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Life Cycle of *Amblyospora indicola* (Microspora: Amblyosporidae), a Parasite of the Mosquito *Culex sitiens* and of *Apocyclops* sp. Copepods

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The life cycle of *Amblyospora indicola*, a parasite of the mosquito *Culex sitiens*, was revealed by field observations and laboratory infection experiments conducted in Australia. In northern Queensland, infected *C. sitiens* larvae were often found breeding in association with two cyclopoid copepods: *Apocyclops dengizicus* and an undescribed species of the same genus. The latter species was found to be an intermediate copepod host of this microsporidium whereas *A. dengizicus* was not. One complete cycle of the parasite extends over two mosquito generations (by transovarial transmission from females with binucleate spores to their eggs) and by horizontal transmission between mosquitoes and copepods. The latter involves horizontal transmission from mosquitoes to copepods via meiospores produced in larval fat body infections and horizontal transmission from copepods to mosquitoes via uninucleate spores produced within infected copepods. Uninucleate clavate spores were formed in *Apocyclops* sp. nov. copepods 7–10 days after exposure to larval meiospores and were infectious to larvae of a microsporidian-free colony of *C. sitiens*. The development of *A. indicola* within mosquito larvae exposed to infected copepods is similar to that of *A. dyxenoides* infecting *C. annulirostris*. It proceeds from stages with a single nucleus to diplokaryotic binucleate cells in oenocytes. These stages persist through pupation to adult emergence after which time a proportion of male mosquitoes and female mosquitoes may develop binucleate spores without the need for a blood meal. A proportion of both male and female larval progeny of infected females with binucleate spores develop patent fat body infections via transovarial transmission and die in the fourth larval instar. Oenocytic infections were not observed in any survivors of patently infected larval batches in which some individuals developed fat body infections. This implies that transovarial transmission only takes place for one mosquito generation after exposure to copepods and that there is an obligate alternation between mosquitoes and copepods during each cycle of the parasite. It is considered that *A. pinensis*, described by Kettle and Piper (1988, *Eur. J. Protistol.*, 23, 229–241), infecting *C. sitiens* in Australia, is not a valid species and that it is a synonym of *A. indicola*.

KEY WORDS: *Amblyospora indicola; Amblyospora pinensis;* microsporidium; *Culex sitiens;* mosquito; *Apocyclops* sp.; copepods; life cycle; horizontal transmission; vertical transmission.

INTRODUCTION

Members of the genus *Amblyospora* are very common obligate parasites of culicine mosquitoes which have been recorded from many localities throughout the world (Hazard and Chapman, 1977). These microsporidia are transmitted vertically via transovarial transmission from infected adult female mosquitoes to their larval offspring and many of these reports have been restricted to observations of fat body infections which develop in transovarially infected larvae. It is only recently that cytological observations on development of *Amblylospora* in mosquito hosts (Hazard and Brookbank, 1984), field investigations of epizootiology (Andreadis, 1983; 1985a), and the discovery that they have intermediate copepod hosts (Sweeney et al., 1985) have revealed their complete life cycles and permitted comprehensive studies of their biology and host/parasite relationships.

Such detailed studies have only been reported for two species: *A. dyxenoides* infecting *Culex annulirostris* mosquitoes and *Mesocyclops albicans* copepods in Australia (Sweeney et al., 1985; 1988; 1989a, b); and *A. connecticus* infecting *Aedes canta-*

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tor mosquitoes and *Acanthocyclops vernalis* copepods in Connecticut (Andreadis, 1983; 1985b; 1988). Similar investigations of other species are required in order to explore the full spectrum of possibilities for the development of this important group of microsporidia within their invertebrate hosts. These investigations will need to focus on such aspects as host specificity in mosquitoes and copepods, expression of the parasite in mosquitoes following transovarial transmission, and epizootiology. These areas of study will facilitate the species identification of the various members of the genus and permit their evaluation for biological control.

A microsporidian parasite found in the larval fat body of the salt marsh mosquito *Culex sitiens* collected at Pondicherry, India, was described as *Amblyospora indicola* (Vavra et al., 1984). This mosquito has a wide distribution in the Australasian, Asian, and African regions (Knight and Stone, 1977). We have collected *C. sitiens* infected with this microsporidium in northern Queensland and in this paper we describe its life cycle in mosquitoes and copepods.

MATERIALS AND METHODS

Field Collections of Copepods and Infected Mosquitoes

Surveys made during 1987 over a 200-km stretch of the north Queensland coast between Cairns and Townsville revealed populations of *C. sitiens* infected with *A. indicola* in brackish situations throughout this area. Larvae with patent fat body infections together with copepods collected from the same breeding sites were airfreighted to Ingleburn at regular intervals. Examination of the copepods from these collections revealed that there were two cyclopoids which were common in association with patently infected larvae. One of these was identified as *Apocyclops dengizicus* whereas the other was an undescribed species of *Apocyclops*. The latter was distinguished from *A. dengizicus* by the morphology of the fifth leg of adult females as well as other characteristics (D. Morton, pers. commun.). Pending its formal description it will be referred to as *Apocyclops* sp. nov. Both copepod species were found together in some collections whereas in other sites only one species was collected. Collections were also made at Homebush Bay, Sydney, New South Wales, in which *Apocyclops* sp. nov. was the only cyclopoid copepod present.

Copepod Infection Experiments

Field collections containing only one of these two copepod species were used for infection experiments by placing ca. 500–2000 adult copepods into plastic trays containing 500 ml of water from their collection sites. They were then held in our laboratory at temperatures of 22–26°C. Dead or moribund *C. sitiens* larvae infected with *A. indicola* were homogenized and added to the trays of copepods at concentrations between 10^4 and 2×10^5 meiospores/ml. For each experiment, 20–40 copepods from the test trays were removed between 6 and 13 days after exposure to meiospores. They were then smeared, stained with Giemsa, and examined for the presence of microsporidian infections. Trays of copepods not exposed to meiospores were included in each experiment as a control and were stained and examined for infection at the same time as the test copepods.

Mosquito Infection Experiments

A colony of microsporidian-free *C. sitiens* was established in our laboratory at Ingleburn from ca. 1000 larvae of this species collected at Cairns, Queensland. The larvae were reared in 30% sea water and the adults were maintained in 0.03 m^3 cages for mating, blood feeding, and oviposition. For mosquito infection experiments batches of 100–200 first-instar larvae from the colony were added to trays of water containing copepods of *Apocyclops* sp. nov. (infected

by exposure to *A. indicola* meiospores as described above) in which the presence of uninucleate spores was confirmed by microscopic examination. These larvae were reared in the trays with infected copepods and the pupae were placed in cages for adult emergence and mating. The females were blooded and then transferred into individual vials of water for oviposition. After the eggs were laid the females were smeared, stained with Giemsa, and examined for the presence of *Amblyospora* infection. The larval progeny of females found to be infected were reared in separate sibling batches. For each experiment, control trays of larvae were reared using our normal insectary procedures without exposure to copepods. The adults which emerged from these trays were Giemsa stained and examined for microsporidian infections.

RESULTS

Copepod Infection Experiments

In four experiments using collections of *A. dengizicus* from north Queensland (from Cairns and Townsville), 110 copepods were examined but none were infected (Table 1). On the other hand, in another four copepod infection experiments using two separate field collections of *Apocyclops* sp. nov. from north Queensland (from Cowley Beach and Cardwell) as well as one collection from Homebush Bay, 92.5% (132/143) of copepods showed the presence of microsporidian infections.

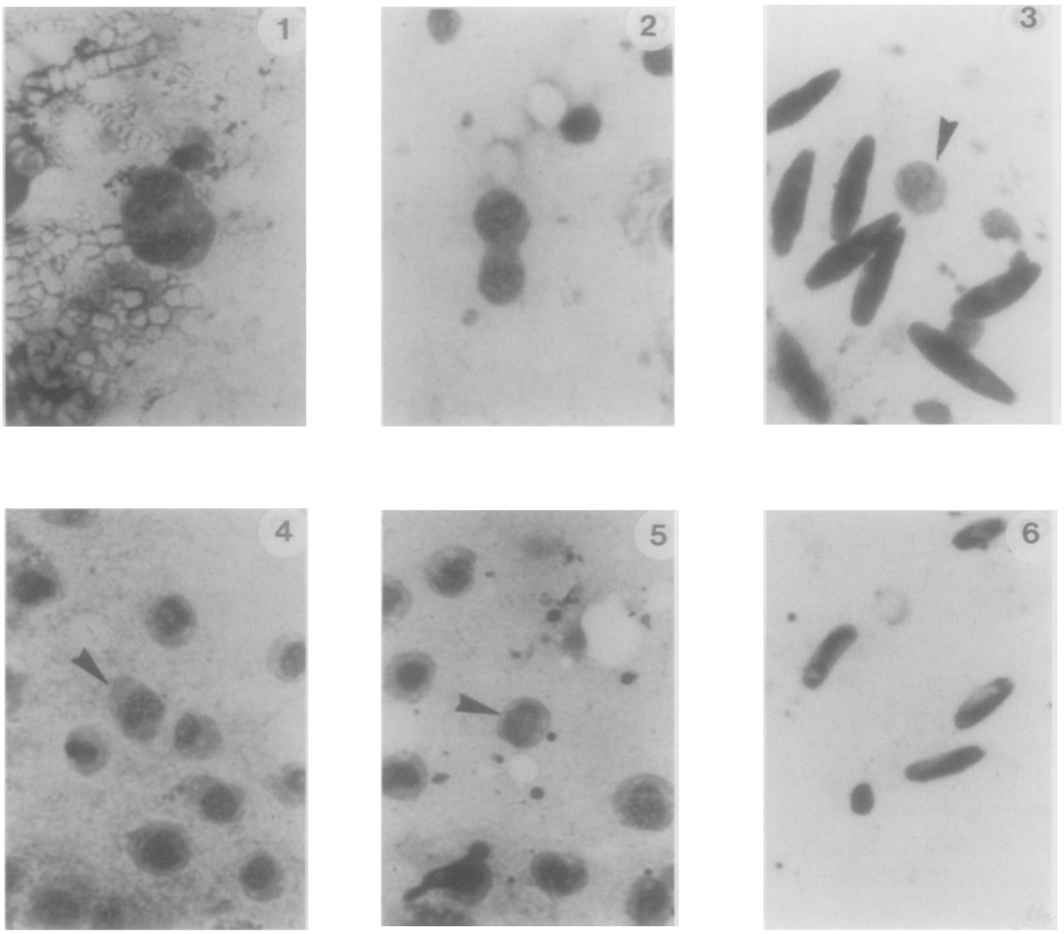
The infections developed within ovarian tissue of copepods (Figs. 1–3). Spherical cells with a large nucleus were seen in Giemsa smears of infected copepods within 5–7 days after exposure to larval meiospores. These multiplied by binary fission to form binucleate and sometimes quadrinucleate stages within ovarian tissue and ultimately formed uninucleate clavate spores 7–10 days after exposure to meiospores. Stained spores measure 12.4 ± 3.8 μm long by 3.8 ± 0.7 μm wide. None of the control copepods were found to be infected with these microsporidian stages.

Copepod Transmitted Mosquito Stages

Uninucleate spherical and pyriform stages were observed in Giemsa smears of early instar larvae of *C. sitiens* exposed to infected copepods. Some of these cells appeared to undergo plasmogamy (similar to the stages of *Culicospora magna* illustrated by Hazard et al., 1985) and subsequently developed to diplokaryotic stages in oenocytes of late instar larvae. Sometimes the uninucleate cells persisted through the pupal stage and subsequently proliferated in both adult females and males (Figs. 4, 5). Examination of Giemsa smears of adult mosquitoes, which were exposed to uninucleate spores from copepods as larvae (Table 2), showed that the infection rate in

TABLE 1
SUSCEPTIBILITY OF *Apocyclops dengizicus* AND *Apocyclops* SP. NOV. COPEPODS TO MEIOSPORES OF *Amblyospora indicola*

Copepod	Collection locality	Meiospore concentration	Number examined	% infected
Apocyclops dengizicus	Cairns	10^4	30	0
Apocyclops dengizicus	Cairns	3×10^4	40	0
Apocyclops dengizicus	Cairns	10^5	20	0
Apocyclops dengizicus	Townsville	5×10^4	20	0
Apocyclops sp.nov.	Cowley	10^4	13	92.3
Apocyclops sp.nov.	Cowley	5×10^4	23	73.9
Apocyclops sp.nov.	Cardwell	5×10^4	77	94.8
Apocyclops sp.nov.	Homebush	2×10^5	30	100



FIGS. 1–3. Light micrographs of *Amblyospora indicola* in *Apocyclops* sp. nov. copepods. (giemsa stained, ×1360). Figures 1 and 2 dividing binucleate stages. Figure 3, uninucleate meront (arrowed) and uninucleate spores.

FIGS. 4–6. Light micrographs of *Amblyospora indicola in Culex sitiens* mosquitoes. (Giemsa stained, Figures 4 and 5 ×1360; Figure 6 ×1020). Figures 4 and 5, uninucleate stages in adult male exposed as larva to infected copepods. Arrows indicate possible sites of cytoplasmic fusion of adjacent gametes. Figure 6, binucleate spores in adult male infected via copepods.

adult male mosquitoes (59/104) was much higher than that in females (12/83). In the majority of infected specimens, mature binucleate spores (Fig. 6) were formed in nulliparous females (which had not been provided with blood meals) and in males as well as in parous females. Some specimens showed the presence of diplokaryotic stages only and in 2 males a mixture of diplokaryotic and early uninucleate stages were observed. None of the control mosquitoes examined in these experiments were found to be infected with microsporidia.

Transovarially Transmitted Mosquito Stages

Some of the larvae which hatched from egg batches laid by infected females developed fat body infections which ultimately resulted in the formation of groups of eight uninucleate meiospores within a sporophorous vesicle as reported by Vavra et al. (1984). The proportion of larvae developing

TABLE 2

INFECTIONS OF *Amblyospora indicola* IN ADULT MALES, NULLIPAROUS FEMALES, AND PAROUS FEMALES OF *Culex sitiens* MOSQUITOES EXPOSED AS FIRST-INSTAR LARVAE TO INFECTED *Apocyclops* SP. NOV. COPEPODS SHOWING OVERALL INFECTION RATES AS WELL AS PERCENTAGES OF INFECTED INDIVIDUALS WITH BINUCLEATE SPORES OR VEGETATIVE STAGES

Adults	Number examined	% infected overall	% infected vegetative stages	% infected spores
Males	104	60.6	7.3	93.7
Nulliparous[a] females	84	16.7	14.2	85.7
Parous females	342	12.2	16.6	83.3

[a] Not provided with blood meals.

such infections was variable with infection rates in the larval progenies of 18 infected female *C. sitiens* ranging from 0 to 88.5% and averaging 20.4%. The larvae from these infected batches which did not develop patent meiospore infections were reared to adulthood. Of those which survived to the adult stage, 266 (142 males and 124 females) were smeared, stained with Giemsa, and examined for microsporidian infection. Oenocytic infections were not observed in any smears of these adult mosquitoes. Only 3 males and 1 female showed the presence of meiospores whereas the remainder were uninfected.

DISCUSSION

In a recent study of parasites of larval culicine mosquitoes in southeast Queensland, Australia *Amblyospora pinensis* was described from *C. sitiens* (Kettle and Piper, 1988). The criteria on which the latter taxon was distinguished from *A. indicola* included differences in size of meiospores, ratio of narrow to wide coils of the polar filament, and geographical location. There were small differences in mean size of fresh meiospores but the size range from the two localities overlapped. Minor apparent differences of the polar filament of the two collections may have been due to intraspecific variability as well as variations in orientation of specimens under the electron microscope. The morphologies of meiospores and vegetative stages of *Amblyospora* parasites in the larval fat body of different mosquito species are very similar. Consequently, it is difficult to assign species identities to these microsporidia based solely on light microscope and electron microscope observations of these stages without considering parasite developmental cycles and host specificity. The question of geographical separation as a taxonomic criterion for these microsporidia has not been addressed at this time but, on the basis of current knowledge, we believe that the *Amblyospora* sp. infecting *C. sitiens* in Australia should be designated as *A. indicola*.

We find it particularly interesting that *Apocyclops* sp. nov. is a susceptible copepod host of *A. indicola* whereas *Apocyclops dengizicus* is not. Both copepods are closely related species which are commonly found together sharing the same breeding sites with *C. sitiens* larvae infected with this microsporidium. It would be interesting to determine the copepod intermediate host of *A. indicola* in other geographical areas including the Type Locality in India.

The present study indicates that the life cycle of *A. indicola* is very similar to that of *A. dyxenoides* (Sweeney et al., 1988). The developmental sequences of both microsporidia in copepods appear to be identical under the light microscope. Moreover, the early uninucleate stages of both parasites within mosquito larvae (derived via horizontal transmission from infected copepods) are very similar in appearance. These develop to diplokaryotic cells within larval oenocytes following gametogenesis and plasmogamy (Hazard et al., 1985). Also, the meiotic sequences in the larval fat body of *C. annulirostris* infected with *A. dyxenoides* appear identical to those in *A. indicola*-infected larvae of *C. sitiens*. Both parasites have the ability to develop mature binucleate spores within infected male mos-

quitoes as well as in females without the need for a blood meal. This characteristic is in contrast to other *Amblyospora* in which sporulation is influenced by host hormones and normally only takes place in females after a blood meal (Lord and Hall, 1983; Hall and Washino, 1986).

The finding that male *C. sitiens* are more susceptible to *A. indicola* infection than females is in accordance with the observations of Andreadis (1988) on the infection of *Aedes cantator* mosquitoes with *Amblyospora connecticus*. On the other hand, both male and female *C. annulirostris* are equally susceptible to infection with *A. dyxenoides* (Sweeney et al., 1989a). The only other difference which we have observed in the mosquito cycles of the two Australian microsporidia is in the transovarially transmitted pathway. A proportion of the larval progeny of *C. annulirostris* females infected with *A. dyxenoides* develop either fat body (patent) infections or benign infections (in which the microsporidium is restricted to larval oenocytes) or are uninfected (Sweeney et al., 1988). On the other hand, the larval progeny of *C. sitiens* females infected with *A. indicola* either are fat body infected or are uninfected. Our observations that oenocytic stages do not develop in transovarially infected *C. sitiens* imply that, in this case, transovarial transmission only occurs for one generation after exposure to infected copepods.

Meiospores formed in fat body infections are responsible for initiating horizontal transmission to copepods whereas larval oenocytic stages persist as benign infections in the mosquito host for another transovarially transmitted cycle. It has been shown by Andreadis and Hall (1979) that these microsporidia cannot be maintained by transovarial transmission alone for more than a few generations so that horizontal transmission is a prerequisite for their survival. Nevertheless, there are considerable differences between different mosquito species in the proportion of transovarially infected progeny which acquire each kind of infection (Kellen et al., 1965). It has been shown that the proportion of transovarially infected *C. annulirostris* acquiring oenocytic infections of *A. dyxenoides* can be increased by selection experiments (Sweeney et al., 1989a) which suggests that the observed differences in *Amblyospora* spp. infecting different mosquitoes may be partly explained by genetic selection. For example, if the copepod host is not always present in the larval habitats of the mosquito host, then the parasite may respond to this situation by increasing the proportion of benign oenocytic infections in mosquitoes. This may assist its maintenance by the transovarial transmission pathway without the need for continuous horizontal transmission.

On the other hand, in mosquitoes infected with some other microsporidia, such as *Aedes taeniorhynchus* infected with *Amblyospora polykarya* (Lord et al., 1981) and *C. sitiens* infected with *A. indicola* (as shown in this study), all transovarially infected progeny develop fat body infections. In these cases, horizontal transmission via copepods is required between each mosquito generation to complete the life cycle and maintain the microsporidium within host populations. One possible phylogenetic explanation for this kind of developmental cycle could be that, in these instances, horizontal transmission is very reliable (due to the continuous presence of the two invertebrate hosts in the aquatic environment) and that selection has led to an increase in the development of fat body infected larvae and the elimination of superfluous oenocytic infections.

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