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NOTES

Host Specificity Studies of *Amblyospora indicola* and *Amblyospora dyxenoides* (Microspora: Amblyosporidae) in Mosquitoes and Copepods

Microsporidia of the genus *Amblyospora* are common mosquito parasites which are characterized by two sporulation sequences within infected mosquitoes (E. I. Hazard, E. A. Ellis, and D. J. Joslyn, 1981, *In* "Microbial Control of Pests and Plant Diseases, 1970–1980," H. D. Burges, Ed., pp. 163–182, Academic Press, New York). One sequence leads to the formation of binucleate spores within adult females and the other leads to the production of meiospores within the fat body of infected larvae. The vegetative stages and spores of *Amblyospora* spp. infecting different mosquito species are morphologically similar and it is not possible to experimentally transmit various isolates between mosquito hosts as the meiospores are not infectious to mosquito larvae. This precluded definitive studies on their taxonomic relationships until the recent discovery that the meiospores infect copepods (A. W. Sweeney, E. I. Hazard, and M. F. Graham, *J. Invertebr. Pathol.* **46**, 98–102, 1985). A third kind of spore formed within the copepod host transmits the microsporidium to mosquito larvae to complete the life cycle. This finding has opened up a new avenue of research for this group of microsporidia by permitting transmission experiments between different mosquitoes via the intermediate copepod hosts. This approach has recently been used to investigate the host specificity of *Amblyospora connecticus* (T. G. Andreadis, *J. Med. Entomol.* **26**, 140–145, 1989). It is also possible to employ this experimental method to determine the specific taxonomic identity of *Ambylospora* spp. infecting closely related mosquito species.

Our previous field and laboratory observations of *Amblyospora* in Australia have revealed the complete life cycle and natural prevalence of *Amblyospora dyxenoides* in its copepod host, *Mesocyclops albicans,* and in its mosquito host, *Culex annulirostris* (A. W. Sweeney, M. F. Graham, and E. I. Hazard, *J. Invertebr. Pathol.* **51**, 46–57, 1988; A. W. Sweeney, S. L. Doggett, and G. Gullick, *J. Invertebr. Pathol.* **53**, 85–92, 1989; A. W. Sweeney, S. L. Doggett, and G. Gullick, *J. Invertebr. Pathol.* **53**, 118–120, 1989). This mosquito is a close phylogenetic relative of the salt marsh mosquito *Culex sitiens* within the subgenus *Culex*. The larval and adult stages of both mosquitoes are very similar in appearance. Also, typical brackish breeding sites of *C. sitiens* in Australia—grassy coastal pools partly inundated with seawater following high tides—resemble the vegetated fresh water pools which are common breeding sites of *C. annulirostris*. Recent studies (A. W. Sweeney, S. L. Doggett, and R. G. Piper, *J. Invertebr. Pathol.* **55**, 428–434, 1990) have shown that the intermediate copepod host of *Amblyospora indicola* infecting *C. sitiens* in Australia is an undescribed species of *Apocyclops* and that the life cycle of *A. indicola* is very similar to *A. dyxenoides*. Also, the developmental stages of these two microsporidia appear very similar in both species of mosquitoes and copepods. Consequently, there was the possibility that they may be conspecific. In order to investigate the relationships of these microsporidia, we conducted experiments on the susceptibility of each mosquito and copepod species to *A. dyxenoides* and *A. indicola.*

The mosquitoes used in these experiments were from microsporidian—free col-

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onies of *C. annulirostris* and *C. sitiens* maintained in our laboratory at Ingelburn. Separate *Amblyospora*-infected colonies of these two mosquitoes were also maintained using procedures described previously (A. W. Sweeney, E. I. Hazard, and M. F. Graham, *J. Invertebr. Pathol.* **46**, 98–102, 1985; A. W. Sweeney, S. L. Doggett, and R. G. Piper, *J. Invertebr. Pathol.* **55**, 428–434, 1990). The intermediate host of *A. indicola, Apocyclops* sp. nov., collected in a salt marsh site at Homebush Bay, Sydney, New South Wales, as well as an unidentified species of *Mesocyclops* from a freshwater site near the same locality were used for a series of infection experiments to determine their relative susceptibility to *A. indicola* meiospores produced in *C. sitiens* and to *A. dyxenoides* meiospores produced in *C. annulirostris*. Water from the collection sites was passed through 300-μm sieves to concentrate a mixture of copepodids and adult copepods which were used for these experiments. The test copepods were placed in trays of water from their respective collection sites and were exposed to 10^5 meiospores/ml using procedures described previously (A. W. Sweeney, S. L. Doggett, and R. G. Piper, *J. Invertebr. Pathol.* **55**, 428–434, 1990). Following the addition of meiospores the test copepods were maintained in the laboratory for 10 days at 22–24°C after which samples of copepods from each experimental tray were removed, stained with Giesma, and examined for the presence of *Amblyospora* infection.

A. dyxenoides is associated with *Mesocyclops* sp. copepods and *C. annulirostris* mosquito larvae in freshwater sites whereas A. indicola is normally found infecting *Apocyclops* sp. copepods and *C. sitiens* larvae in brackish water. There was the possibility, therefore, that the meiospores of the former parasite may have been adversely affected by salinity during exposure of *Apocyclops* sp. copepods. Conversely, meiospores of the latter parasite may have been affected by fresh water during exposure to *Mesocyclops* sp. copepods. An experiment was performed to investigate this possibility in which a field collection of each copepod species was made at Homebush Bay and the water was removed by passing through a 300-μm sieve. The *Apocyclops* sp. copepods were placed into trays of river water (from a freshwater stream near Sydney which is used for rearing our mosquito colonies) and exposed to meiospores of *A. dyxenoides*. The *Mesocyclops* sp. copepods were placed into trays of 25% seawater (by diluting water from the sea near Sydney with river water) and were exposed to meiospores of *A. indicola*. The conditions under which the copepods were exposed to the meiospores, maintained in the laboratory, and examined for infection were similar to those of the previous experiment.

In order to further investigate the relationship between these two microsporidia, infection experiments were made by exposing *C. sitiens* larvae to *Mesocyclops* sp. copepods infected with *A. dyxenoides* and by exposing *C. annulirostris* larvae to *Apocyclops* sp. copepods infected with *A. indicola*. For each experiment, batches of 100–200 first instar larvae from the appropriate mosquito colony were added to trays of water containing copepods (*Apocyclops* sp. or *Mesocyclops* sp.) which were infected by exposure to meiospores as described above and in which the presence of uninucleate spores was confirmed by microscopic examination. These larvae were reared in the trays with infected copepods for the duration of larval development (7–10 days) and the pupae were placed in cups for adult emergence. The adults were smeared, stained with Giemsa, and examined for the presence of *Amblyospora* infection. Giemsa-stained specimens of the three kinds of spores of *A. indicola* and *A. dyxenoides* were measured with an ocular micrometer to determine their comparative lengths and widths.

The results of the copepod infection experiments (Table 1) showed that meiospores of *A. indicola* formed in *C. sitiens*

TABLE 1

RESULTS OF LABORATORY INFECTION EXPERIMENTS SHOWING SUSCEPTIBILITY OF *Apocyclops* sp. nov. AND *Mesocyclops* sp. COPEPODS TO MEIOSPORES OF *Amblyospora dyxenoides* PRODUCED IN *Culex annulirostris* AND TO *Amblyospora indicola* MEIOSPORES PRODUCED IN *Culex sitiens* WITH COPEPODS HELD IN EITHER FRESH WATER OF BRACKISH WATER

Microsporidium	"Host" mosquito	"Test" copepod	Water	No. examined	% Positive
A. indicola	*C. sitiens*	*Apocyclops* sp.	Brackish[a]	149	36.2
A. indicola	*C. sitiens*	*Mesocyclops* sp.	Fresh[a]	84	0
A. dyxenoides	*C. annulirostris*	*Mesocyclops* sp.	Fresh[a]	66	77.3
A. dyxenoides	*C. annulirostris*	*Apocyclops* sp.	Brackish[a]	120	0
A. indicola	*C. sitiens*	*Apocyclops* sp.	Fresh[b]	34	85.3
A. dyxenoides	*C. annulirostris*	*Mesocyclops* sp.	Brackish[c]	20	90

[a] Copepods held in water from their field collection sites.
[b] Water from copepod collection sites replaced with river water.
[c] Water from copepod collection sites replaced with 25% seawater.

were able to infect *Apocyclops* sp. copepods but were not infectious to *Mesocyclops* sp. copepods. On the other hand, meiospores of *A. dyxenoides* derived from *C. annulirostris* were infectious to *Mesocyclops* sp. copepods but were not infectious to *Apocyclops* sp. copepods. Infection rates of the natural copepod host of each microsporidium were higher in the second experiment when the field site water was replaced with river water (in the case of *Apocyclops* sp.) and with 25% sea water (in the case of *Mesocyclops* sp.). The age and sex composition of the copepod collections used for these experiments were not known. This may have influenced the infection rates in the two experiments as it has been shown that only females of the copepod *Acanthocyclops vernalis* are susceptible to *A. connecticus* whereas males are not susceptible (T. G. Andreadis, *J. Invertebr. Pathol.* **52**, 73–77, 1988). Nevertheless, it is apparent that the infectivity of the meiospores was not adversely affected by the salinity of the water containing the test animals.

In the infection experiment involving exposure of mosquito larvae to *Apocyclops* sp. nov. copepods infected with *A. indicola,* 77 of 166 *C. sitiens* test specimens (46.4%) became infected whereas none of 110 *C. annulirostris* mosquitoes developed microsporidian infections. Similarly, in the experiment involving exposure of mosquitoes to *Mesocyclops* sp. copepods infected with *A. dyxenoides,* 3 of 80 *C. annulirostris* became infected but none of 72 *C. sitiens* developed *Amblyospora* infections. In the latter case, it is presumed that the low infection rate (3.8%) of *C. annulirostris* to its natural parasite was due to poor infectivity of the batch of infected copepods used as inoculum. Consequently, the failure to infect *C. sitiens* in this instance does not offer conclusive evidence that it is refractory to spores of *A. dyxenoides* produced in copepods. Nevertheless, the results of the copepod infection experiments with both microsporidia as well as the mosquito infection data obtained with *A. indicola* imply that these two *Amblyospora* species are not conspecific.

The three types of spores (meiospores and binucleate spores in mosquitoes as well as the spores produced in copepods) of *A. indicola* looked the same and were within the same size ranges as those of *A. dyxenoides* (Table 2). We have previously reported that the morphological appearance of the vegetative stages of *A. indicola* in *C. sitiens* mosquitoes and *Apocyclops* sp. nov. copepods appeared identical to those of *A. dyxenoides* in *C. annulirostris* mosquitoes and *Mesocyclops* sp. copepods (A. W.

TABLE 2
COMPARATIVE SIZE OF THREE KINDS OF GIEMSA-STAINED SPORES OF *Amblyospora indicola* AND *Amblyospora dyxenoides* FROM THEIR RESPECTIVE MOSQUITO (LARVA AND FEMALE) AND COPEPOD HOSTS (n = 25, ± SD)

	Microsporidium			
	A. indicola		*A. dyxenoides*	
Spore type (host)	Length	Breadth	Length	Breadth
Meiospores (larva)	5.2 ± 0.4	4 ± 0.4	5.4 ± 0.4	4 ± 0.3
Binucleate (female)	9.6 ± 1.2	3.3 ± 0.3	9.6 ± 0.9	3.1 ± 0.4
Uninucleate (copepod)	12.4 ± 3.8	3.8 ± 0.7	11.3 ± 3.1	3.9 ± 0.8

Sweeney, S. L. Doggett, and R. G. Piper, *J. Invertebr. Pathol.* 55, 428–434, 1990). The transmission experiments reported above indicate that the two microsporidia are different species but this could not be inferred by microscopic examination of infected specimens. This highlights the difficulties of relying on gross morphology (size and shape of spores) as a taxonomic criterion for this group of microsporidia.

We have previously shown that *Apocyclops dengizicus* is not susceptible to *A. indicola* even though it is closely related to *Apocyclops* sp. nov. and is often found in breeding sites of *C. sitiens* infected with this microsporidium (A. W. Sweeney, S. L. Doggett, and R. G. Piper, *J. Invertebr. Pathol.* 55, 428–434, 1990). Several species of *Mesocyclops,* as well as the type host (*M. albicans*), are susceptible to *A. dyxenoides* (A. W. Sweeney and S. L. Doggett, unpubl.). However, the host range of this latter microsporidium in copepods has not been fully clarified at this time as the taxonomic status of all members of the genus *Mesocyclops* in Australia have not been resolved (D. Morton, pers. commun.).

Many studies are required to investigate host specificity ranges of other *Amblyospora* spp. in mosquitoes and copepods. For example, *A. connecticus,* which infects *Aedes cantator,* was successfully transmitted to four other *Aedes* mosquitoes via exposure to spores from the intermediate copepod host (T. G. Andreadis, *J. Med. Entomol.* 26, 140–145, 1989). However, in all of these species, the microsporidium was not able to infect the ovaries and complete its life cycle via transovarial transmission. These studies imply that future host range investigations of this microsporidian genus will need to include observations of vertical transmission in the mosquito host as this work on *A. connecticus* has shown that susceptibility of mosquitoes to a particular *Amblyospora* sp. via horizontal transmission is not necessarily related to the ability of the microsporidium to complete its life cycle within this host. Our previous work on this subject has been facilitated by investigations of microsporidia infecting mosquitoes which are capable of laboratory colonization but such studies may be difficult or impossible in *Amblyospora* spp. parasitizing mosquitoes which will not blood feed, mate, or oviposit in the laboratory.

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