because none are transmitted with 100% efficiency and there is no assistance from the male mosquitoes (i.e., no paternal-mediated vertical

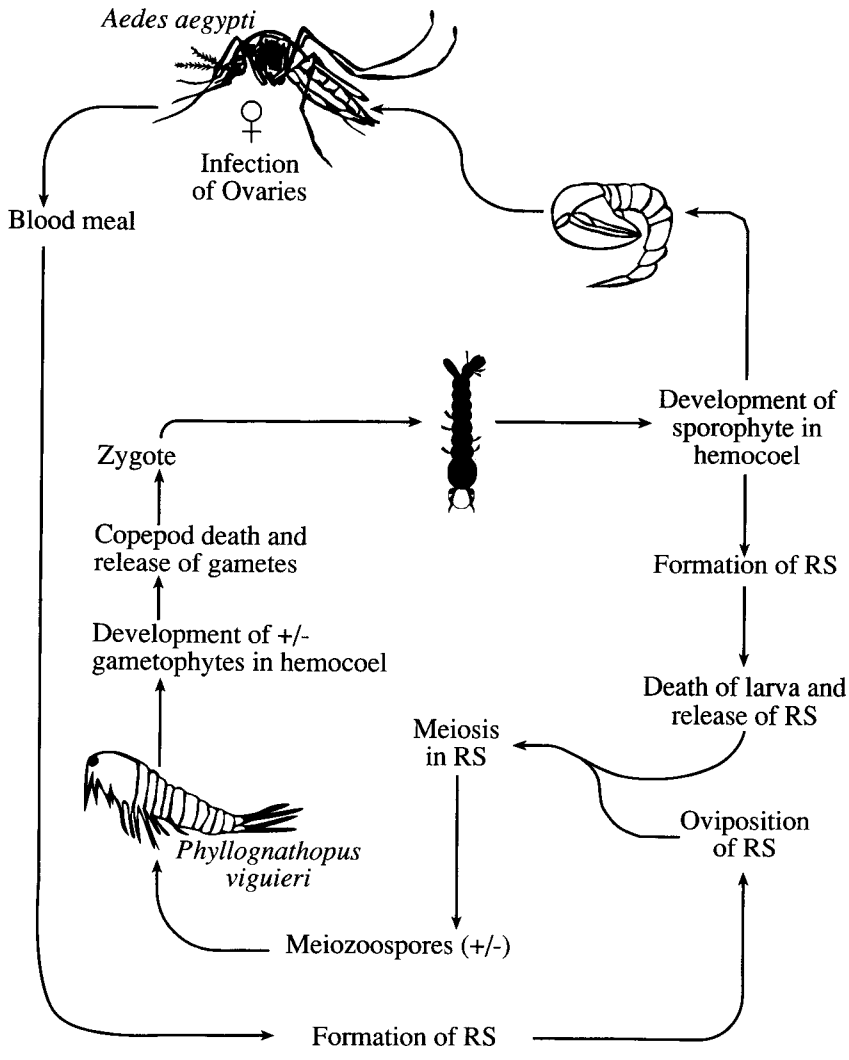

Fig. 2. Life cycle of *Coelomomyces stegomyiae.*

transmission) (Andreadis and Hall 1979b; Andreadis 1983, 1985b; Sweeney et al. 1988, 1989).

Hurst (1991, 1993) and Sweeney et al. (1989) have hypothesized that in those aquatic habitats where copepods are abundant at the appropriate time, the most effective long-term strategy for *Amblyospora* may be to increase meiospore production by producing such infections in female as well as male progeny, even though there would be a corresponding decrease in the frequency of transovarial transmission to the next generation. One might also predict that where copepods are sparse, natural selection would favor the evolution of benign oenocytic infections in females that would allow for continuous transfer of the microsporidium through female progeny. Sweeney et al. (1989) have shown that the proportion of a mosquito population that develops each type of infection (patent with meiospores and benign with transovarial transmission) can be influenced by genetic selection in laboratory experiments in as few as 4 generations. This suggests that the expression of each of these 2 types of infection may be quite variable. However, the degree to which such shifts in parasite development occur in natural mosquito populations and are driven by the abundance of scarcity of copepods is presently unknown.

The evolution of polymorphism and the production of binucleate spores that function solely in ovarian infection within the female host mosquito would suggest a strong selective pressure

toward reliance on this method of transmission for survival in *Amblyospora*. This is in contrast to other species of vertically transmitted microsporidia, such as *Nosema* for example, that utilize the same spore for both horizontal and vertical routes (Canning and Hulls 1970). These microsporidia generally rely more heavily on horizontal pathways and are typically less efficiently transmitted via the egg.

The mechanisms involved in ovarian infection by species of *Amblyospora* are not entirely understood. In those instances where the process has been examined in the female mosquito (Andreadis and Hall 1979a, Andreadis 1983), binucleate spores have typically been observed in close proximity to the developing oocytes. In some host mosquitoes the spores have actually been found lying between the ovarioles and oocytes within the ovariole sheath (Andreadis 1983). It is generally presumed that ovarian infection and transovarial transmission result from the direct inoculation of individual oocytes with sporoplasms from germinated spores. However, Hall (1985) has proposed an alternative hypothesis wherein the spores germinate and release the sporoplasms into the hemolymph from which they are then carried to the ovaries.

Transovarial transmission is also an important strategy for long-range dispersal of *Amblyospora* to new habitats and for establishing new foci of infection in host populations. It provides an effective mechanism for the introduction of the parasite into an aquatic environment where it can then be disseminated by horizontal means, provided an appropriate copepod host is present. *Amblyospora,* thereby, relies on the movements and oviposition activities of its definitive host mosquito, which is highly mobile.

Coelomomyces is not known to be transovarially transmitted. Instead, fungal infections in adult ovaries result in castration. Even though eggs are not produced, adult females attempt oviposition and deposit RS of the fungus instead of eggs (Lucarotti 1987). As in the case of *Amblyospora,* infected adult female mosquitoes play a role in the dissemination of the fungus to new habitats, especially in those species of *Coelomomyces* that infect small-container-breeding mosquitoes.

Transovarial transmission provides *Amblyospora* with a means of intraspecific transmission not available to *Coelomomyces.* Despite reports to the contrary (Dubitskij and Nam 1978), *Coelomomyces* does not appear to have a diploid repeating stage that would horizontally short cycle the fungus through the mosquito host. The absence of such a stage in *Coelomomyces* is particularly interesting in light of the fact that the related saprophytic fungus *Allomyces* has such a stage (Emerson 1941). As a saprophyte, however, *Allomyces* is not dependent on the presence of suitable hosts, only on the availability of substrate and appropriate environmental conditions. Diploid mitozoospores, which emerge from thin-walled sporangia, repeat the diploid stage enabling the fungus to exploit resources while available. Thick-walled RS for overwintering are produced on the same thallus with the thin-walled sporangia.

HORIZONTAL TRANSMISSION AND THE ROLE OF THE INTERMEDIATE HOST

All species of *Amblyospora* and *Coelomomyces* that have been investigated thus far appear to have evolved an indirect method of horizontal transmission that requires intermediary development in the microcrustacean host. The adaptive significance of involving another living host in the life cycle is undoubtedly complex, and is probably driven by several factors that collectively increase the likelihood of producing viable parasite progeny.

Assuming that the rate at which the parasite is acquired and disseminated horizontally within a host population is directly proportional to the number of encounters made between susceptible hosts and the parasite inoculum (i.e., spores or zoospores) (Anderson and May 1981), then a clear selective advantage can be seen in those host–parasite–host interrelationships that involve univoltine mosquitoes or multivoltine mosquitoes with distinct nonoverlapping generations. In these instances, larval mosquito hatch is usually quite synchronous and development to pupation is fairly uniform. As a result, very few if any mosquito larvae are normally present within the pool of water when mature spores for intraspecific horizontal transmission are eventually liberated from dying 4th-instar larvae. However, in many instances, copepods are still quite abundant (Andreadis 1990, Apperson et al. 1992a). The rationale for *Amblyospora* species that infect multivoltine mosquitoes with overlapping generations, where potential larval mosquito hosts are omnipresent, is less apparent.

In this regard, it is appropriate to examine and compare the transmission strategy of a closely related microsporidium, *Edhazardia aedis* (Kudo), a parasite of *Ae. aegypti.* This microsporidium is also vertically and horizontally transmitted, but unlike *Amblyospora,* it has effectively evolved a mechanism for bypassing the intermediate host by producing a perorally infectious spore directly in the mosquito host (see Becnel et al. 1989 for life cycle details). These spores are formed in transovarially infected lar-

vae via nuclear dissociation of diplokaryotic sporonts and are structurally and functionally similar to spores of *Amblyospora* that are produced in the copepod host. Meiosis and the formation of meiospores may also occur in infected larvae but this appears to be abortive, perhaps representing an earlier vestige (Becnel 1994). The selective advantage in eliminating the intermediate host is most probably driven by the biology of the host mosquito, *Ae. aegypti.* This mosquito develops almost exclusively in artificial containers and has a very limited flight range that is typically restricted to the immediate vicinity of the larval habitat. Breeding is continuous, with overlapping generations throughout much of the year in warmer climates and, under favorable summer conditions, larvae can mature in as few as 6–10 days (King et al. 1960). The omnipresence of susceptible host larvae within the container habitat thus affords ample opportunity for continuous horizontal transmission via spores that would be released with the death of infected larvae and hence there is no need for an intermediate host. The close affinity of *E. aedis* to *Amblyospora* has recently been affirmed in the finding of a new species, *Amblyospora trinus* Becnel and Sweeney, in wild populations of the predacious mosquito *Culex halifaxii* Theobald (Becnel and Sweeney 1990). The microsporidium concurrently produces both spore types (dissociation and meiospore) in more uniform proportion, thus suggesting that formation of one spore type or another may be evolutionarily labile.

Unlike *Amblyospora, Coelomomyces* overwinters free of any living host as RS. Apperson et al. (1992a) have shown that infection of *Anopheles quadrimaculatus* Say larvae by *Coelomomyces punctatus* Couch is positively correlated only to the abundance of the intermediate host *Acanthocyclops robustus* (G. O. Sars) and not to any other copepod species or measured abiotic factor. Here, and in other instances, randomly dehiscing RS would provide a continual source of inoculum against the copepod host. The only prerequisite for infection of susceptible mosquito larvae by species of *Coelomomyces* is the overlapping presence of the intermediate host. Slow continual release of meiozoospores over the mosquito and copepod breeding seasons will ensure that the fungus survives in the environment because the meiozoospores are capable of actively seeking even limited numbers of their copepod hosts (Federici and Lucarotti 1986), unlike *Amblyospora* which relies on chance encounters and passive ingestion.

Development in an intermediate host further provides *Amblyospora* with a mechanism for survival in a living host throughout most of its life cycle rather than in the abiotic environment (Andreadis 1990). This strategy eliminates the need to divert energies toward the production of a resistant free-living stage that is capable of surviving in a relatively unstable environment that is subject to periodic flooding and drying. Unfortunately, we have little knowledge of the survival capabilities of *Amblyospora* in the extra corporeal environment. Spores are highly susceptible to desiccation and have been shown (Andreadis 1991) to exhibit a significant loss in viability after storage in water for 5 months at 4°C, thus suggesting that they are relatively short-lived. The ability to invade another host also enables *Amblyospora* to more fully exploit its biotic environment and therein increase the opportunity for genetic variability and recombination.

Development in an intermediate host provides *Coelomomyces* with the means to be placed in close proximity to its primary host in the water column. The process of gametogenesis and gamete release is triggered by a temporal gating mechanism (Federici 1983, Lucarotti and Federici 1984a, Apperson et al. 1992b). This gating mechanism operates in such a way that the gametes of the 2 mating types are released simultaneously at around dusk when mosquito larvae and copepods are both near the water surface (Apperson et al. 1992b). Synchronous release increases the chance of encounters between gametes of the 2 mating types and the timing and placement of release increases the likelihood that the resultant zygotes will encounter a suitable host. The success of this gating mechanism combined with slow, continuous release of meiozoospores from RS may well have been instrumental in eliminating the need for a diploid repeating stage in the life cycle of *Coelomomyces.*

PARASITE DEVELOPMENT AND HOST PATHOLOGY

Definitive host: Horizontal transmission of *Amblyospora* to the definitive mosquito host is achieved by direct ingestion of spores formed in the copepod. Spore germination occurs within the anterior portion of the midgut of susceptible larvae and the microsporidium actively invades cells of the gastric caecae and midgut (Andreadis 1985b, 1988a). The factors governing larval host specificity and infectivity for *Amblyospora* are unknown. It has been suggested (Undeen 1976, Becnel and Johnson 1993) that resistance to microsporidian infection in larval mosquitoes may be due to the inability of the spore polar filament to penetrate the host peritrophic membrane. Differences in peritrophic membrane permeability and structure have been noted among certain species of larval mosquitoes (Clements 1992),

but these differences have yet to be associated with pathogen infectivity.

In a process that is also not understood, the microsporidium then spreads to oenocytes and muscle tissue where it enters its sexual phase of development. Gametes are produced and these undergo plasmogamy to form diplokaryotic cells (diploid). This process takes place during larval development from one stadium to the next with no apparent pathology. This adaptation provides a mechanism for transstadial transmission to the adult stage where the microsporidium can then infect the ovaries of female hosts and be transovarially transmitted. One enigmatic aspect in this phase of the life cycle is the apparent lack of function of horizontally transmitted infections in male mosquitoes. Laboratory (Andreadis 1988a; Sweeney et al. 1989, 1990) and field studies (Andreadis 1985b, 1990) have shown that males are equally or even more susceptible to infection following the ingestion of spores. However, the microsporidium is unable to invade gonadal tissue and there is no venereal or paternal-mediated vertical transmission. Therefore, males are dead-end hosts for horizontally acquired infections. We can only assume that this lethal trait in male hosts is accordingly offset by the reproductive success in female hosts and the highly efficient nature of transovarial transmission.

In many species of *Amblyospora,* such as *A. connecticus,* sporulation and subsequent ovarian infection in adult female mosquitoes do not occur until a blood meal is taken and appear to be controlled by titers of host reproductive hormones, specifically 20-hydroxyecdysone (Lord and Hall 1983). This phenomenon is a highly significant adaptation that clearly serves to increase transovarial transmission efficiency by ensuring that spore formation is well synchronized with oocyte development. Parasites reproduce asexually in these female hosts but produce no apparent pathology that might have a detrimental effect on those factors such as host longevity, mating success, or fecundity that would, in turn, negatively impact the transfer of the parasite to the next generation (Andreadis and Hall 1979b; Andreadis 1983, 1985b; Sweeney et al. 1989). There is also little or no multiplication within the embryos of most transovarially infected eggs (Andreadis and Hall 1979a, Andreadis 1983). This is an equally significant strategy because it ensures that host mosquitoes do not succumb to infection while still within the egg and thus defeat the purpose for which transovarial transmission has evolved (Canning 1982). Many species of *Amblyospora* that infect northern *Aedes* mosquitoes have further adapted to successfully overwinter in diapausing eggs as well (Andreadis 1985b, 1990). In some species of *Amblyospora* sporulation may proceed independently of the physiological and nutritional status of the host and is not intimately related to egg development or bloodfeeding (Sweeney et al. 1989). These species, however, do not appear to be as efficiently transmitted as those that require a host blood meal for sporulation. It is not presently known whether the former represents a more primitive condition or whether it results from greater overall reliance on horizontal routes of transmission for these particular species.

Parasite development in larvae infected with *Amblyospora* is generally restricted to fat body tissue where the microsporidium undergoes meiosis and a prolonged sporulation sequence to produce large numbers of meiospores as the larvae progress through 3 successive molts (Andreadis and Hall 1979a, Andreadis 1983). The confinement to fat body tissue results in little or no disruption of metabolic processes that are essential for host survival. This allows *Amblyospora* to produce a greater number of spores in older, larger larvae. Hurst (1991, 1993) has argued that the killing of male larvae at the 4th stadium is an optimizing strategy for maximizing spore production and the potential for horizontal transmission, because vertical transmission is not possible with males anyway. However, this strategy does place other limitations on horizontal transmission, as spore release into the environment can only take place with the disintegration of infected tissues following death of the host. This contrasts directly with development within the alimentary tract where more rapid and continuous expulsion of infective inocula (spores) would be possible through all instars.

Gametes of *Coelomomyces* pair by mating type (+ and −) (Whisler et al. 1975) and seek the appropriate mosquito host. Kerwin (1983) has implicated larval cuticular carbohydrates in the zygote host-recognition process. Zygotes of *Coelomomyces psorophorae* Couch were found to encyst most frequently at the bases of the anal gills, at intersegmental membranes, and on the head than on other parts of the body (Travland 1979, Zebold et al. 1979). There appears to be no difference in susceptibility to infection between male and female larvae. The cyst forms a small appressorium from which a penetration tube punctures the larval cuticle. The fungal cytoplasm migrates through the tube and into an epithelial cell (Travland 1979). From the epithelial cell, the fungus enters the hemocoel and develops at the expense of the host fat body (Couch 1968, Roberts 1974). Resting sporangia are produced at hyphal tips and branches (Iyengar 1935, Umphlett 1964, Martin 1969) toward the end of development. Depending on the host, time of infection, and species of *Coelomomyces,* between 10,000 and 60,000 RS may be formed in a 4th-

instar larva (Couch 1972, Federici and Chapman 1977).

In adult female mosquitoes, *Coelomomyces* occupies the interstitial spaces of the ovaries (Lucarotti and Klein 1988). The migration of hyphae from the hemocoel to the ovaries occurs during the late pupal stage and in the young adult (Lucarotti 1992). The only mosquito cells known to be directly invaded by hyphae are epithelial (Travland 1979, Lucarotti 1992). Lucarotti (1992) has suggested and provided evidence that the transfer of at least some of the hyphae from the hemocoel to the ovaries is effected when hyphae invade epithelial cells that penetrate into developing ovaries to produce tracheoles (Wigglesworth 1991). In *Ae. aegypti,* females are competent to mate and take a blood meal within 72 h of eclosion (Lea 1968, Gwadz and Spielman 1973). Within 72 h of eclosion the ovaries of infected adult females are full of *C. stegomyiae* hyphae but the hyphae will not develop to RS without a blood meal (Lucarotti and Klein 1988).

The maturation of *C. stegomyiae* hyphae into RS is also stimulated by the pulse of 20-hydroxyecdysone that peaks 18 h after a blood meal has been taken by *Ae. aegypti* (Shapiro et al. 1986, Lucarotti 1992). In order for *Coelomomyces* to utilize the mating, feeding, and oviposition behavior of the adult female mosquito for dispersal, it is essential that it does not damage the cells of the ovary and thereby interrupt the hormonal feedback system that controls these behaviors (for a review see Hagedorn 1986). The hormonal feedback system is required beyond the first blood meal because not all of the hyphae mature with a single blood meal (Lucarotti and Klein 1988). It is significant that maturation of the RS takes 72 h (Lucarotti and Klein 1988), the same time as for eggs in healthy females (Christophers 1960). Infected adult female *Ae. aegypti* oviposit RS of *C. stegomyiae* instead of eggs and the meiozoospores that emerge from the RS must infect *P. viguieri* (Lucarotti 1987).

Adult male *Ae. aegypti* can also harbor *C. stegomyiae* infections but hyphae are not localized in the gonads, the fungus cannot be venereally transmitted, and RS are present even though males do not take blood meals (Lucarotti 1987).

As an intracellular parasite, *Amblyospora* is sheltered from host immune responses by the host cell that it inhabits. *Coelomomyces,* on the other hand, is an extracellular parasite in constant contact with host hemolymph and hemocytes and yet is neither encapsulated by hemocytes nor melanized. The hyphae of *Coelomomyces* lack a cell wall (Martin 1969, Whisler et al. 1972). Lucarotti and Federici (1986) have shown, however, that the hyphae are covered by a fibrous coat that is apparently free of polysaccharides. Circumstantial evidence indicates that the infected larvae are still capable of responding to foreign polysaccharides but that, as the walls of the RS are formed, wall polysaccharides are laid down beneath the fibrous coat (Lucarotti and Federici 1986). It would appear then that the hyphae are somehow masked from internal host defenses. Once RS have begun to form, the larva is unable to pupate and eventually dies and decomposes, releasing the RS to the environment. It is not known what factors at the onset of infection affect the course to larval death or pupation and eclosion of infected adults in interactions such as those between *C. stegomyiae* and *Ae. aegypti* but, larval instar at the time of infection, inoculum, larval nutrition, and ambient temperature have all be suggested.

Intermediate host: As in the mosquito host, a strategy of tissue selectivity and delayed mortality occurs in the intermediate copepod host infected by *Amblyospora.* Here, parasite development is unikaryotic and comparatively simple (Andreadis 1985a, 1988b; Sweeney et al. 1988; Becnel 1992). In most copepods, infections are localized within female ovarian tissue. This inhibits egg production but does not seriously impede the normal metabolic processes of its host. This allows the microsporidium to multiply and produce larger numbers of spores that will directly facilitate horizontal transmission to the mosquito host and thereby enhance its survival (Andreadis 1988b). Likewise, *Coelomomyces* develops in the hemocoel of the copepod, occupying virtually all available space (Whisler et al. 1975). The copepod nauplius is most susceptible to infection but mature gametophytes of the fungus are found in the adults (Federici 1980). Thus, the fungus is able to maximize production of gametes by developing in the open hemocoel until the copepod has reached its optimal size.

CONCLUDING REMARKS

Evidence from ribosomal RNA sequences suggests that the lineage leading to the microsporidia separated very early in evolutionary time from the branch leading to other eukaryotes (perhaps as early as 2.9–2.7 billion years BP) (Vossbrinck et al. 1987). The Chytridiomycetes may have diverged from the main lineage of terrestrial fungi 550 million years BP (Berbee and Taylor 1993). Thus, *Amblyospora* and *Coelomomyces* belong to 2 distinct and unrelated assemblages of Protista (Corliss 1984) and have adopted the same definitive and intermediate hosts through convergent evolution. Species of *Amblyospora* and *Coelomomyces* can be found in the same habitats and could, potentially, be in competition for the same hosts.

The evolution of polymorphism and the production of 3 different spore types, each with a highly specialized function, has enabled members of *Amblyospora* to more fully exploit each host stage and maximize both vertical and horizontal transmission efficiency. Meanwhile, *Coelomomyces,* with motile meiozoospores and gametes, is able to seek out appropriate hosts even when host numbers are limited. As with many symbiotes, the development of these 2 parasites is well synchronized with development of each respective host stage.

The impetus to study natural enemies of mosquito vectors has been the need to find alternatives to chemical control. *Coelomomyces* and *Amblyospora* are probably not suitable for use as microbial insecticides at this time, as neither can be cultured *in vitro* and both have complex life cycles with a requirement for an intermediate host. However, *Coelomomyces* and *Amblyospora* are part of a complex of biotic and abiotic factors that affect the natural dynamics of mosquito populations. Both may have potential for use in classical biological control because they have been reported to cause epizootics, they can cycle in the environment, and, via infection of adult female mosquitoes, they have the potential for long-range dispersal.

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