THE BIOLOGY OF DIMORPHIC MICROSPORIDIAN PARASITES OF MOSQUITOES

by

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Thesis submitted for the Degree of Doctor of Philosophy

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AUGUST 1989

ABSTRACT

Literature on the life cycle and sexuality of polymorphic microsporidia from mosquitoes (Genera: Amblyospora, Parathelohania and Polydispyrenia in particular) and the ecology of mosquito hosts is reviewed.

A survey was made to detect microsporidian infections in mosquitoes from the field in Britain and in Tanzania with a view to using collections in experimental studies of the life cycle of polymorphic species and the involvement of alternate hosts.

In Britain microsporidia were found in mosquitoes from 5 sites. Two of these infections were octosporous and two multisporous. The sporogonic sequence of the fifth type was indeterminate as the host had died before examination. All species were described photomicroscopically and in the case of one of the multisporous species ultrastructurally also. Their taxonomic position and prevalence of infection are discussed.

In Tanzania 6 sites were found with microsporidian infections in larvae, and infected adults were collected at 2 sites. Infections in Anopheles gambiae larvae and adults were identified as Parathelohania africana Hazard and Anthony (1974). The stages in An. gambiae adults were diplokaryotic: these were transmitted to 100% of the F_1 generation, which became heavily infected with octospores. The octospores were used in transmission experiments.

Three of the sites, Kisiwani, Muheza Sisal Estate and Mamakingi, provided Mansonia africana larvae infected with an octosporous species at levels of 13.2%, 6.9% and 21.5% respectively and a multisporous species at levels of 2.5%, 5.7%, and 2.3% respectively. Benign diplokaryotic infections were seen in other Ma. africana larvae from the same sites at levels of 3.8%, 1.7% and 0.08% respectively. In adults collected near the Muheza Sisal Estate 2 types of diplokaryotic microsporidian infections were detected which differed in size. The larger type (Species B) was found in 5.7% of adult females and the smaller type (Species A) in 10.9% of adult females. W h e n progeny of adults infected with Species A were raised all infections were multisporous. It was therefore suggested that Species B in adult mosquitoes might give rise to octosporous infections in progeny. Octospores from larvae of Ma. africana and An. gambiae were not directly transmissible to larvae of the same species. All infections are described photomicroscopically and/or ultrastructurally and their generic status is discussed.

Copepods, Mesocyclops rarus, from Muheza Sisal Estate and Mamakingi were infected with a microsporidium in which spores of two sizes but similar morphology were present. Prevalences at the two sites were 24.3% and 13.5% respectively. The infection was described photomicroscopically and ultrastructurally. Transmission experiments failed to link this infection either with multisporous or octosporous infection in mosquito larvae.

The general low level of microsporidian infections in field conditions and the implications of microsporidia in biological control are discussed.

ACKNOWLEDGEMENTS

Firstly, I should like to thank my supervisor, Professor E.U. Canning for her guidance and help which has been invaluable throughout this study.

I would also like to acknowledge the financial support provided by N.E.R.C. and W.H.O. for this study and the field trips to Tanzania.

I am indebted to Dr. G.B.White, Dr.M.Gillies, Mr.C.Ramsdale, Mr.G.Smith and Mr.E.Green for their willing advice and help with collection of mosquitoes in Britain. I should also like to thank Professor W.L.Kilama and Dr.S.Irare of N.I.M.R., Tanzania, for allowing me to work at Muheza, and to Dr.C.F.Curtis, Dr.J.D.Lines, Mr and Mrs T.J.Wilkes, Mr. C. Chambika and all the team at the Ubwari Field Station for helping to make my visit so pleasant and productive.

Thanks are also due to Dr. G. Boxshall (British Museum, Natural History) for his identification of copepods, to Mr. J.P.Nicholas, Mrs. V.Walters, Mr. R.Hartley, and Mr. F.Wright for their technical assistance and to Miss X. Christophers for proof reading.

Finally, I should like to thank Mum, Dad, Jonat and Paresh for always being there.

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INTRODUCTION

Mosquitoes are known to be hosts of numerous species of microsporidia including those with complex dimorphic lifecycles, involving two host generations, e.g. Microsporidium aedis, Culicosporella lunata and Culicospora magna, (Becnel, 1986; Becnel et al., 1988). Amblyospora dyxenoides from, Culex annulirostris has been shown to have an additional generation in a copepod alternate host, Mesocyclops albicans (Sweeney et al., 1985, 1988). This alternation of hosts has also been found for Amblyospora connecticus in Aedes cantator and the copepod Acanthocyclops vernalis (Andreadis, 1985a, 1988b), for A. californica in Culex tarsalis and on unidentified copepod (Becnel, 1988) and for A. campbelli in Culiseta incidens and the copepod Diaptomus sp. (Dickson, 1988).

The genus Parathelohania is closely related to Amblyospora and is therefore likely to have a similar alternation of generations. In addition the genus Polydispyrenia from blackflies having similar features to Parathelohania and Amblyospora (diplokaryotic meronts and early sporonts but uninculeate spores), can be implicated as another possible dimorphic species (Canning and Hazard, 1982).

The aim of this study was to find a heavy dimorphic infection in mosquitoes, preferably a vector species, describe it ultrastructurally, elucidate its mode of transmission and compare it with those previously found. The ecological significance of microsporidia in natural populations, and implications in biological control were also considered.

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION TO THE MICROSPORA

Microsporidia have been well known for over 100 years. The first to be named was Nosema bombycis Nageli in 1857. N. bombycis is the cause of "pebrine" or silkworm disease, which, in part, caused the decline of the silk industry in Europe. Interest in microsporidia increased and in 1924 Kudo produced an authoritative monograph listing 14 genera and 150 species. Due to their diminutive size however, it was not until the onset of electron microscopical techniques, that the early problems of morphological identification were resolved. Microsporidia are now considered as a separate phylum of protistan organisms (Sprague, 1976; Weiser, 1977). The number of known species has now reached about 800, from almost all invertebrate phyla, and all 5 classes of vertebrates (Canning, 1989). Arthropods, then fish are the commonest hosts. Some microsporidia have occasionally been reported as pathogens of man, e.g. Encephalitozoon cuniculi and Enterocytozoon bieneusi (Canning, 1975; Desportes et al., 1988; Rijpstra et al., 1988).

Microsporidia are obligate intracellular parasites of enormous reproductive potential, characterised by their possession of thick walled spores containing a polar filament and the infective agent or sporoplasm. All microsporidian cells are structurally simple and non-specialised except for the modified spores. Each cell has one or more nuclei arranged singly or as diplokarya, surrounded by cytoplasm with all the usual organelles, except mitochondria and lysosomes, which are never present. The cell membrane is of the simple unit type in early developmental stages, becoming more complicated as development proceeds (Vavra, 1976). They are typically eukaryotic, though they have been shown to possess ribosomal RNAs with prokaryotic properties (Curgy et al., 1980). Spore morphology can be highly variable, from simple ovoid or pyriform to highly ornamented types. Spores measure from 2-12um in length. They are refractile and appear greenish in transmitted light (Canning, 1977a). The spore is the last stage of development, adapted for transmission to, and infection of, new hosts. There are 3 major components of the spore, the sporoplasm or infective agent, the extrusion apparatus (polar filament, polaroplast, posterior vacuole) by which the sporoplasm is introduced into new cells and the spore envelope consisting of endospore and exospore.

Ultrastructural studies by Ishihara (1968) and Weidner (1972) confirmed that the sporoplasm is inoculated into uninfected host cells through the everted polar filament. In a further 2 papers, Weidner (1976; 1982) reported that the polar filaments discharged as tubes with subsecond velocity and sufficient plasticity to allow the passage of the sporoplasm. In 1984 Weidner et al., using fluorescent probes showed that the single plasma membrane within the spore remained there after discharge, but that the sporoplasm quickly acquired another membrane originating from the polaroplast organelle.

It has long been known that it is possible to demonstrate spore extrusion in vitro using a variety of stimuli, for example, pH, osmotic pressure and concentration and species of cation (Ishihara, 1967). These cocktails of stimuli often relate to the environment found within the host organisms gut after ingestion of spores. Dall (1983) suggested that alkaline environmental conditions establish a proton gradient across the spore plasma membrane driving a cation/proton exchange, which would then initiate a similar exchange between the sporoplasm and organelles (e.g. polaroplast and posterior vacuole). This would lead to a rapid inflow of water and the associated pressure increase would cause the explosive discharge of the polar filament. Clearly, there is still debate over the subject of sporoplasm extrusion. Once entry to the new host cell has been gained, two phases of development ensue, merogony and sporulation.

Merogony (or schizogony) is a vegetative process of repeated binary or multiple fissions, responsible for the increase in parasite numbers within the host. All meronts are structurally simple, surrounded by a unit membrane, with little cytoplasmic differentation. The nuclei may be single or paired as diplokarya. Each nucleus of a diplokaryon has its own intact envelope, although the adjacent surfaces of the two nuclei lie so close together that they become flattened against each other (Canning, 1977a).

Sporulation is the phase responsible for the production of spores, and is comprised of a division sequence called sporogony and spore morphogenesis. Sporonts can usually be distinguished from meronts by the presence of an electron dense surface coat secreted on to the plasma membrane as the meront transforms into the sporont. In some genera an additional layer also develops around the sporont called the sporophorous vesicle. Sporont division, which can involve meiosis, results in sporoblasts which undergo spore morphogenesis, resulting in mature spores. During morphogenesis the thick spore wall is laid

down. This wall is composed of two layers, a proteinaceous exospore and a chitinous endospore, within which the organelles develop. The resulting spores are the only stages capable of survival outside the host. The number of sporoblasts derived from a sporont is different in various genera of microsporidia, and this feature has been used in their taxonomy (Vávra, 1976; Canning, 1989).

Transmission generally occurs directly between hosts of the same species, by ingestion of spores which have contaminated the environment, and to a lesser extent by transovarial transmission (Canning, 1971). However, some of the dimorphic/polymorphic species are transmitted transovarially and in a few an alternate host is needed for completion of the life cycle (Sweeney *et al.*, 1985; Andreadis, 1985). In these species there are 3 different spore types, in comparison with just one, as found in directly transmissible species.

1.1.1 Dimorphism, polymorphism and sexuality in microsporidia

In dimorphic species there are two different sequences of sporogony culminating in two types of spore differing in shape, size and internal structure. In the genus *Vairimorpha*, dimorphism is temperature dependent. At 25°C *V. necatrix* undergoes disporoblastic sporogony, resulting in free, binucleate spores. At 20°C an additional octosporoblastic sporogony, resulting in the formation of sporophorous vesicles containing 8 uninucleate spores occurs (Pilley, 1976). The genus was named by Pilley (1976) to accommodate microsporidia with temperature dependent dimorphism as described above. Previously, the two types of development in *V. necatrix* had been regarded as different species. Kramer (1965), identified them as *Nosema necatrix* (free spores) and *Thelohania diazoma* (octospores). *V. plodiae* was also originally described as two species; *Nosema plodiae* and *Thelohania nana* (Kellen and Lindegren, 1968, 1969), until Maddox and Sprenkel (1978), showed them to be a single species.

An ultrastructural study by Malone and Canning (1982) of *V. plodiae* showed that meronts had 2 or 4 nuclei in diplokaryotic arrangement preceding both sporogonic sequences. Disporoblastic sporonts were ovoid or fusiform with 2 or 4 nuclei still in the diplokaryotic arrangement. However, the octosporoblastic sporonts were spherical with 2, 4 or 8 isolated nuclei. Synaptonemal complexes were found in the binucleate octosporoblastic sporonts.

Malone and Canning (1982) also discovered that both spore types were equally infective to their lepidopteran hosts. This is also true of V. necatrix (Fowler and Reeves, 1974). Two other species, V. ephestiae (Weiser and Purrini, 1985) and V. invictae (Jouvenaz and Ellis, 1986) have subsequently been described at the ultrastructural level.

A different form of temperature dependent spore dimorphism is seen in the genus Burenella. In B. dimorpha, a parasite of Solenopsis geminata, a tropical fire ant, binucleate free spores develop at temperatures between 20°C and 32°C, at relatively constant levels. Numbers of uninucleate octospore are maximal at 28°C (35.9% of spores were octospores), and are progressively reduced at higher and lower temperatures (Jouvenaz and Lofgren, 1984b). Solenopsis geminata pupae which have died of infection are cannibalised by adult ants. However, the spores are not ingested, but diverted to the infrabuccal cavity, where they form a pellet. This pellet is then fed to 4th instar larvae by the adults. Earlier instars do not possess the specialised spines for holding solid food while feeding and are therefore not infected. Both spore types are ingested by the 4th instar larvae, but only the binucleate free spores are infective and hatch. The octospores pass out in the faeces and their function remains unknown (Jouvenaz et al., 1981). Work by Jouvenaz and Lofgren (1984a) showed that B. dimorpha could be cross-transmitted to S. invicta, S. richteri and S. xyloni, but could not be propagated in the long term in these species. Unlike the octosporoblastic sporonts of Vairimorpha species, synaptonemal complexes have not been demonstrated in B. dimorpha (Jouvenaz and Hazard, 1978), though their existence has not been discounted.

A third type of dimorphism, not reliant on temperature is exemplified by the genera Amblyospora and Parathelohania, of the family Thelohaniidae. In the same family there are a further 9 genera, Thelohania, Pegmatheca, Inodosporus, Cryptosporina, Pilosporella, Agmasoma, Systenostrema, Hyalinocysta and Chapmanium (Hazard and Oldacre, 1976). When the family was first described, only Parathelohania and Amblyospora were known to be dimorphic, though subsequent work has shown Pilosporella chapmani (from Aedes triseriatus) also to be dimorphic (Becnel et al., 1986). The genera within the family are characterised by producing uninucleate octospores within sporophorous vesicles. In the dimorphic genera there is one cycle in adult females resulting in binucleate free spores. These spores hatch, inoculating their sporoplasms into developing

eggs. The progeny usually develop fatal infections with masses of octospores by 4th instar.

Amblyospora and Parathelohania are very closely related genera and are particularly common, widely distributed parasites of Diptera (Hazard and Oldacre, 1976; Hazard and Anthony, 1974). Indeed, 16 out of 66 species of mosquitoes in Florida have been found to be infected with Amblyospora type infections (Hall and Lord, 1982). Much experimental and ultrastructural work has elucidated many aspects of the biology of these enigmatic genera, particularly Amblyospora, forming a good basis for comparison with any new parasites which may be found. Many of the papers describing new species have been concerned solely with ultrastructural details of the octosporous sequence in larvae, and in only a few has the sequence in adult females been described. It was not until the work of Sweeney et al. (1985) and Andreadis (1985) that the complete life cycle of at least two Amblyospora species was understood. They discovered that there was an additional spore type which developed in an intermediate copepod host. An unnamed copepod was also shown by Becnel (1988) to be the alternate host of Amblyospora californica in Culex tarsalis. All these copepods were cyclopoid, and so it is interesting that Dickson (1988) found a calanoid species, Diaptomus spp., to be the alternate host of Amblyospora cambelli in Culiseta incidens. Using Amblyospora connecticus from Aedes cantator (mosquito) and Acanthocyclops vernalis (copepod) (Andreadis, 1985a; 1988b), and Amblyospora dyxenoides from Culex annulirostris (mosquito) and Mesocyclops albicans (copepod) (Sweeney et al., 1985; 1988) as examples, the general life cycle of Amblyospora species can be seen as follows:

Unikaryotic spores, produced in the intermediate copepod host are directly infective per os to mosquito larvae. The initial infection of larval midgut cells by uninucleate stages from the copepod has only been seen in Amblyospora connecticus and a species in Aedes stimulans (Andreadis, 1985b) and not yet in Amblyospora dyxenoides. In Amblyospora connecticus development initially begins in the epithelial cells of the midgut and gastric caecae of young larvae. Repeated binary fissions occur resulting in the rupture of host cells and release of uninucleate meronts which spread to the muscles and oenocytes. Pyriform or fusiform stages which were considered by Andreadis to be gametes, undergo plasmogamy to form diploid diplokaryotic zygotes. Gametes were also seen by Sweeney et al. (1988) in Amblyospora dyxenoides. Sporulation ensues and binucleate spores are produced which are responsible for trans-

ovarial transmission. Spontaneous hatching of the spores in adults releases diplokaryotic sporoplasms which invade the host ova, infecting all or a percentage of the F_1 generation. In the progeny of infected females patent fat body infections develop, ultimately producing the characteristic octospores.

The earliest stages seen in Amblyospora dyxenoides were diplokaryotic meronts, which multiplied in fat body cells, then commenced sporulation. During early sporogony meiosis occurred, with nuclear configurations similar to those seen by Hazard and Brookbank (1984) in another Amblyospora sp. from Culex salinarius. Synaptonemal complexes were clearly visible, signifying meiosis. The wall of the developing sporont becomes thickened and a sporophorous vesicle develops. Subsequent divisions occur to form quadrinucleate and octonucleate sporonts. Nuclear and cytoplasmic division produces uninucleate sporoblasts which undergo spore morphogenesis, the wall becoming thickened and the organelles, including an anisofilar polar filament, developing within. Papers by Larsson (1981), Toguebaye and Marchand (1984) Vávra et al. (1984) Maurand and Loubès (1978) and Vávra and Undeen (1980) Lord et al. (1981), Weiser and Ibrahim (1985) report on, or describe the ultrastructure of octosporous development cycles in other Amblyospora spp..

During the course of octosporous infection, the fat body becomes filled with masses of octospores, the larvae generally dying by the 4th instar. Such larvae appear sluggish, swollen and opaque white in colour. These fat body infections occur in both sexes, the rate of infection varying from 0-100%. On the death of a patently infected larva, octospores are released into the water of the breeding ground, where they are infectious to copepods. The first stages observed in copepods are spherical meronts, with a single large nucleus. Binary fission occurs to form binucleate and quadrinucleate cells and multinucleate plasmodia. Multiplication occurs and sporulation begins. Sporonts have thickened cell walls and can appear in a rosette formation finally undergoing cytokinesis to form uninucleate sporoblasts. Sporonts secrete a sporophorous vesicle in which all sporogonic stages, and finally spores develop. The most prominent organelles are the vesicular polaroplast and polar filament, which in the mature spore has many isofilar coils. These infections are fatal to the copepods and on ingestion by mosquito larvae give rise to the stages responsible for transovarial transmission. Adult female and juvenile copepodid stages of A. vernalis were susceptible to Amblyospora

connecticus but males and larval nauplii were not. Development occurred within the median ovary and oviducts which interferred with normal egg production (Andreadis, 1988a). A summary, in terms of chromosome cycle is given by Canning (1988). This is the basic lifecycle of all members of the genus Amblyospora, however, there are many variations between the different species.

For instance, in Aedes stimulans the gastric caecae cells, presumed to have been infected with uninucleate stages from the copepod, are often sloughed off, thus reducing the overall infection finally found in the oenocytes (Andreadis, 1985b). This is not so in A. connecticus where identical levels of infection were found in larvae and adults. Andreadis (1988b) suggested that this might mean that A. connecticus has had a longer standing relationship with the mosquito host, which may also help to explain why transovarially induced epizootics have been seen in the field (Andreadis, 1983a,b).

In A. connecticus diplokaryotic stages are found in males and females but sporulation occurs only in females. The same pattern of parasite development was seen previously by Andreadis (1983a) in Aedes cantator. In contrast, in A. dyxenoides (Sweeney et al., 1988) and in an unidentified Amblyospora sp. from Aedes stimulans (Andreadis, 1985b) sporulation occurs in both sexes. In A. connecticus spore formation coincides with the development and maturation of the host oenocytes. This response is thought to be hormonal rather than due to the presence of bloodmeal products. A hormonal response was shown in an Amblyospora sp. from Culex salinarius (Lord and Hall, 1983). In this case sporulation was induced, in the absence of a bloodmeal, by injection of 20-hydroxyecdysone, a hormone the precursor of which is produced by ovaries of bloodfed mosquitoes. Sporulation of A. californica in Culex tarsalis continues in synchrony with egg production in the absence of a bloodmeal, because the mosquito is autogenous (Hall and Washino, 1986). In comparison, the formation of binucleate spores from diplokaryotic stages in A. dyxenoides does not proceed synchronously within a batch of infected adults. For instance, within 48 h of emergence 6/12 infected females and 7/12 infected males had mature spores. Conversely, some individuals of both sexes 2-3 weeks after emergence had only diplokaryotic meronts or immature spores. In females mature spores could be produced before or after a bloodmeal (Sweeney et al., 1988).

The octosporous sporogony occuring in the progeny of infected adults also varied between species. In A. dyxenoides cytokinesis resulting in uninucleate sporoblasts can occur at either the binucleate, tetranucleate or octonucleate stage which is in contrast to A. connecticus where cytokinesis only occurs at the octonucleate stage. Some larvae of both species can survive to the pupal stage and in A. dyxenoides a few even to adulthood. Some do not develop fat body infections at all, but instead benign infections of diplokaryotic stages within oenocytes. These infections persist to adulthood, resulting in binucleate spores which in females initiate another transovarial transmission cycle. In some rare cases both types of sporulation proceed simultaneously resulting in adults with octospores in their fat bodies and binucleate spores in their oenocytes. In A. callosa a parasite of the caddis fly Rhacophila fuscula, this is the normal occurrence (Hazard and Oldacre, 1976; Larsson, 1985). Experimental work by Sweeney et al. (1989a) suggested that the proportion of mosquito populations acquiring each type of infection can be influenced by genetic selection.

The sequence in copepods also varies, for instance the multinucleate plasmodia are rare in A. dyxenoides but common, possessing as many as 12 nuclei, in A. connecticus. Bioassay experiments on the dose response of Mesocyclops albicans copepods to Amblyospora dyxenoides octospores have shown that infection rates in copepods alive after 2 weeks were directly proportional to the concentration of octospores applied. An IC_{50} was estimated as $9.9x10^3$ octospores/ml with 95% fiducial limits of $7.3x10^3 x 1.3x10^4$ octospores/ml (Sweeney et al., 1989b)

Until a recent paper by Avery (1989), horizontal transmission had not been found in Parathelohania spp.. Avery (1989) showed that pyriform spores concentrated from the water of a mosquito breeding ground, where larvae were infected with Parathelohania obesa octospores, were infective to 1st instar Anopheles quadrimaculatus. The origin of the pyriform spores was unknown, but presumed to be from an alternate host. The infections that developed in An. quadrimaculatus larvae were of 2 types. The first was benign and diplokaryotic, as seen in Abmlyospora infections, but the second produced uninucleate octospores. Production of octospores in larvae infected with spores from the alternate host hever occurs in Amblyospora infections.

Meiosis discerned by the presence of synaptonemal complexes was first seen by Loubès et al. (1976) in Gurleya chironomi , and was subsequently

demonstrated in a further 8 genera, Thelohania, Pleistophora, Tuzetia, Baculea, Glugea (Loûbes, 1979), Amblyospora and Parathelohania (Hazard et al., 1979) and Stempellia (Desportes, 1976). If meiosis occurs, then karyogamy must also occur somewhere in the life cycle and this was demonstrated by Hazard and Brookbank (1984) for an Amblyospora sp.. In 1985 Hazard et al. also demonstrated gametogenesis and plasmogamy in Hazardia milleri, Culicospora magna and a Microsporidium sp.. Becnel (1986) described the life cycles of 4 sexual, polymorphic microsporidia, Amblyospora californica, Microsporidium aedis (now placed in the new genus Edhazardia as E. aedis Becnel et al. 1989), Culicospora magna and Culicosporella lunata (previously described using light microscopy by Hazard et al., 1984), comparing developmental and morphological similarities between them.

A. californica had a life cycle similar to that already described for A. connecticus and A. dyxenoides. M. aedis (Edhazardia aedis) is transovarially transmitted to progeny, where diplokaryotic sporonts develop. These then undergo two different sporulation sequences, one sexual, resulting in 8 haploid spores within a sporophorous vesicle and the other producing haploid sporonts which eventually become uninucleate spores. These spores are morphologically similar to those from copepods in the previous species, and are responsible for horizontal transmission. When ingested by mosquito larvae the ensuing developmental sequence involves gametogenesis and plasmogamy with the production of thin walled binucleate spores in adults, which are responsible for vertical transmission to progeny. Very few mature octospores are produced, and this sequence seems redundant.

In Culicospora magna vertical transmission is again effected by binucleate spores. In the infected progeny a single sporulation sequence occurs producing uninucleate spores in both sexes. The sporulation begins with the dissociation of the 2 nuclei of the diplokaryon finally producing uninucleate sporonts which develop into large, haploid, pyriform spores each within a vesicle. These are responsible for horizontal transmission. Development in larvae infected horizontally involves a sexual sequence of gametogenesis and plasmogamy which restores the diplokaryotic and diploid state of the cell. Binucleate spores are formed, which are responsible for transovarial transmission.

In Culicosporella lunata two spore types of a possible polymorphic life cycle are known. Within larvae two sporulation sequences occur. Some diplo-

karyotic meronts undergo a sexual sequence with meiosis, which when successful (which is not common) produces haploid octospores as in Amblyospora. Other meronts produce sporonts which in turn give rise to binucleate spores, within vesicles which are responsible for horizontal transmission.

Dimorphism was suspected to occur by Canning and Hazard (1982) in another species - Polydispyrenia simulii. This parasite from blackflies was originally assigned to the genus Pleistophora as it produces a large and variable number of spores within a sporophorous vesicle. However, there were fundamental differences between the two genera. Pleistophora typicalis, the type species in a fish Myoxocephalus scorpius, has unpaired nuclei throughout its development, with no sexual cycle. In addition the persistant thick sporophorous vesicle is formed during merogony. By comparison Polydispyrenia simulii (Pleistophora simulii) has diplokaryotic meronts which undergo meiosis (Loubes, 1979), producing haploid spores, and a fine sporophorous vesicle produced at the onset of sporogony. As meiosis occurs it follows that karyogamy must also occur, perhaps in an, as yet unkown, developmental sequence within adults or an alternate host. There is evidence that the spores cannot be transmitted directly to another blackfly host, as with Amblyospora and Parathelohania, and so the possibility of an alternate host remains feasible.

Clearly, there are common themes amongst these parasites. All are dimorphic if not polymorphic and have sexual development in the form of gametogenesis and/or meiosis, although there is not always a necessity for an alternate host.

Microsporidia have developmental cycles closely synchronised with their hosts. Andreadis (1983a,b) showed that epizootics sometimes occur in favourable conditions and it is this fact that makes them candidate biological control agents.

1.2 INTRODUCTION TO THE CULICIDAE

Mosquito is the common name, derived from the Spanish (muscato, muskitto or musqueto being some of the early spellings) meaning "little fly", for haematophagous insects belonging to a single family of Diptera, the Culicidae (Gillett, 1971). They are easily recognisable from other Nematocera, as they possess an elongate proboscis as adults. They are nuisance pests and well known vectors of disease, due to the blood-sucking habits of the adult females. There are three subfamilies Anophelinae, Culicinae and Toxorhyn-

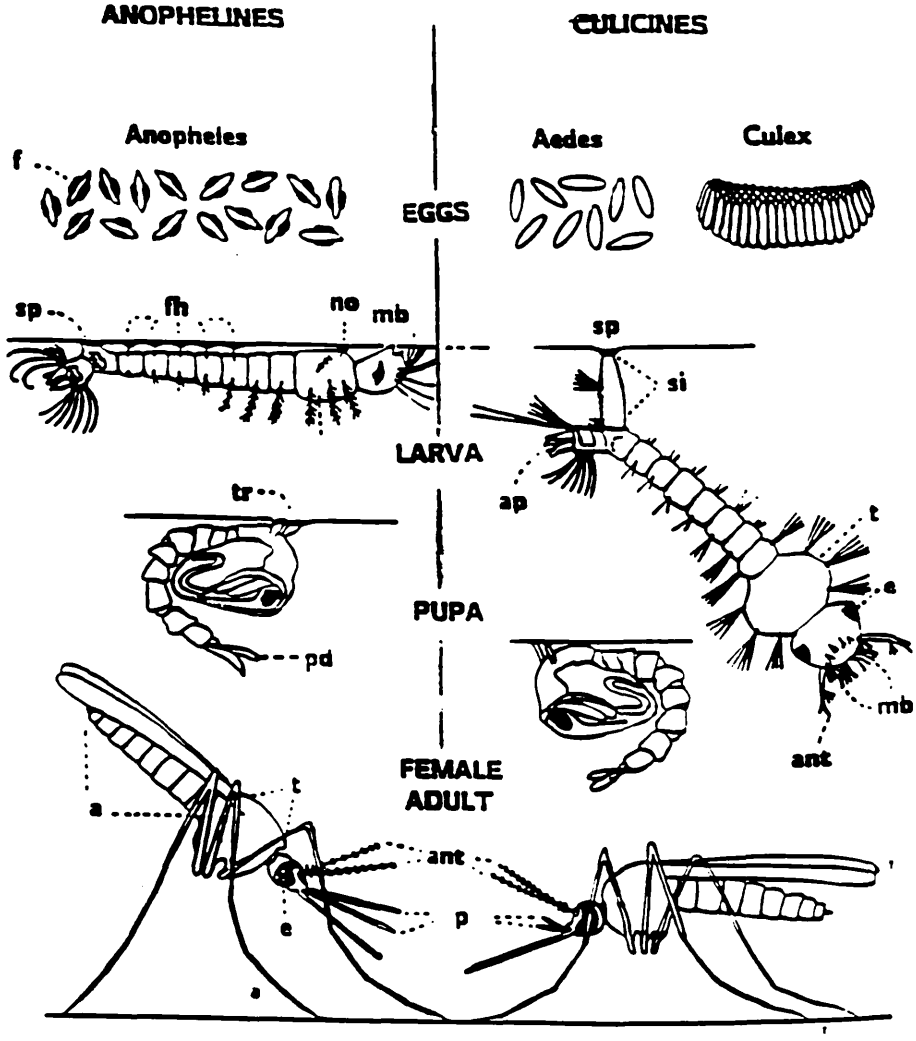
chitinae, classified as follows into 35 genera and over 3,200 species (White, 1982).

Subfamily	Tribe	Principle Genera
Anophelinae	-	Anopheles
Culicinae	Aedeni	Aedes
		Armigeres
		Eretmapodites
		Haemagogus
	Culicini	Culex
	Culisetini	Culiseta
	Mansoniini	Coquillettidia
		Mansonia
	Sabethini	Heizmannia
		Limatus
		Sabethes
		Trichoprosopon
		Tripteroides
		Wyeomyia
Toxorhynchitinae	-	Toxorhynchites

For mosquito species, eggs are generally laid singly, as in Anopheles and Aedes spp. (Anopheles spp. also having floats on each egg), or in floating "rafts", as in Culex and Culiseta spp. (See Diagram page 13) for characteristic differences between anophelines and culicines at all stages of the life cycle). The only exceptions are members of the genus Mansonia which lay their eggs in clumps on the undersides of floating vegetation. Eggs can be laid on damp soil and vegetation or directly onto the water, although in all genera except Aedes they become non-viable if allowed to dehydrate completely.

Larvae are the growth phases of the life cycle and moult through 4 instars before pupating. Their lifetime may be only a week, as in many tropical species, or many months as an overwintering form in more temperate species. Depending on species, they can be found exploiting almost every type of still water body, from pit latrines to forest pools, hoof prints, old tyres, tree holes or brackish water ditches.

DIAGRAM: BASIC MORPHOLOGY OF LIFE STAGES OF ANOPHELINE AND CULICINE MOSQUITOES



Key: a - abdomen, ant - antenna, ap - anal papillae
e - eye, f - float, fh - float hair,
mb - mouth brush, no - notched organ, p - palp,
pd - paddle, sp - spiracle, t - thorax,
tr - trumpet. (from Cranston et al., 1987)

Larvae are filter feeders, using rhythmically beating brushes to draw food into the mouth. Respiration occurs directly or indirectly with atmospheric air. Anophelines have two spiracles, flush with the dorsal surface of the eighth abdominal segment, which lead to the tracheal system. Thus, when they lie flat on the water surface with the head swivelled by 180^{o} they can both respire and feed at the water film. Culicines in comparison have spiracles set at the top of a chitinous siphon. For this reason they hang at an angle to the water surface, feeding below the water film. Members of the tribe Mansoniini are exceptions in that they respire via the roots of water plants. Larvae and pupae are agile swimmers escaping quickly from the surface if threatened. They are particularly sensitive to changes in light intensity, although they become habituated to repeated fluctuations.

The pupa is a comma shaped individual, entirely different in form to both the larva and subsequent adult. It moves differently, in dorso ventral kicks rather than lateral whips and respires through trumpets at the head end as opposed to spiracles at the tail end. It is during the pupal stage that the greatest transformations in morphology occur, although the pupa remains mobile until minutes before the final emergence of the adult. Once eclosion has begun it takes only a matter of minutes for the new adult to emerge. The adult is able to make its first flight after only ten minutes.

Both sexes feed on nectar, the sugar solution being sucked up through the proboscis and held in the crop, where it is released to the stomach as required. The female also requires blood for the production of eggs. The only exceptions to this are members of the genus Toxorhynchites which never feed on blood and a few autogenous species which can produce one batch of eggs on the reserves laid down by the larva. Foraging females fly upwind searching for the scent trail of a suitable host, using sensitive hairs on their antennae. Visual cues are also used although most feeding occurs either at twilight or at night.

The proboscis is highly specialised comprised of a sheath surrounding a complicated set of stylets and sensory structures. Once applied to the skin it is pushed downwards, the sheath folding back as the cutting stylets sink deeper into a capillary. Fluid from the salivary glands containing an anti-coagulant, passes into the capillary via the probosis. The blood passes up directly into the stomach via the oesophagus where it coagulates and is digested. The saliva also acts to increase the blood supply at the region of

"the bite" and an itchy immune response ensues. There is usually a degree of host preferences, for instance some Culex and Coquillettidia species are specifically bird feeders.

The bloodmeal provides the stimulus for egg production, and on occasion nourishment for the adult. Mating has usually occurred by the time the bloodmeal is taken, the sperm being stored in the spermathecae. In the case of Deinocertes cancer and Opifex fuscus mating occurs during emergence of the adult female. Males wait near female pupae on the verge of eclosion and mate with them as soon as they begin to emerge. Members of the genus Eretmapodites mate only when the female is warm and swollen with a fresh bloodmeal which is in contrast to the usual. Generally there is enough sperm deposited in a single mating to fertilise all subsequent eggs produced by that female and although subsequent matings can occur females are usually unwilling to mate again. Although males emerge before females, they are not ready to mate for at least 24 hours until their terminalia have rotated by 180^{o} (hypopygial circumversion). In addition females are not receptive for a few days after eclosion.

Males usually recognise receptivity in females as a change in the pitch of their flight tone, with a few exceptions. The flight tone is detected using sensory hairs on the palmate antennae of the males which are modified for the reception of stereophonic sound. Once mating has begun (in flight) the pair will usually land and rest on a suitable surface, uncoupling after only a few seconds.

Mosquitoes are also known to swarm; that is to fly actively, while remaining in one stationary mass. These groups are usually solely comprised of males, although mixed male and female swarms have been reported. Swarming is thought to be linked with mating, as any females entering the swarms are seized by males. Swarms of particular species fly at certain times and in certain places, which are located by, as yet, unknown markers. In addition females of different species fly at different heights, and male swarms of the same species fly at the corresponding height, thus introducing a vertical component to swarming (Gomulsky, 1988).

Having taken a bloodmeal the female rests, during which time egg maturation occurs. The duration of time between the bloodmeal and the female becoming gravid varies between species, particularly between those in tropical and temperate environments. The female then searches for a suitable site for

egg laying, by exploring regions that are darker than their surroundings, have a higher relative humidity, and a more reflective surface. Once water or moisture is found; the pH, level of organic material, relative amounts of light and shade and presence of water plants become important factors in choice of site. After mating, females sometimes make dispersal flights of many kilometres during which time they may take a bloodmeal. In this way they are able to find new breeding grounds.

When faced with overwintering (temperate species), or complete drying out of breeding grounds (temperate and tropical species), mosquitoes have the ability to hibernate or aestivate. This can occur at all stages of the lifecycle, depending on species. Overwintering is not only controlled by falls in temperature, but also by reductions in day length (Gillett, 1971; Cranston et al.,1987).

1.2.1 Description of species found in this study infected with microsporidia

BRITISH SPECIES

Woodland species: Aedes cantans and Aedes punctor

There are two species of mosquito commonly found associated with temporary woodland pools in Britain and these are Aedes cantans and Aedes punctor. Both species are univoltine (Marshall, 1938) and bite both man and other mammals. Ae. cantans has been implicated as a vector of Tahyna virus in certain areas (Service, 1977). For both species eggs, laid in soil or leaf litter in and around dried out woodland ditches in the summer, undergo an obligatory diapause and do not hatch until late December, or early January of the following year (Service, 1977). Ae. cantans lay their eggs randomly in dry woodland depressions (Lakhani and Service, 1974), but Ae. punctor lay their eggs in a narrow horizontal band 30-40cm from the deepest part of the prospective larval site (Fallis and Snow, 1983b)..

Eggs require conditioning by a sequence of stimuli before hatching occurs. The final hatching stimulus for both Ae. punctor and Ae. cantans was shown by Fallis and Snow (1983a) to be a gradual reduction in the oxygen content of the medium. Hatching occurs within 4 hours of complete depletion of dissolved oxygen. In natural sites this gradual depletion of oxygen is caused by growth of bacteria in the surrounding medium, which provides an excellent

food source for larvae when they hatch. For both species eggs do not hatch simultaneously, a phenomenon termed "instalment hatching" (Service, 1977), and in some cases, particularly when rainfall levels are below average, eggs of both species will remain in diapause until a subsequent season (Service, 1969).

Larvae of Ae. cantans are generally found in densely shaded non-saline pools and not in open, sunlit waters. Larval development is usually finished by June/July when the pools are beginning to dry out. Any remaining larvae risk becoming stranded when drying out is complete. Mortality during larval development is greatest during the first two instars (Lakhani and Service, 1974; Service, 1977). Mortality is due in small part to diseases caused by viruses, fungi and microsporidia, and to predators such as Coleoptera and Diptera, but in the main part to unknown factors, perhaps competition for food and space.

Ae. cantans adults first begin to appear in late April and early May, the highest levels being found in July. They disappear by October. Ae. punctor adults have a similar season, being found throughout the year in low numbers, with greatest numbers in July but very few persist later in the year.

As the number emerging increases, the number of predatory flies also grows and these have been seen to prey on emerging Ae. cantans particularly at dusk when maximum mosquito emergence takes place (Service, 1969).

In Ae. cantans bloodmeals are not taken in the first 3-4 weeks, adults feeding only on nectar. Service (1971c) speculated that during this time, food was required for swarming and dispersal and only after this were adults likely to be orientated towards reproduction and the necessity of taking a bloodmeal.

Fallis and Snow (1984) reported that male (and a few female) Ae. cantans in Epping Forest could be found swarming between 18.00 h and 21.30 h (sunset being 21.20 h) from late May to late July. They demonstrated that females were inseminated before the first male swarms were seen suggesting that either swarming had no function in mating or that it was necessary for females to re-mate later in the season. The swarms varied in shape and number of participants, but were always between 75 and 125 cm from the ground. Service (1971,a) found swarms to be even nearer ground level, 30-40 cm. The results of Fallis and Snow (1984) are in contradiction to those found by Nielsen and Greve (1950) for Ae. cantans in Denmark, who found swarms earlier in the

season, from the end of April. In addition they also saw swarms ascending to the tree tops.

Females seek out hosts for bloodmeals at dusk, though they will feed in an opportunistic way during the day if a host enters their immediate vicinity. They are capable of detecting bait at a distance of 7 - 10m, and will approach it directly without resting on vegetation beforehand. The total time spent on the host varies between 2 and 4 minutes. After feeding the female flies away with apparent ease. Indeed, bloodfed adults have been collected 3-4 km from the nearest hosts. Resting ensues amongst scrubby or grassy vegetation, in a patchy distribution, even when the area is apparently uniform. Both males and females spend a high proportion of their lifetime inactive, resting on vegetation (Service, 1971b).

Digestion of blood takes 5-8 days in summer after which suitable oviposition sites are found. Oviposition occurs at dusk, the female spending 1-3 minutes testing the soil or leaf litter before beginning to lay. Ae. cantans can lay 30 eggs in 2.5 - 4.5 minutes.

An interesting fact is that gynandromorphs have been found in populations of both Ae. punctor (Packer et al., 1986) and Ae. cantans (Campbell and Service, 1987).

Saltmarsh species: Aedes detritus and Aedes flavescens

Aedes detritus is a multivoltine species in contrast with Aedes flavescens, which is univoltine in Great Britain. However in Ae. flavescens there is occasionally a second egg hatch in the summer. These summer egg hatches occur if the eggs are subjected to intermittent immersions, showing that diapause is not necessarily obligatory, but rather environmentally controlled.

Ae. detritus is a largely coastal or estuarine species, the aquatic stages being tolerant of high salinities; up to a concentration of 133% sea water (Marshall, 1938). Most other species of mosquito larvae are killed by high salt levels, and it is obviously a valuable adaptation of these species to exploit brackish water. Even in Ae. detritus the 1st and 2nd instars are less tolerant to the higher levels (Service, 1968c). There have been rare inland observations of Ae. detritus, for instance by Marshall (1933). These have all been in brine deposits except one flourishing population breeding in freshwater near Leeds, some 91 km from the sea (Service, 1973b). Ae. flavescens is not as closely linked to brackish water and, although common in

some areas (Hearle, 1929), is rare in Britain. Service and Smith (1972) described a firmly established population on the Isle of Grain. Here, the salinity varies, usually up to 50% sea water, the larvae preferring open sunlit sites.

Eggs of Ae. detritus can remain viable for a year or more (Marshall, 1938) and often require multiple immersions to hatch. Most hatch between the 2nd and 5th immersions (Service, 1968c). Oviposition sites for Ae. detritus are areas flooded by both rain water and sea water, either being flooded by exceptionally high tides or in low lying areas protected by a sea wall where flooding occurs by percolation or breach by sea water. Eggs of both species are laid on the mud and decaying plant material in the drying larval sites, rather than on surrounding vegetation (Service, 1968c; Service and Smith, 1972).

Eggs of Ae. detritus hatch at any time of year, whenever the sites are sufficiently flooded, though Service (1968c) found that 1st instar larvae were not found in summer. He suspected that this was due to the short duration of this stage at warmer temperatures. Larval development can be completed within 3 weeks, though pupae are never found after September, as larvae reaching 4th instar at this time remain so until March of the following year. Thus, in cold weather development is greatly retarded. Interestingly, 1st, 2nd and pupal stages tend to aggregate at the edges of marshy pools and late instars migrate throughout the site (Service, 1968c).

Adult Ae. detritus appear from March onwards and remain abundant until November after which only old females persist. Emerging adults tend not to fly far, the maximum distance being 8-11 km (Service,1973d). In comparison Ae. flavescens adults begin emerging at the beginning of May, numbers build up to a maximum in June after which the numbers diminish until there are very few left in July. Adults of both species rest on vegetation near the breeding sites.

At certain times of the year in Poole, Dorset, Ae. detritus have been found to require two bloodmeals to complete one gonotrophic cycle (Service, 1968c). The occurrence of this phenomenon was greater in cooler months. Some Ae detritus in southern France are autogenous, being able to complete their first gonotrophic cycle and produce eggs without a bloodmeal, however, even in these autogenous members, a bloodmeal increased their fecundity (Guilvard et al., 1979).

As these mosquitoes are vicious biters they can become a great nuisance to people living and working near saltmarshes. For this reason control attempts using chemical and Bacillus thuringiensis insecticides (Bti) are often used, for instance at Sandwich in Kent (Hargreaves, 1986).

It is useful at this point to mention another common saltmarsh species Aedes caspius, which is often found associated with organically polluted brackish waters and is thought to have been out competed by Ae flavescens in some areas such as the Isle of Grain (Service and Smith, 1972).

Culiseta annulata

Culiseta annulata is one of the commonest species of mosquitoes to be found in Britain. Egg rafts and larvae can be found in a variety of water bodies, sunlit or shaded, natural or man-made, freshwater or brackish (Marshall, 1938). The size of the egg raft is highly variable (Service, 1968a) and, once laid, the eggs do not undergo diapause and cannot withstand desiccation. They can begin to hatch after 4 days (Marshall, 1938), although during the winter this time may be greatly extended, e.g. to 24 days in one case (Service, 1968a). The whole process of hatching, once begun can also be extended over several days. Larvae are highly tolerant of a variety of different water conditions but are most commonly found in water with a high level of organic pollution.

Adults rest in sheltered areas, indoors or outdoors, in barns, caves and even hollow trees. They feed on a variety of hosts particularly cattle and birds, but also man (Service, 1971b,c). Overwintering is unusual in this species in that egg rafts, larvae, pupae and adults can all be found throughout the year. Adult females (parous and nulliparous) can be found throughout the winter and most are gonoactive (Ramsdale and Wilkes, 1985), with varying amounts of fat body. Service (1968a) showed that gonoactivity was temperature rather than photoperiod dependent, bloodmeals being used in fat production at low temperatures and for ovarian development at higher temperatures. The adult females therefore seem to be able to survive cold periods by switching from gonoactivity and oviposition to cold-induced quiescence when fat body production occurs. Gynandromorphism has been seen in Cs. annulata (Classey, 1942).

AFRICAN SPECIES

Mansonia africana and Mansonia uniformis

Members of the genus Mansonia are well known vectors of human filariasis caused by Wuchereria malayi, W. bancrofti and Brugia malayi and B. timori species (Mattingly, 1969; Laurence, 1960; Antonipulle et al., 1958; Wharton, 1957a,b). Indeed, M. uniformis is one of the most efficient vectors of W. bancrofti in New Guinea. Van Dijk (cited in Van den Assem & Metselaar, 1958) found an infection rate of 91% (n = 56) in specimens collected from the Mappi district. These mosquitoes are also known to transmit Dirofilaria and viral diseases. Even in areas where they are not vectors they are still a significant pest insect, as they are vicious human blood feeders.

In Africa the genus is represented by two closely related species Ma. africana and Ma. uniformis. Ma. africana, as the name suggests is restricted to Africa, but the distribution of Ma. uniformis ranges from eastern China through Cambodia, Thailand, India and Sri Lanka to the Seychelles Islands and Africa. Both species exploit the same breeding grounds, and larvae can often be found together in stagnant water bodies where floating vegetation is abundant.

Early work on these species was greatly hampered by the difficulties of finding larvae. Bonne-Wepster and Brug (1939) related several stories of failed attempts at larval collection that happened even when adults were plentiful. Larvae are well known for their unusual method of respiration. The siphon is highly modified in all but the first instar and includes a highly sclerotized saw which allows it to pierce submerged plant tissue, finding air cavities where gaseous exchange can take place (Hopkins, 1952). Laurence (1960) showed that, once larvae were attached, they became very difficult to dislodge, so on removal of plants the roots must be shaken vigorously in fresh water and examined carefully to find larvae. Slight disturbances of the roots are only sufficient to stop larval feeding briefly. Water bodies with high organic contents are preferred sites, Ma. africana being particularly sensitive to organic content and hence mud and organic matter from plant roots often fills up collecting bowls and dislodged larvae may become hidden. For this reason various collecting techniques have been developed to gather quantitative information on larvae (Bidlingmayer, 1954; McDonald, 1970; Walker and Crans, 1986). Cultures can be reared in a strong infusion of guinea-pig droppings (Jayewickreme and Niles, 1952)

Newly hatched larvae do not have such a modified siphon, but will readily attach to plant roots in the presence of bacterial and fungal food items (Laurence, 1960), contrary to the belief of van den Assem (1958) that they respired at the surface like other culicine larvae. All instars will readily attach to air filled surfaces including paper, plastic foam, cork and floating leaves (Laurence and Smith, 1958). Pistia stratiotes is the common plant used for attachment, but is by no means the only one (van den Assem and Metselaar, 1958) and in choice experiments (Laurence, 1960) unusual plants such as Juncus, Carex and Limnobium were also found to be acceptable sites. When deprived of attachment sites respiration occurs at the surface, but the larva cannot moult. Pupae also attach to plant roots and detach within 12 h of eclosion. When moulting, the old cuticle is left attached to the roots and the larvae re-attach elsewhere. Larvae of both species develop at similar rates, although Ma. uniformis is slightly faster and has a faster rate of growth in the egg.

All mosquito larvae are filter feeders, but Mansonia species are generally found well below the water surface. Their root piercing lifestyle, while affording some protection against predators (van den Assem, 1958) has drawbacks. It is thought that the oxygen level, although adequate, is lower than that available to surface-dwelling larvae. Selective pressure has resulted in sluggish larvae with a slow rate of development. From egg hatch to emergence of the adult takes 24.3 to 25.5 days (Laurence, 1960).

At 25^{o} - $30^{o}C$ larvae moult at intervals of 5 or more days, the final pupal period lasting 4 - 5 days. Eclosion takes 15 - 20 minutes.

Adult Mansonia females are ready to take a bloodmeal after 24 h, whether mated or not, and will feed again after every oviposition. The range of hosts is wide amongst mammals and birds.

It has been shown in Uganda that bloodfeeding in both species is nocturnal (Haddow, 1945), biting activity increasing and decreasing in cycles throughout the night (Haddow, 1954), reaching a peak between 02.00 and 05.00 local time (Gillett, 1957). Both young and old females feed at the same time.

Gillies and Wilkes (1972) showed that females were attracted to a bait of 2 calves from a distance of 20 - 30 metres (slightly less for an equivalent source of CO_2). At a distance they are attracted by olfactory cues, although certain visual features of a target are also important (Gillies and Wilkes, 1982) and these act even on moonless nights. For instance, they respond

positively to dark entrances. Interestingly, males are also attracted to calf bait, from distances of 3 metres, for the purpose of mating (Gillies, 1975; Gillies and Wilkes, 1975; McIver et al., 1980). Hence, they are also guided at distance by olfactory cues. Nearer the target they use wing beat tone of the females. Males do not swarm and both sexes are low flying, usually taken in traps below 1m from ground level (Gillies and Wilkes, 1976; Snow, 1979).

Having taken a bloodmeal the female rests until gravid (about 4 days) and then oviposits. The eggs are laid in compact masses on the undersides of floating leaves (particularly those leaves resting at a slight angle to the water), by the female positioning herself with her abdomen curled under the leaf. Eggs are not laid on larger leaves, or those in open water (Laurence, 1959). The eggs hatch after 5 - 6 days and the cycle begins again.

Control of these mosquitoes has generally been aimed at adults, by the use of residual insecticides inside houses, although they are strongly exophilic. Larviciding is less effective as the larvae are not surface dwellers (Yap et al., 1968). Herbicides can be used to destroy the Pistia but so many other attachment sites can be used that this may be ineffective. The most recent work on Mansonia mosquitoes has studied the ecology of oviposition with the aim of assessing future possibilities for control (Gass et al., 1983; Lounibos and Linley, 1987). For this reason biocontrol using candidate microsporidia is an interesting possibility.

Anopheles gambiae sensu lato

Anopheles gambiae was formerly regarded as a single species until Davidson (1962, 1964) showed it to be a complex of six species. These include the freshwater species An. gambiae sensu stricto, An. arabiensis, An. quadriannulatus (zoophilic) and an unnamed species D. The saltwater species are An. melas (West Africa) and An. merus (East Africa).

Malaria caused by Plasmodium spp. is endemic in Africa, transmitted principally by An. gambiae s.l. and An. funestus (White, 1974). It is thought that the global incidence of malaria is presently about 200 million cases per year. A more realistic estimate for 1986 was made by Sturchler (1988) as 489 million clinical cases, 234 million due to Plasmodium falciparum, of which 2.3 million are fatal. In addition An. gambiae s.l. is also an efficient vector of filarial worms (Wuchereria bancrofti).

In Muheza, Tanzania, the An. gambiae s.l. population is comprised mainly of a mixture of An. gambiae s.s. and An. arabiensis with some An. merus nearer the coast. Larvae occur in a wide variety of water types, but prefer open sun-lit pools. These areas can be naturally formed, or more commonly appear as a result of human activity, showing the close association this species has with man. An. gambiae s.l. is known for its highly opportunistic nature, being able to take advantage of short-lived temporary sites, such as water-filled ruts in the road, formed after rains. Being such a pioneer species they achieve a tremendous advantage by colonising areas before other competitors or predators are able to do so. Such pools are therefore highly productive over short periods of the year, after the rains, until they dry up and An. gambiae s.l. is forced to use less attractive, permanent sites. Unlike many other opportunistic species, the eggs are not resistant to desiccation and are liable to frequent catastrophic mortalities (Gillies & De Meillon, 1968).

The water in the breeding grounds can be clear or muddy, though pools with high levels of colloidal matter do seem to be preferred. An. gambiae s.l. are not associated with pools with high organic matter levels of vegetable or animal origin. The species complex can survive a wide range of temperatures, from 17°C to 42°C and, at mean temperatures of 25° - 28°C, it can complete the egg to adult cycle in 6 days - a very rapid rate of growth.

An. arabiensis tends to be associated with more arid regions, though it is commonly the predominant species in rice paddy fields. This is thought to be because the irrigated rice paddy fields were set up in previously arid regions, and are therefore likely to become infested with the commoner form of An. gambiae s.l. from these regions, (Service, 1985). Between the two species some differentiation in breeding sites would be expected. Preliminary work by Service (1985) showed that within the same pool in Ghana, each species tended to aggregate in different areas. He suggested therefore that seasonal changes occurring in the ecological quality of habitats may confer an advantage on one species over another.

As would be expected the adult density tends to follow the seasonal rainfall pattern. In savanna zones, with a single rainy season the numbers rise explosively soon after the rains, peak and then fall to a stable level. In equatorial zones, with two wet seasons, there are two peaks. This is a general view of what happens and the situation is not always so simple. In some cases a heavy rainfall will flush out pools, followed by a period with very low numbers of adults.

Emergence occurs in the early evening, followed by a short flight to a suitable resting place. Few mosquitoes fly more than 1.5 - 2.5 kilometres, unless the regions are very dry and without arresting features, such as settlements. Long range transport in planes and trains has been recorded (Curtis and White, 1984). Males have been seen to swarm, usually at dawn, about 1 - 2 m off the ground.

An. gambiae s.s. is much more anthropophilic (man-biting) than An. arabiensis, which is easily diverted to cattle. Although both species rest indoors, a proportion of An. arabiensis populations exhibit greater exophilic tendencies than An. gambiae s.l., leaving houses soon after feeding, without resting. Some An. arabiensis which are found resting inside houses have fed outside, on cattle for instance, and use the house as a safe refuge. For this reason An. gambiae s.l. is theoretically the better vector. This is not true in rice growing regions, where An. arabiensis predominates (Service, 1985; Service, 1982; Gillies, 1967). In addition, due to its exophilic tendencies An. arabiensis is more difficult to control with residual insecticides.

In humid areas such as Muheza, An. gambiae s.l. can survive to a much greater age, 20% being 3-parous or older at any one time and 1% being 7-parous and older (Gillies and Wilkes, 1965). Using mark-release-recapture techniques, the same workers showed that the first gonotrophic cycle lasted 3-4 days and later cycles 3 days. Sporozoite rates increased with age; 80% of malaria infections being transmitted in the 5th, 6th and 7th cycles.

Females of all ages feed at approximately the same time during the night, entry into houses peaking between 2200 and 0200 hours. After entering the house, some considerable time can elapse before the host is attacked. Once the bloodmeal is taken a further period of time is spent resting inside the house (less true for An. arabiensis). Remaining within the house is significant, as it is relatively free from predators and therefore safe. Populations of An. gambiae s.s. found resting outside are usually low and difficult to find and are comprised predominantly of unfeds and gravids. They are usually to be found in dark areas, pits, gullies, even deep cracks in the earth. One bloodmeal is usually sufficient for maturation of the ovaries.

Control of adults has largely been restricted to the use of residual insecticides sprayed onto the walls of houses. This is less effective against the exophilic An. arabiensis and has encouraged the selection of resistant strains of An. gambiae s.s.. Recent work by Curtis and Lines (1986) and

MacCormack and Snow (1985) using bednets impregnated with pyrethroids has given encouraging control at village level.

Chemical and biological insecticides have also been applied to larval breeding grounds, though it is impossible to treat every temporary water body that forms. For these reasons interest in other control possibilities is growing.

1.3 INTRODUCTION TO THE COPEPODA

There are three orders in the Crustacean family of Copepoda, these being the Cyclopoida, the Calanoida and the Harpacticoida. Copepods are distributed worldwide and found utilising practically every type of water body. They are of great economical importance, not only as food for fish, but as intermediate hosts of human parasitic worms, for instance the Guinea worm Dracunculus medienensis (Gurney, 1931). Recently they have also been implicated as intermediate hosts of dimorphic microsporidia (Andreadis, 1985; Sweeney et al., 1985).

The Harpacticoida are generally benthic and the Calanoida planktonic. The Cyclopoida are a very much more diverse group, some benthic, some planktonic, their life histories varying enormously. It is the Cyclopoda that are generally implicated in parasite transmission.

Typically the development of all copepods passes through 5 or 6 nauplius stages and 5 copepodid stages. The nauplius stages are generally very small and do not resemble the final adult at all. Copepodid stages do however resemble small adults. The adult females are easily distinguished by their clearly visible egg sacs. Harpacticoid adults are generally flattened in adaption to their benthic life style, in comparison with the other 2 orders, which have pear shaped bodies 1-3mm in length. Females of the latter two orders are easily distinguished as Calanoids have only 1 egg sac and Cyclopoids have 2, one on either side of the genital segment (Gurney, 1931).

Dormancy and encystment have been observed in the Copepoda. This is a phenomenon exhibited by a few Harpacticoid species and many more species of Cyclopoids. It occurs under variable conditions and in response to different stimuli, including temperature. It is thought to occur much more widely than thus far known, and to be accompanied by the ability to resist drought (Gurney, 1931, 1932, 1933; Fryer and Smyly, 1954).

Food and feeding mechanisms in copepods are also very diverse, some being active predators, others detritivores, herbivores and some parasitic. Fryer (1957,a) observed the gut contents of a number of species including Mesocyclops leukarti, which was found to feed predominantly on animal material. In all cases copepods tend to find their food by chance encounter (Fryer, 1957,b), though herbivorous species can discriminate between edible and inedible particles.

M. leukarti is a common cyclopoid species found in Europe and the western part of Northern Asia. It was thought also to occur in Africa until its taxonomic status was redefined by Van de Velde (1984a). The species found in Africa and often mistaken for M. leukarti include M. rarus, the species found infected with microsporidia in this study. As they are closely allied species, much of the work on M. leukarti can be related to M. rarus.

Gophen (1977) and Jamieson (1980) demonstrated that the early stages of M. leukarti (naupliae and copepodid stages I-III) were dominantly herbivorous while the later copepodid stages (IV-V) and adults were preferentially carnivorous. The rate of predation was higher at cooler temperatures (15-22^{o}C c.f. 22-27^{o}C), and under starvation conditions, females survived longer (Gophen, 1977). In addition, the rate of development was also slower with increased temperature (Jamieson, 1980). Jamieson (1980) also showed that the size of prey items was important. Small prey, such as protozoa were sufficient for development, but very few copepodids were reared successfully on larger prey (Cerodaphnia dubia). With mixed diets, the time spent at copepodid stage V was shortest, suggesting that large food items were not an important source of food until this stage.

Habitats supporting diverse communities of copepods are often the same habitats that harbour mosquito larvae. As copepods can be predaceous on small larvae and act as alternate hosts of microsporidia and fungi, they have a strong potential influence over mosquito populations (Nasci et al., 1987).

CHAPTER 2

MATERIALS AND METHODS

2.1 DESCRIPTION OF SITES

Collections of mosquitoes were made at 134 British sites and 51 Tanzanian sites. Microsporidian infections were only found at 14 of these sites, and it is these sites which are described.

2.1.1 British Sites

Englemere

This area consisted of a small permanent lake in which no mosquitoes were found and an area of deciduous woodland (predominantly oak and beech) 3 miles from Ascot, Berkshire. There were many hollows where trees had fallen, which formed temporary pools in the spring. The size of these pools varied from 1 metre diameter to 3 metres in diameter. Most were approximately 30 cm deep.

In addition to the temporary pools there were shallow ditches (25–30cm deep) at the edges of the path, which also filled with water in the spring. Aedes cantans and Aedes punctor were collected in these temporary pools.

Smithfield Marsh

This area on the Isle of Grain, Kent, consisted of two semi-permanent brackish-water ditches 1–4 metres in diameter and 25–50 cm deep, fed by rain-water and seepage of salt water from the sea. The areas of ditch sampled were enclosed within a field of rough pasture, where horses grazed. Aedes detritus and Aedes flavescens larvae were collected from these ditches, and it was presumed that the horses provided the principal hosts for the adult females. This site also provided a habitat for dragonfly larvae, which were predacious on the mosquito larvae.

Sandyride

This area consisted of two small permanent pools in the garden of a large house near Ascot, Berkshire. One was 1m in diameter and 10cm deep, and the other, yielding more larvae, was just 0.5m diameter, but 40–50cm deep. At this site, larvae of Culiseta annulata, Culiseta morsitans and Culex pipiens were collected.

Swinley Brick Pits

These disused brick pits were set among diciduous woodland 3-4 miles from Ascot, Berkshire, on the A332. The area is of special scientific interest as it provides habitats for numerous bird, animal and insect species. Temporary and permanent pools are formed in the woodland, and provide breeding sites for many mosquito species including Culiseta annulata and Culiseta fumipennis, which were collected.

Bookham Common

Bookham Common in Surrey is an area similar to Englemere, comprised of deciduous woodland with areas forming temporary pools in the spring. These pools were formed from old gun pits and trenches dug during the second World War as a training ground for the army (Bangerter & Castell, 1948), and now provide excellent breeding grounds for Aedes cantans and Aedes punctor, which were collected.

2.1.2 African sites

Roadside Pool (Tanga/Pangani)

This pool/ditch was small (0.5m by 2m) and at the roadside between Tanga and Pangani. The surrounding land was rough scrub land grazed by cattle. Culex spp. were collected.

Kariakoo ditch

This ditch was between houses in a street in the Kariakoo area of Dar Es Salaam. The ditch was produced by an accumulation of rain water and water leaking from drains. Culex quinquifasciatus larvae were collected.

Mombo Irrigated Paddy Fields

These paddy fields were on the outskirts of the town of Mombo, and were run by T.I.R.D.E.P. All fields were flooded forming excellent breeding sites for mosquitoes. Anopheles gambiae s.l. and Culex spp. were collected.

Jitengeni Swamp

This was an extensive area of swamp land near to the village of Jitengeni, 1 mile from Muheza. The surface was completely covered in water lettuce (Pistia stratiotes), and in some areas with dense rush-like vegetation. This site was sampled in 1985, but by 1987 it had disappeared as the dam holding the water had broken. Mansonia spp. were collected.

Kisiwani Pond

In 1985 this pond, 300 m from the village of Kisiwani, was approximately 4m in diameter and completely covered in water lettuce plants (Pistia stratiotes). Debris from an overhanging coconut palm fell into the pond, producing an abundance of decaying material on which many organisms in the water, including Mansonia spp. of mosquito, fed. Monitor lizards and dragonflies, amongst others, were also seen taking advantage of the habitat. The surrounding land was under cultivation. In 1987, the pond had become reduced in size by approximately 50%, although it was still covered by water lettuce plants and still provided an excellent habitat for many organisms, including Mansonia spp. The surrounding land had fallen fallow and had become quite overgrown. During both years Mansonia spp. were collected.

Muheza Sisal Estate

This too was an extensive area of swampy water, 1 mile distant from Muheza town and within 200m of the Sisal Estate Village. It was created when the dam that held Jitengeni swamp burst, and was therefore only sampled in 1987. The swamp was divided into two by a road, and collections were only made from one side, as the other was inaccessible. The water surface was completely covered by water lettuce plants, and rushes, in which weaver birds were nesting. Mansonia spp. were collected.

Mamakingi Pond

This pond was similar to the Kisiwani pond as it was small, approximately 4m diameter, and completely covered in water lettuce plants. The pond was situated about 300 m from the village of Tengeni (2 miles from Muheza). Surrounding land was under cultivation, and although the pond water was rich in flora and fauna, it was regularly used for drinking and washing. This pond was only sampled in 1987. Mansonia spp. were collected.

Jitengeni Village

Jitengeni is a small village 1 mile from Muheza, where adult Anopheles gambiae s.l. were collected from huts.

Kumbamtoni Village

Kumbamtoni is a large village 15 miles from Muheza, where adult Anopheles gambiae s.l. were collected from huts.

2.2 HOST SAMPLING

Mosquito larvae, heavily infected with microsporidia were usually easy to distinguish from healthy individuals as their thoracic and abdominal segments appeared swollen and opaque. Larvae and adults could also carry latent infections.

2.2.1 Collection of larvae

Surface dwelling mosquito larvae

Larvae at a particular site were first examined for overt infections and when found, more detailed collections were made of larvae, adults and copepods (putative alternate hosts). A white enamel bowl 25cm in diameter and 5cm deep, skimmed across the water surface (Service, 1976) was used to sample all mosquito larvae and pupae except those in tree-holes or pit latrines and the Mansonia spp. Samples were taken in light and shaded, open and edge sites to maximise the micro-habitats sampled. Care was taken not to cast shadows over the water whenever possible and to create as little disturbance as possible before dipping. For the same reason, when it was necessary to wade into the water, the surface was allowed to settle for several minutes before dipping was resumed. A pipette was used to transfer larvae from the bowl to a screw top container to be transported to the laboratory. Any copepods in the bowl were also collected. The water remaining in the bowl was emptied away from the area of collection, or onto the bank of the site, where it could trickle back into the pond slowly, creating less disturbance. The screw top containers were only sealed just before being transported to the laboratory, and always kept out of direct sunlight to reduce mortality of the larvae.

In the laboratory these larvae were either smeared and examined for infection, or maintained in trays until signs of infection developed (as in the African collections of 1985).

Tree-hole Mosquitoes

Water-filled tree holes were too small to use the shallow bowl method. All water and larvae (Aedes geniculatus and Anopheles plumbeus) were extracted by pipette from each hole.

Mosquitoes in pit latrines

Culex quinquifasciatus larvae were sampled from pit latrines in Tanzania (1985) using a ladle on a long cane dipped into the latrine. The liquid was then tipped into a tray and larvae removed using a pipette.

Mansonia species

At all sites where Mansonia spp. larvae were collected, the water plants Pistia stratiotes with which they were associated, covered a high proportion of the water surface. Plants at the edges could be lifted out by hand and put into a tray with a little water before many larvae became dislodged and lost. The roots were shaken vigorously to dislodge larvae which were then collected using a pipette and held in a screw top pot. Plants varied in their attractiveness as attachment sites, some having many larvae, and some none at all. For this reason it was necessary to sample from many plants on each day, at each site. Plants that were out of reach were removed from the water using the pond net (see copepod collection) care being taken that the plant was disturbed as little as possible before being lifted from the water. Any larvae that were dislodged as the plant was transferred to the tray were caught in the net. As Mansonia respire via the roots of water plants it is not ideal for them to be held in pots for long periods of time. Transport from field sites was often extended (walking 2 miles) and so a tiny plant or leaf with roots on was always put into the pot with the larvae to aid survival. In the same way larvae could be maintained overnight in trays with plants.

In the case of all larval and copepod collections, if the same collecting bowls were used at different sites, they were sterilised before re-use. Sterilisation was achieved by microwaving pots and wiping with alcohol in Britain and by immersion in boiling water and wiping with alcohol in Africa. The same was true of the enamel collecting bowl, and pond net.

2.2.2 Collection of adults

Adults resting on vegetation

At the Smithfield site, resting adults were caught using a sweep net. The net was swept through the tall grass and other vegetation at either side of the site. Adults trapped in the net were then transferred to a cage using an aspirator.

Adults resting in animal houses and huts

In Britain, resting anopheline and culicine adults were collected using an aspirator from stables and dairies, and transported in test tubes.

In Tanzania, Anopheles gambiae s.l. and An. funestus adults were collected in the same way while resting in the eaves of huts, or in bed-nets. In many cases the huts were dark and a torch was necessary to see the adults before they were caught.

Night - biting catches

The human-bait catch was the method adopted for the collection of adult Mansonia spp. at Muheza (1985 and 1987). Catches were not made at specific times during the night, but whenever was convenient to the catchers, as Mansonia spp. are known to bite continually throughout the night.

Four or five people sat near to the larval site, each with a torch and an aspirator. If a mosquito was felt to alight (they had rolled up trouser legs and/or sleeves), it was found by torch and aspirated before it had bitten. It was then transferred to a small holding cage. The group of catchers as a whole provided a strong attractant to all mosquitoes in the proximity of the site. The holding cage was returned to the laboratory and held on a tray which was floating in another larger tray of water, to protect the mosquitoes from predatory ants until the following morning when the mosquitoes were fed using an anaesthetised guinea-pig. Sugar solution on cotton wool was provided to sustain them overnight.

Light traps

Standard C.D.C. light traps (Service, 1976), run on a 6V battery were used on two occasions in the village of Kisiwani (1987) to collect Mansonia spp. mosquitoes from the Kisiwani pond as an alternative to night biting

catches. Cotton wool soaked in sugar solution was put into the cage section of the trap to sustain the mosquitoes overnight.

2.2.3 Collection of copepods

Collection of copepods was facilitated using a fine mesh pond net. The mesh of the net was fine enough to enable both adult copepods and copepodids of all species to be collected. A universal tube was attached to the net and all organisms not passing through the mesh accumulated in it, in a small volume of water. Several net-fulls of water were filtered before the universal and its contents were emptied into a screw top pot to be transported to the laboratory. Up to 15 pots were filled in this way. Other organisms trapped in the net were removed using a pipette to another pot to inhibit predation on the copepods. Water was sampled from light and dark, open and edge, deep and shallow areas, to enhance the numbers collected. On some days the numbers seemed reduced and collection time had to be extended to collect sufficient copepods. All screw top pots were kept shaded during collection before being transported to the laboratory.

2.3 TECHNIQUES USED IN IDENTIFICATION OF HOSTS AND MICROSPORIDIAN PARASITES

2.3.1 Mosquitoes

The identification of larval culicine mosquitoes was achieved by viewing the siphon and/or head for distinct characteristics and of anophelines the head alone. Siphons and heads were removed and preserved in Berlese fluid under a coverslip when species identification was to be made. Keys by Marshall (1938) and Cranston *et al.* (1987) were used to identify British species and by Gillies & DeMeillon (1968) (Anophelines), Hopkins (1952), Evans (1938) and Edwards (1941) (Culicines) for African species. Adults held in test tubes were stunned by tapping the tube on the palm of the hand and then transferred to a microscope slide where they were examined for characteristic features.

2.3.2 Copepods

Copepods were preserved in 70% alcohol and identified by Dr.G.Boxshall (British Museum, Natural History).

2.3.3 Microsporidia

A heavy microsporidian infection in larvae is usually easily recognised as abdominal segments become swollen and opaque white in colour. A thin smear of an infected larva viewed fresh at x1000 magnification shows the refractile spores. Larvae must be smeared while alive for more accurate identification of the microsporidian species as, at death, developmental stages begin to degenerate and spores held within sporophorous vesicles will be released, thus making identification impossible.

Giemsa staining

All larvae from a site were dissected after identification and one part was smeared, air dried and stained in 10% Giemsa (Gurr's improved R66) buffered at pH 7.2 for 30 min and then screened for infection. A prevalence level was determined. Using light microscopy gross features of the microsporidian can be seen, e.g. whether it was multisporous, octosporous or had isolated spores, but accurate generic and specific identification was only possible using electron microscopy. The remaining pieces of larvae were stored at $4^{o}C$ on damp cotton wool then fixed for electron microscopy if found to be infected, or stored in 100 units/ml penicillin/streptomycin for use in field trials.

Electron microscopy

Small pieces (1 mm lengths) of infected tissue were prepared for transmission electron microscopy, according to the schedule below:-

1. Modified Karnovsky's fluid, 10 min, room temperature
2. Fresh modified Karnovsky's fluid, 1 hour, $4^{o}C$
3. 0.12 M sodium cacodylate, 15 min, $4^{o}C$
4. 0.12 M sodium cacodylate, 15 min, $4^{o}C$
5. 2.5% (w/v) OsO_4 in 0.1 M sodium cacodylate, 1 hour, $4^{o}C$, on rotator
6. 0.1 M sodium acetate, 10 min, $4^{o}C$, on rotator
7. 0.1 M sodium acetate, 10 min, $4^{o}C$, on rotator
8. 0.25% (w/v) uranyl acetate in water, 1 hour, $4^{o}C$
9. 0.1 M sodium acetate, 10 min, $4^{o}C$, on rotator
10. 0.1 M sodium acetate, 10 min, $4^{o}C$, on rotator
11. 35% acetone, 5 min, $4^{o}C$

12. 50% acetone, 5 min, $4^{o}C$
13. 70% acetone, with 1% uranyl acetate and 1% phosphotungstic acid, overnight, $4^{o}C$
14. 90% acetone, 10 min, room temperature
15. 100% acetone, 15 min, room temperature
16. 100% acetone, 15 min, room temperature
17. 100% acetone, 15 min, room temperature
18. 50/50 acetone/Spurr's resin, overnight, room temperature, on rotator
19. Gold sections cut on a Reichart ultramicrotome and transferred to grids
22. Grids stained in uranyl acetate for 5 min and washed
23. Grids stained in Reynold's lead citrate for 5 min and washed
24. Grids viewed on a Phillips 300 T.E.M.
25. Photography

Material fixed in Tanzania was held at stage 4 in the schedule until returned to the laboratory in Britain where the schedule was completed. The method used to make up modified Karnovsky's fluid is in Appendix 2.

Scanning Electron Microscopy

Spores from dead, heavily infected larvae, stored in 100 units/ml penicillin/streptomycin solution were prepared for scanning electron microscopy in Britain according to the following schedule.

1. Larvae macerated in a tube of penicillin/streptomycin solution with glass beads on a whirlymixer.
2. The homogenate was filtered through 4 layers of muslin to remove large debris and the filtrate spun for 10 min at 1000 g.
3. Pellet resuspended in 0.5 ml of distilled water.
4. This contaminated suspension of spores was layered onto a solution of 50% Percoll and spun at 1000 g for 10 min.
5. The layer containing the spores was resuspended in 100 units/ml penicillin/streptomycin solution.
6. The spores were fixed as for transmission electron microscopy in modified Karnovsky's fluid, and washed in 0.12 M cacodylate buffer.
7. Spores were dehydrated through a series of acetone solutions to 100% acetone.

8. Critically point dried onto cover slips.
9. Mounted on stubs.
10. Sputtered with gold for 10 min.
11. Viewed on PSEM Scanning e.m.

2.4 CULTURE TECHNIQUES

2.4.1 Mansonia uniformis and Mansonia africana

Two colonies of Ma. uniformis from Sri-Lanka and Tanzania were maintained in the laboratory for 14 and 4 generations respectively. It was intended that they should be used to study the transmission of microsporidia collected from wild-caught Mansonia spp. This was not achieved as the conditions for infectivity to Mansonia larvae were not met in the laboratory in Britain. Colony records are reported in Appendix I, Table C. Preliminary tests to detect species differences between the two colonies by comparing DNA sequences were made. The results were inconclusive.

These mosquitoes (subgenus Mansonioides) and other members of the subgenus are difficult to culture owing to their close association with water plants. The method used was based on the technique of Laurence et al. (1962) with some modifications: Salvinia sp. and Pistia sp. plants were used as attachment sites for larvae, rather than wet strength papers. The company making the original papers went out of business and although other papers were tested none provided suitable attachment sites.

Larval bowls contained 5 litres of guinea pig food infusion (25 g in 5 litres), a turf of grass to maintain a constant pH and Salvinia sp. to provide attachment sites for larvae. Yeast was added every 3 days to maintain a high level of micro-organisms, on which the larvae fed, and plants were replaced regularly when they died. Adults which began emerging from the bowls 25-40 days after oviposition were then transferred to a $30cm^3$ cage. The cage was held in a polythene bag, with damp absorbent paper to maintain a high humidity (>70%). Adults fed on sugar solution (8g fructose in 100ml distilled water) and mating occurred quickly. The first blood feed (either human, or guinea pig) was carried out 3-5 days after the first emergences. Bloodfed females were transferred to an "ovicage", where they rested for 2 days, until fully gravid. They were then transferred to "ovipots" where egg batches were laid. Ovipots consisted of a paper cup with a glass slide inside, on which the mos-

quito could rest and two Salvinia leaves floating on water, under which eggs could be laid. Sugar solution on cotton wool was applied to the netting held over the pot to sustain the mosquito. All ovipots were stored in a large humid cage until oviposition had occurred. Resulting egg batches were allowed to hatch into a small container before being introduced into a recently prepared larval bowl. Up to 8 or 10 egg batches were put in each bowl.

The culture technique was also used in the field for Ma. africana in attempts to infect them with microsporidia. Modifications are described in the section on field trials (see 2.5).

2.4.2 Mesocyclops rarus

The technique was modified from Brandl (1973) and Van de Velde (1984a,b), and used in an attempt to culture copepods in the laboratory in Britain after transport from Africa.

Adult, egg-bearing females were removed from those returned from Tanzania and held separately until they dropped their eggs. The females were returned to a stock tank and the eggs to a second tank. As soon as the eggs hatched Chlorella were added as food, then Paramecium were added as copepodid stages began to appear. When the copepodid stages became larger they were removed using a 250 um sieve and introduced into a third tank, with more Paramecium. As the adults appeared they too were removed using a 600 um sieve and put into the stock tank. Adults in the stock tank were fed with Artemia nauplii. The Artemia fed on algae and did not compete for food with adult copepods. The water was lightly aerated to prevent the formation of a bacterial film and changed regularly. Both Chlorella and Paramecium were cultured in the laboratory for several months (See Appendix II). The different developmental stages were reared separately in case of cannibalism. Modifications to this system were used in the field trials (2.5).

2.5 EXPERIMENTAL FIELD TRIALS

2.5.1 Adult collections : Study of transovarial (vertical) transmission

Adult bloodfed An. gambiae s.l. and An. funestus collected in huts at Jitengeni and Kumbamtoni were individually introduced into pots to lay eggs. These pots contained damp cotton wool and filter paper onto which eggs were

laid. Field collected adult Ma. africana and Ma. uniformis from Muheza Sisal Estate were bloodfed and held in an ovicage for two days before being transferred to ovipots to lay eggs (See 2.4.1). Ovipots were similar to those used for the colony in Britain. However, only small numbers of Salvinia plants were available, so small round pieces 1cm diameter were cut from Pistia leaves and polystyrene insulated wall lining, to be used as additional oviposition sites.

Each day, those that had laid eggs (of all species) were identified and the thorax and a small part of abdomen was smeared, stained with Giemsa and examined for microsporidian infection. The remaining piece of abdomen was stored on damp cotton wool at $4^{o}C$. If the thorax was infected, the abdomen was fixed for electron microscopy.

Egg batches laid by infected females, when hatched, were introduced into larval bowls and reared to 3rd-4th instar. After identification they were smeared and stained for transovarially transmitted infections. Egg batches from healthy females were set up as controls.

Ma. africana larval bowls were smaller (containing 2 litres of water) than those used for the colony in Britain and Pistia stratiotes plants were used as attachment sites. These plants were collected from Kwamihayo pond (1 mile from Muheza) where, although repeated collections were made, no Mansonia spp. were ever found. Plants were thoroughly washed in running water before use, to remove mud and predaceous larvae, and regularly replaced as they died. All larval bowls were covered in a fine meshed netting to prevent other species from laying eggs in them.

2.5.2 Larval Collections : Study of horizontal transmission

Larvae from the field were examined for infection. Larvae which were overtly infected were smeared after removal of the siphon and last body segment for identification. The remaining part of the larva was either dissected and fixed for electron microscopy, or stored intact in 100 units/ml penicillin/streptomycin solution for future use in transmission experiments.

All apparently healthy larvae were identified (by dissection of the siphon) and the remainder divided into two. Half was smeared, stained and examined for infection, and the other half stored on damp cotton wool at $4^{o}C$. If Giemsa staining revealed infections, the remaining half was fixed for electron microscopy.

Experiments on transmission of octospores and multispores to copepods (Mesocyclops rarus) from mosquitoes (Mansonia africana and Anopheles gambiae s.l.).

Copepods collected from field sites in screw top pots were tipped into white enamel trays, care being taken not to disturb the sediment at the bottom of the pots. This sediment was resuspended in fresh water and viewed separately. Adult copepods (a mixture of sexes) and some copepodids to make up the numbers, were pipetted from the tray into the trial pots (paper cups) and held in 50ml of water. On occasions they were held in this way overnight before trials were initiated. Before the trials all pond water and debris was removed and replaced with tap water. Copepods were sustained during trials by daily feeding with dead healthy mosquito larvae, chlorella, and paramecium suspension. (For chlorella and paramecium culture see Appendix II).

In 1985 a preliminary trial using no controls was carried out. One larva from Kisiwani infected with octospores was fed to copepods held in 20 ml of water. All were smeared, stained and viewed after 7 days. A similar trial was also made with copepods collected from Mombo irrigated paddy and one infected An. gambiae s.l. larva. The results from the trial using spopres from Ma. africana suggested that an infection had been established in the copepods but was only in its early stages after 7 days. In trials carried out in 1987 the copepods were held in 50-75ml of water and smeared and examined at 7 day intervals.

This experiment was repeated several times with various modifications. Firstly the pots were screened daily, then the holding volume was increased and finally the copepods were held separately to prevent cannibalism. The same trials were repeated using the spores of a multisporous species.

Experiment on the transmission of octospores from Mansonia africana to British copepods

In Britain, copepods were collected from Virginia Water, Surrey, and used in infection trials with octospores from Ma. africana and Culex annulirostris (kindly donated by Dr.A.W.Sweeney). Thirty five copepods were held in 50 ml of water with spores from 1 disrupted, infected larvae, and smeared after 7 days.

Experiments on direct transmission of octospores between mosquitoes of the same species

Using octospores from Anopheles gambiae s.l.

Spores of an octosporous microsporidian species (octospores) from the progeny of two infected An. gambiae s.l. adults from Kumbamtoni were fed to approximately 100 healthy 1st instar An. gambiae s.l. larvae, held in approximately 50 ml of water for 30 min. These larvae were from a colony in culture in Muheza. They were then transferred to covered larval bowls to develop. The larval bowls were the same as used for Mansonia culture, but had only 1 inch depth of water. A few strands of grass were left floating on the surface and larvae were fed a yeast, dried milk and ground fish mixture daily. 4th instar larvae, pupae and emerging adults were smeared and examined for infection. Controls held without spores in 50 ml water for 30 min and then reared, were also examined.

Using octospores from Mansonia africana

Octospores collected in the field from Ma. africana larvae were fed to uninfected 1st instar Ma. africana larvae, in 50 ml of water with a small piece of Pistia stratiotes plant (1 infected, disrupted larva). One group of larvae (from one egg batch) were held in this way for 30 min and another for 2 h (termed, short hold and long hold), before being transferred to larval bowls to develop. Larvae were identified, smeared and stained for infections after 21 days. No controls were used in this experiment as few 1st instar larvae were available at the time. However, the experiment was repeated with using 'short hold' conditions and controls on return to Britain.

Experiment on transmission of field collected copepod infections to 1st instar mosquito larvae

50 live and 50 dead field collected copepods were held with 1st instar Ma. africana larvae from 1 egg batch for 30 min. The larvae were then reared for 21 days in larval bowls and examined for infection.

CHAPTER 3

RESULTS

3.1 MICROSPORIDIAN INFECTIONS IN FIELD COLLECTIONS OF BRITISH MOSQUITOES

Microsporidian infections were found in 5 mosquito breeding grounds only (Table 1), out of a total of 26 breeding grounds (94 sites). A further 63 sites were visited where no larvae were found (Appendix I Table A).

3.1.1 Prevalence of infections

Englemere

Two 4th instar Aedes sp. from a pond where Aedes cantans and Aedes punctor were breeding out of approximately 475 larvae and pupae collected, were found with an octosporous microsporidian infection. The prevalence in the two mosquito species could not be determined as the larvae were not identified to species.

The larvae displayed no gross symptoms of infection, i.e., chalky white, swollen bodies and appeared healthy, although the thorax and abdomen were packed with spores and other developmental stages in both specimens. The infections appeared to be lethal as the larvae were moribund when collected.

Smithfield

One 3rd - 4th instar larva of Aedes detritus, out of approximately 200 larvae and pupae of a mixed population of Ae. detritus and Aedes flavescens sampled from Ditch 2, was found with an octosporous microsporidian infection. The prevalence cannot be determined, as larvae collected were not all identified to species. However, when adult collections were made 7 days after the octosporous infection was found in larvae, all but one of those collected were Ae. flacescens. Adults emerging from a site, rest in nearby vegetation and therefore the relative proportions of adult Ae. detritus to adult Ae. flavescens at Smithfield is likely to reflect the relative proportions of the two species as larvae at the same site. It is likely that the one infected Ae. detritus larva found within a week of the adult collections was one of only a few Ae. detritus larvae present at the time. Collections made later in the year, however, in a nearby area of marsh (within 10 metres of the original ditch) were exclusively Ae. detritus and still no infections were found. Copepods present at the site were identified as Acanthocyclops bicuspidatus lubbocki.

TABLE 1: Details of British sites where microsporidian infections were found

Date	Site	No. larvae Collected	No. larvae Examined	Instar	Species	Parasites	
						Microsporidia	Other
ENGLEMERE 9.4.86	Random	>80	80	3rd-4th	Aedes sp.	One 4th instar larva found infected with an octosporous species.	0
12.4.86	i)Pool formed by fallen tree	>100	80-100	2nd-4th	Aedes sp.	0	0
	ii)Small ditch by pond	>50	40-50	2nd-4th	Aedes sp.	0	0
	iii)Ditch along path.						
	Stretch A	>75	65-75	3rd-4th	Aedes sp.	0	0
	Stretch B	>75	65-75	3rd-4th	Aedes sp.	0	0
	Stretch C	>75	65-75	3rd-4th	Aedes sp.	One 4th instar larva found infected with an octosporous species.	0
	Stretch D	>75	65-75	3rd-4th	Aedes sp.	0	0
19.5.86	Stretch B	78	78	3rd-4th	Aedes sp.	0	0
		9	9	Pupae	Aedes sp.	0	0
	Stretch E	9	9	3rd-4th	Aedes sp.	0	0
		6	6	Pupae	Aedes sp.	0	0
	Stretch F	10	10	3rd-4th	Aedes sp.	0	0
		6	6	Pupae	Aedes sp.	0	0
	Stretch G	3	3	3rd-4th	Aedes sp.	0	0
	iv) Alders	7	7	3rd-4th	Aedes sp.	0	0
2.9.86	Ditch	0	0	-	-	-	-
10.4.87	Ditch	30	30	3rd-4th	Aedes sp.	0	0
	v) Carr	17	17	Pupae	Aedes sp.	0	0
SMITHFIELD	i)Ditch 1	>30	25=30	3rd-4th	Aedes sp.	0	0
22.4.86	ii)Ditch 2	>30	25-30	3rd-4th	Aedes sp.	0	0
22.5.86	i)Ditch 1	20	20	3rd-4th	Aedes sp.	One Aedes detritus larvae infected with microsporidian species with isolated spores	0
		16	16	Pupae	Aedes sp.	0	0

TABLE 1: Continued (2)

Date	Site	No. larvae Collected	No. larvae Examined	Instar	Species	Parasites Microsporidia	Parasites Other
	ii)Ditch 2	59	59	3rd-4th	Aedes sp.	One Aedes detritus larva found infected with an octosporous species	0
		9	9	Pupae	Aedes sp.	0	0
	iii)Pool	64	64	3rd-4th	Aedes sp.	0	0
		4	4	2nd	Aedes sp.	0	0
		24	24	Pupae	Aedes sp.	0	0
29.5.86	i)Ditch 1	2	2	4th	Aedes sp.	0	0
		11	11	3rd	Aedes sp.	0	0
		1	1	2nd	Aedes sp.	0	0
		7	7	pupae	Aedes sp.	0	0
	ii)Ditch 2 Stretch 1	8	8	4th	Aedes sp.	0	0
		2	2	3rd	Aedes sp.	0	0
		1	1	2nd	Aedes sp.	0	0
		10	10	pupae	Aedes sp.	0	0
	Stretch 2	14	14	4th	Aedes sp.	0	0
		6	6	3rd	Aedes sp.	0	0
		4	4	pupae	Aedes sp.	0	0
	Stretch 3	8	8	4th	Aedes sp.	0	0
		2	2	3rd	Aedes sp.	0	0
		3	3	pupae	Aedes sp.	0	0
	Stretch 4	1	1	3rd-4th	Aedes sp.	0	0
		4	4	pupae	Aedes sp.	0	0
	Stretch 5	1	1	4th	Aedes sp.	0	0
		2	2	3rd	Aedes sp.	0	0
		1	1	2nd	Aedes sp.	0	0
		4	4	pupae	Aedes sp.	0	0

TABLE 1: Continued (3)

Date	Site	No. larvae Collected	No. larvae Examined	Instar	Species	Parasites Microsporidia	Parasites Other
	Stretch 6	1	1	4th	Aedes sp.	0	0
		5	5	3rd	Aedes sp.	0	0
		2	2	2nd	Aedes sp.	0	0
		3	3	pupae	Aedes sp.	0	0
	Stretch 7	6	6	4th	Aedes sp.	0	0
		13	13	3rd	Aedes sp.	0	0
		3	3	pupae	Aedes sp.	0	0
	Around Ditch 2	53	53	Adult	Ae. flavescens	0	0
		1	1	Adult	Ae. detritus	0	0
9. 7.86	Ditch 1 & 2	-	-	-	-	-	-
11. 9.86	Area of marsh not previously sampled	23	20	3rd	Ae. detritus	0	0
		2	2	pupae	Ae. detritus	0	0
12. 5.87	Ditch 2	-	-	-	-	-	-
SANDYRIDE 13. 6.86	Lower Pool	9	9	3rd	Culiseta annulata	3 larvae found infected with a multisporous species	0
24. 7.86	Lower Pool	4	4	3rd	Culex pipiens	0	0
		1	1	2nd	Cx. pipiens	0	0
		1	1	1st	Cx. pipiens	0	0
		4	4	4th	Cs. annulata	0	0
		5	5	3rd	Cs. annulata	2 larvae found infected with a multisporous species	0
		1	1	1st	Cs. annulata	0	0
		1	1	3rd	Cs. morsitans	0	0
		1	1	Pupa	-	0	0
	Upper Pool	2	2	4th	Cx. pipiens	0	0
		5	5	3rd	Cx. pipiens	0	0
		1	1	Pupa	-	0	0
14. 5.87	Lower Pool	1	1	4th	Cx. pipiens	0	0
		3	3	3rd	Cs. morsitans	0	0
18.11.87	Lower Pool	0	0	-	-	-	-

TABLE 1: Continued (4)

Date	Site	No. larvae Collected	No. larvae Examined	Instar	Species	Parasites Microsporidia	Parasites Other
8. 4.88	Lower Pool	5	5	4th	Cs. annulata	0	0
		3	3	3rd	Cs. annulata	0	0
		1	1	2nd	Cs. annulata	0	0
9. 9.88	Lower Pool	3	3	4th	Cs. annulata	0	0
		7	7	3rd	Cs. annulata	One larva found infected with a multisporous species	0
SWINLEY	Pool 1	0	0	-	-	-	-
8. 9.86	Pool 2	0	0	-	-	-	-
	Pool 3	0	0	-	-	-	-
	Pool 4	1	1	2nd	Cs. annulata	0	0
	Pool 5	0	0	-	-	-	-
	Pool 6	12	12	2nd	Cs. fumipennis	0	0
	Pool 7	22	22	4th	Cs. annulata	One larva found infected with a multisporous species	0
		4	4	3rd	Cs. annulata	0	0
		4	4	2nd	Cs. annulata	0	0
		8	8	Pupae	Cs. annulata	0	0
BOOKHAM	Pool 1	100-300	30-40	1st-2nd	Aedes sp.	0	0
27. 3.86	Pool 2	50-100	20-25	1st-2nd	Aedes sp.	0	0
	Pool 3	40-50	10-15	1st-2nd	Aedes sp.	0	0
17. 6.86	Pool 2	3	3	4th	Aedes sp.	0	0
		2	2	3rd	Aedes sp.	0	0
		5	5	2nd	Aedes sp.	0	0
		9	9	Pupae	Aedes sp.	0	0
		1	1	Dead 4th	Aedes sp.	Larva found infected with species with isolated spores	0

Sandyride

Five Culiseta annulata larvae, out of 28 examined (17.8%) were found infected with a multisporous microsporidian species. The larvae displayed no gross signs of infection. Copepods present at the site were identified as Eucyclops agilis. Culex pipiens and Culiseta morsitans collected at the same site were not infected.

Swinley

One Culiseta annulata larva out of 38 collected was found infected with a multisporous infection (2.6%). Again no gross symptoms of infection were seen. No material was fixed for electron microscopy, but light microscopy showed that the parasite resembled that found in the Sandyride collections. The site was in an area of special scientific interest which prevented further collections being made.

Bookham Commom

At Bookham Common one 4th instar Aedes sp. collected from a pond where Aedes cantans and Aedes punctor were breeding was found infected with microsporidian spores. The larva was dead on collection and by the time it was examined only spores remained, so no information on development, including whether or not spores developed within vesicles could be obtained.

3.1.2 Morphology of Infections

Octosporous species in Aedes sp. (Englemere)

No material was examined by electron microscopy, but light microscopical examination revealed that the parasite had several features characteristic of the family Thelohaniidae.

Figures 1 - 4 show sequential sporogonic stages of the parasite. The earliest stages seen were early sporonts (Fig. 1) in which the two nuclei of a single diplokaryon were present. A sporophorous vesicle is laid down at the onset of sporogony. Although the vesicle could not be seen by light microscopy, its presence was presumed, as sporoblasts (and spores) were held together in groups of 8. Sporonts with variable numbers of nuclei (Figs. 2,3) to a maximum of 8 (Fig.2), were also seen. In these stages, metabolic products

FIGS. 1-4 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF AN OCTOSPOROUS INFECTION IN *Aedes* sp. LARVAE (ENGLEMERE)

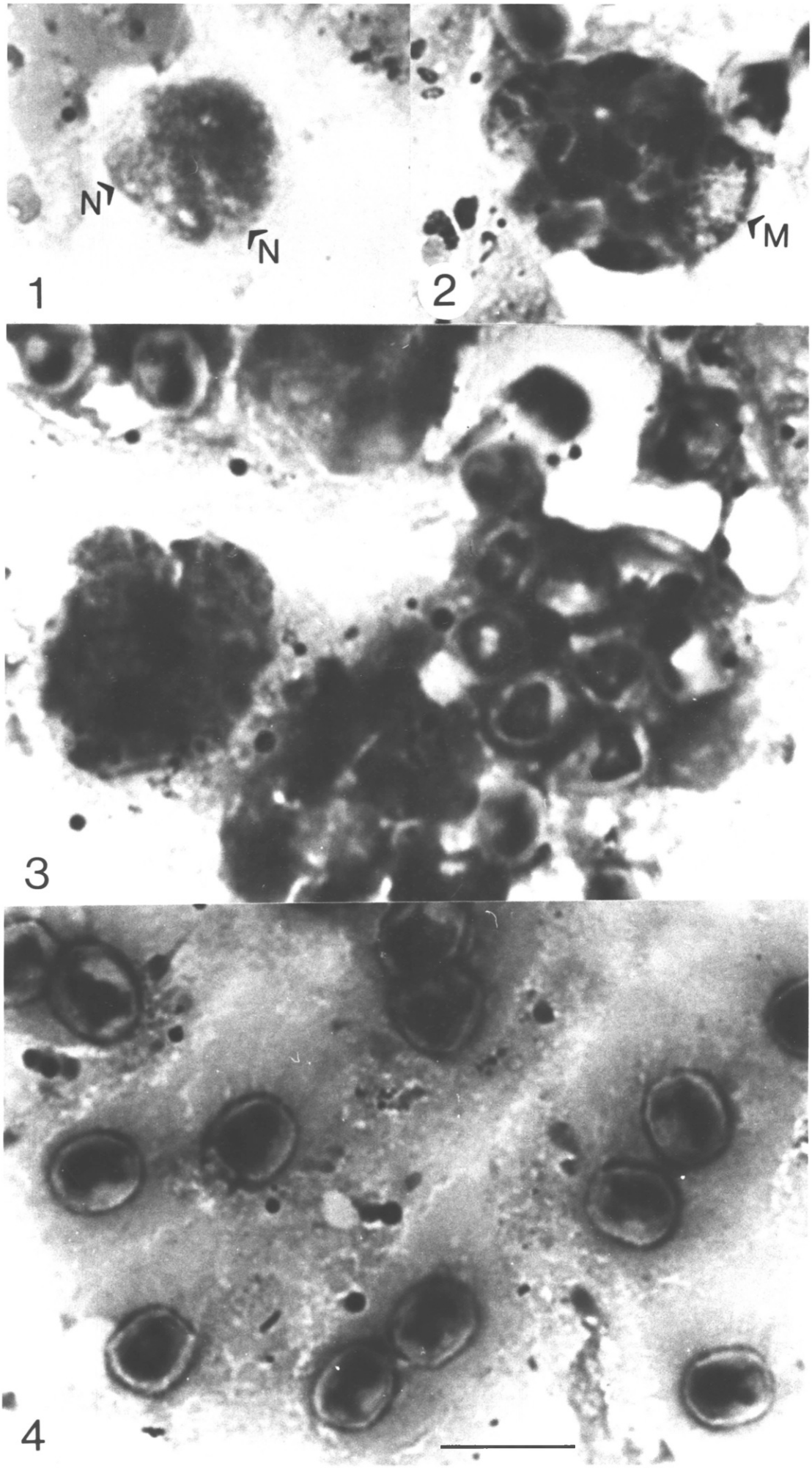
Scale bar = 10μm (Figs.1-4)

1. Early sporont; with 2 nuclei of the diplokaryon (N = nucleus)

2. Sporogonic stage with 8 nuclei; metabolic products (M) give the impression of supernumery nuclei.

3. Sporogonic stages with variable numbers of nuclei and sporoblasts in groups of 8.

4. Isolated spores presumed to have been released from a fine, easily disrupted sporophorous vesicle.


N
N
M
1
2
3
4

resembling nuclei but with different staining properties were visible within the vesicle (Fig.2). Division of the sporonts produced sporoblasts (Fig. 3) and spores (Fig. 4). The spores were generally isolated in the smears, suggesting that the sporophorous vesicle is of a fine, easily disrupted nature. Mature spores measured 6.8µm ± 0.4µm x 5.2µm ± 0.4µm (n=10) when fixed and stained and the sporophorous vesicles measured 21µm ± 1.1µm (n=5) diameter around the sporoblasts.

Octosporous species in Aedes detritus (Smithfield)

As with the infection at Englemere the larvae appeared normal. Electron microscopy was not carried out on the material, but light microscopy showed that the parasite had features characteristic of the family Thelohanidae. No merogonic stages were seen, but there were numerous sporonts with variable numbers of nuclei, up to 8 (Figs.5,6). Sporoblasts and spores in groups of eight were also seen (Fig.7). In comparision with the Englemere infection the sporophorous vesicle did not rupture as easily. The spores measured 5.8µm ± 0.2µm x 4.2µm ± 0.2µm (n=8), within a vesicle of average diameter 18.5µm ± 2.1µm (n=7).

One larva was found with spores not bound together in sporophorous vesicles. The larva was already dead when smeared so that developmental stages were lost and it was not possible to determine whether the spores normally developed within sporophorous vesicles. The spores measured 5.7µm ± 0.3µm x 4.3µm ± 0.2µm (n=10) and were not obviously different from the octospores found in Ae. detritus at the same site.

Multisporous species in Culiseta annulata (Sandyride)

Light microscopic appearance

The earliest stages seen were uninucleate and binucleate meronts or early sporonts (Figs.8,9). Subsequent nuclear divisions finally produced multinucleate stages (Figs.10,11). Nuclear and cytoplasmic division appeared to occur simultaneously as paler areas resembling lines of cytoplasmic cleavage were present between groups of nuclei (Figs.10,11). Sporonts developed into sporoblasts (Fig.12) and spores (Fig.13). Spores appeared oval and measured 2.3µm ± 0.2µm x 1.3µm ± 0.3µm (n=11). The number of spores per vesicle varied between 30 and 60 and the diameter of these vesicles ranged from 12.6µm to 21.0µm.

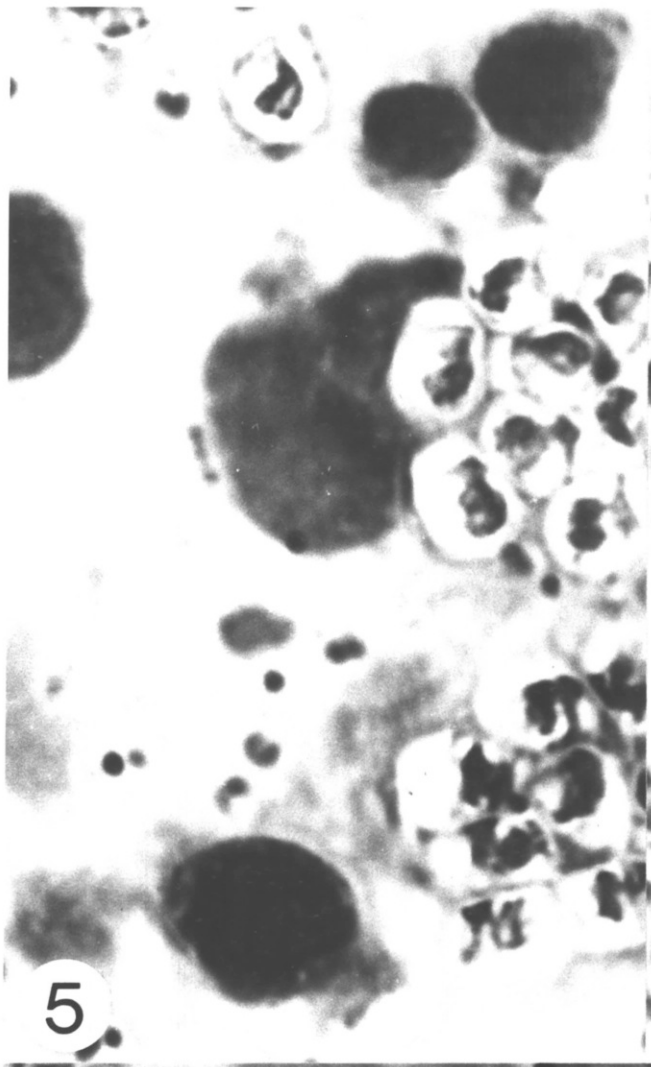
FIGS. 5-7 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF AN OCTOSPOROUS INFECTION IN Aedes detritus LARVAE (SMITHFIELD)

Scale bar = 10μm (Figs. 5-7)

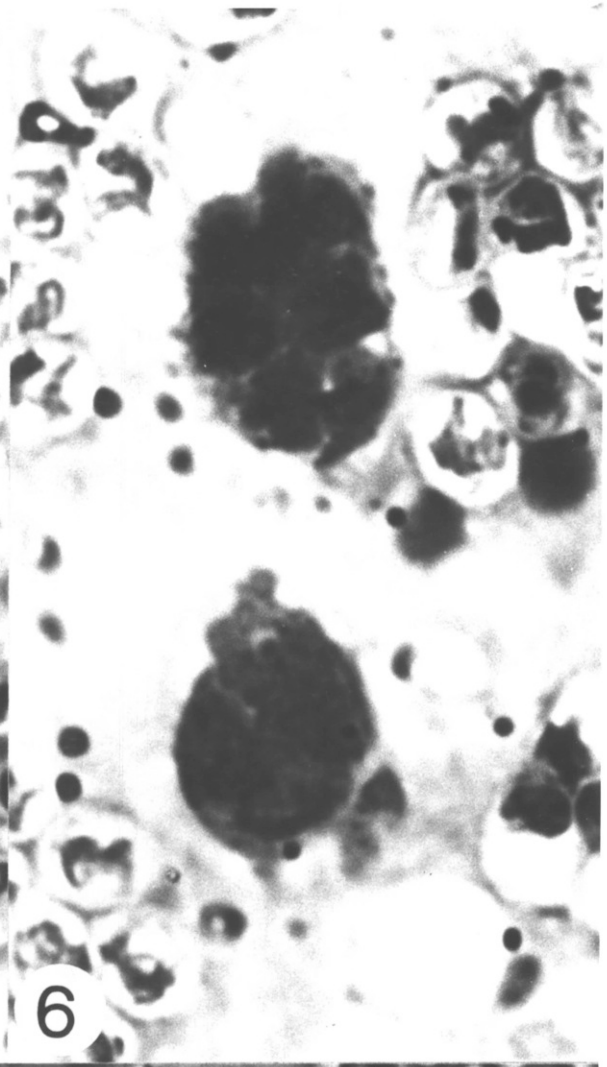
5. Sporogonic stages with variable numbers of nuclei.

6. Sporogonic stages with variable numbers of nuclei.

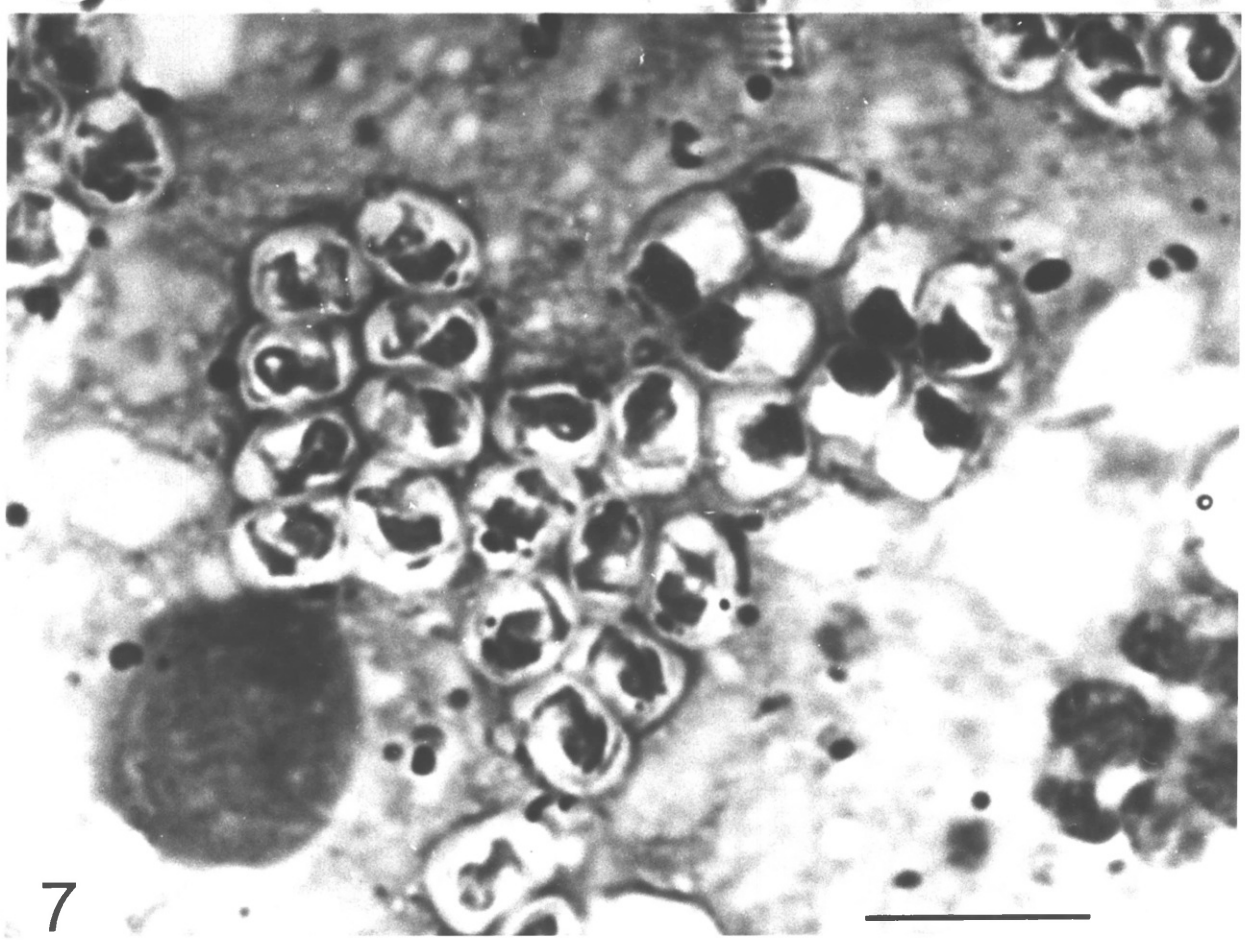
7. Sporoblasts and spores in groups of 8.



5



6



7

Ultrastructural appearance

Only sporogonic stages were found, and these were well advanced in sporogony, all within a fine membranous sporophorous vesicle (Fig.14). There was evidence of the patchy deposition of host ribosomes on the external surface of this membrane (Fig.15). Several segments of the original sporont each with one or more nuclear profiles packed the vesicle (Fig.14). The surface membranes of these segments were thickened externally in places by deposition of electron dense material (Fig.15) and additional patches of this material lay close to the parasites but free in the vesicle cavity. The space between the segments was occupied by finely granular material and coils of the same electron dense material as that covering the sporont division products. The nuclei contained patches of condensed chromatin but there was no evidence of synaptonemal complexes. Rough endoplasmic reticulum was abundant in the cytoplasm.

At a slightly later stage, the electron dense material formed a complete covering over the segments which were still at a uninucleate or binucleate stage as seen in one plane (Fig.16). At this stage the ribosomal covering of the sporophorous vesicle was almost complete. The final divisions of the segments gave uninucleate sporoblasts (Fig.17). The coiled metabolic products in the vesicle cavity were now dispersed amid the finely granular material and an occasional profile of a large tubule was present. The tubules increased in numbers as the sporoblasts matured (Fig.18). Late sporoblasts with an 0.05nm thick surface were crenated in outline and showed traces of the development of the polar filament. The vesicles containing maturing spores (Figs.19,20) appeared to be bounded by two membranes without ribosomes but the origin of the second membrane could not be determined. The cavity of the vesicle now contained little except a few of the large tubular structures. Spores were not well preserved in the material but immature spores showed signs of the interpolation of the lucent endospore layer under the exospore. Traces of endoplasmic reticulum and a single nucleus were the only spore organelles visible (Fig.20). Spores measured 2.28μm $\pm$ 0.12μm x 1.1μm $\pm$ 0.1μm (n=4).

Multisporous species in Culiseta annulata (Swinley)

Earliest stages seen were uninucleate and binucleate (Fig.21). Subsequent nuclear divisions produced tetranucleate (Fig.22) and multinucleate stages (Figs.23,24). Sporoblasts (Fig.23) developed within sporophorous

FIGS. 8-13 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF A MULTISPOROUS INFECTION IN Culiseta annulata LARVAE (SANDYRIDE)

Scale bar = 10μm (Figs. 8-13)

8. Uninucleate meront or early sporont.

9. Binucleate meront or early sporont.

10 & 11 Multinucleate sporogonic stages. The paler regions (arrowed) resemble lines of cytoplasmic cleavage between nuclei

12. Uninucleate sporoblasts.

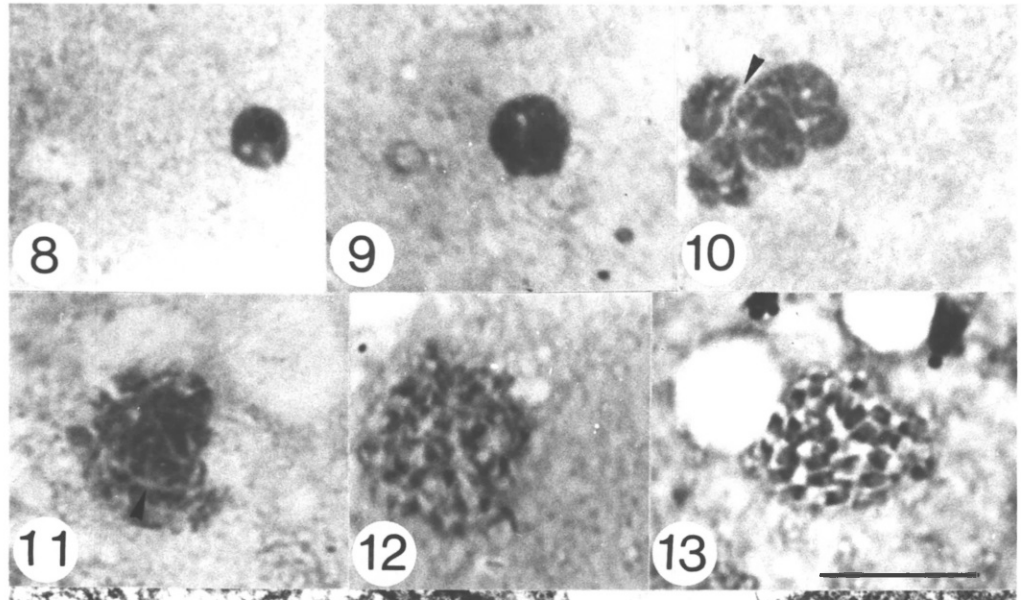
13. Uninucleate spores.

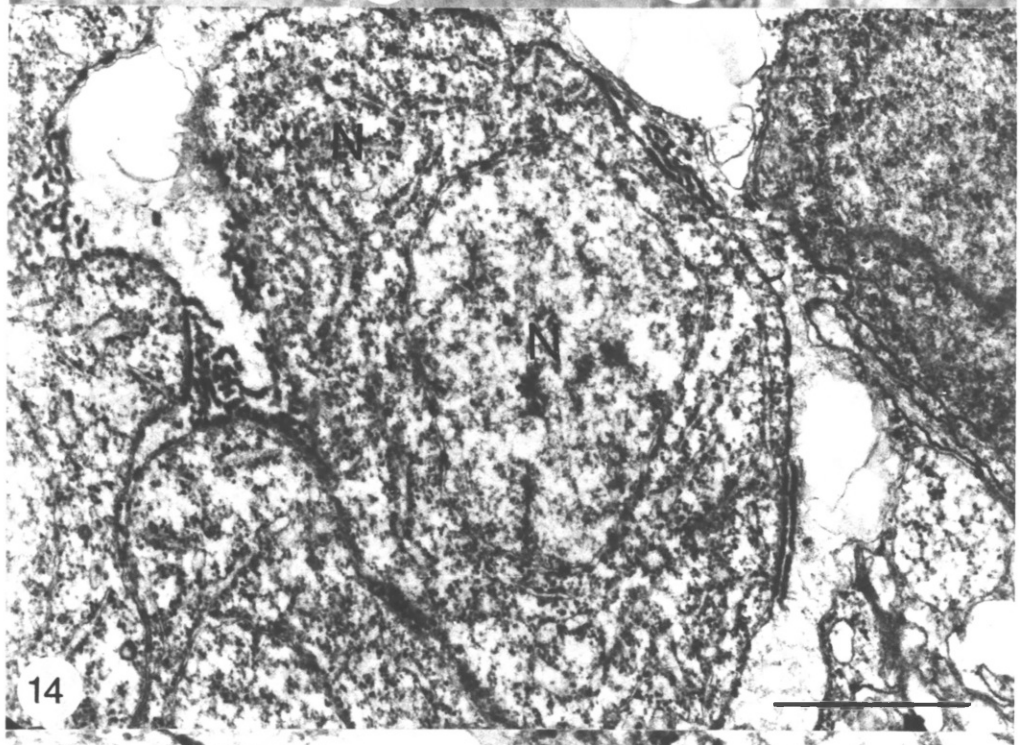
FIGS. 14-20 TRANSMISSION ELECTRON MICROGRAPHS OF A MULTISPOROUS INFECTION IN Culiseta annulata LARVAE (SANDYRIDE)

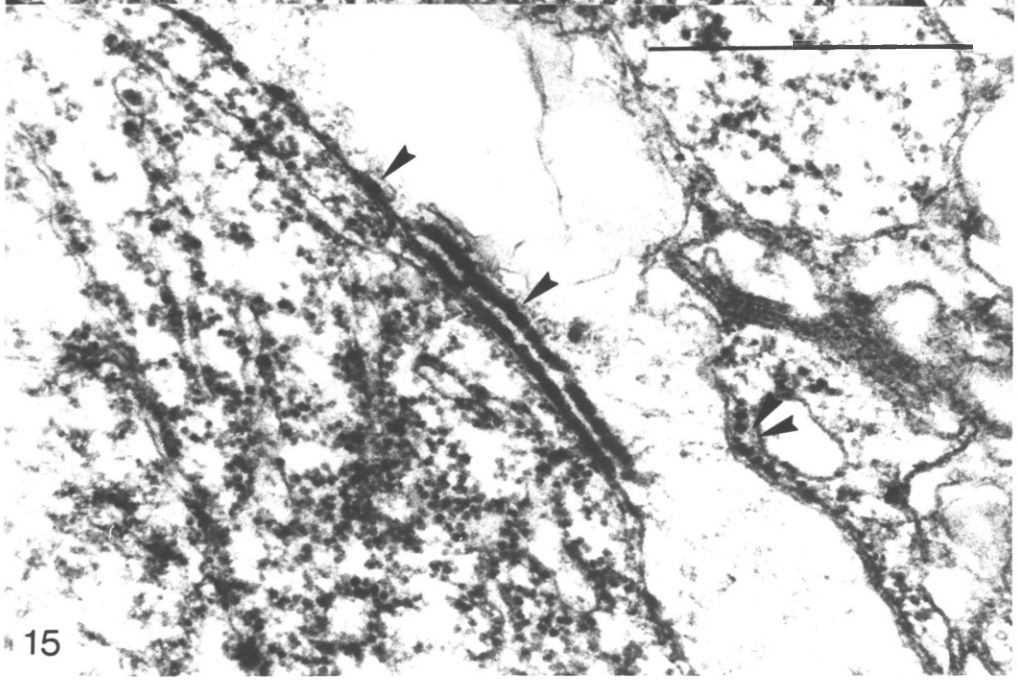
Scale bars = 1μm (Figs. 14-20)

14. Sporont segments, each with more than 1 nuclear profile within a fine sporophorous vesicle. (N = nucleus)

15. Detail of the plasma membrane and sporophorous vesicle in Fig.14. The plasma membrane is thickened externally in places, (indicated by a single arrow), and some of this electron dense material is sloughed off into the vesicle cavity (also indicated by a single arrow). Host ribosomes are also being patchily deposited onto the sporophorous vesicle (indicated by the double arrows).


8
9
10
11
12
13


N
N
14

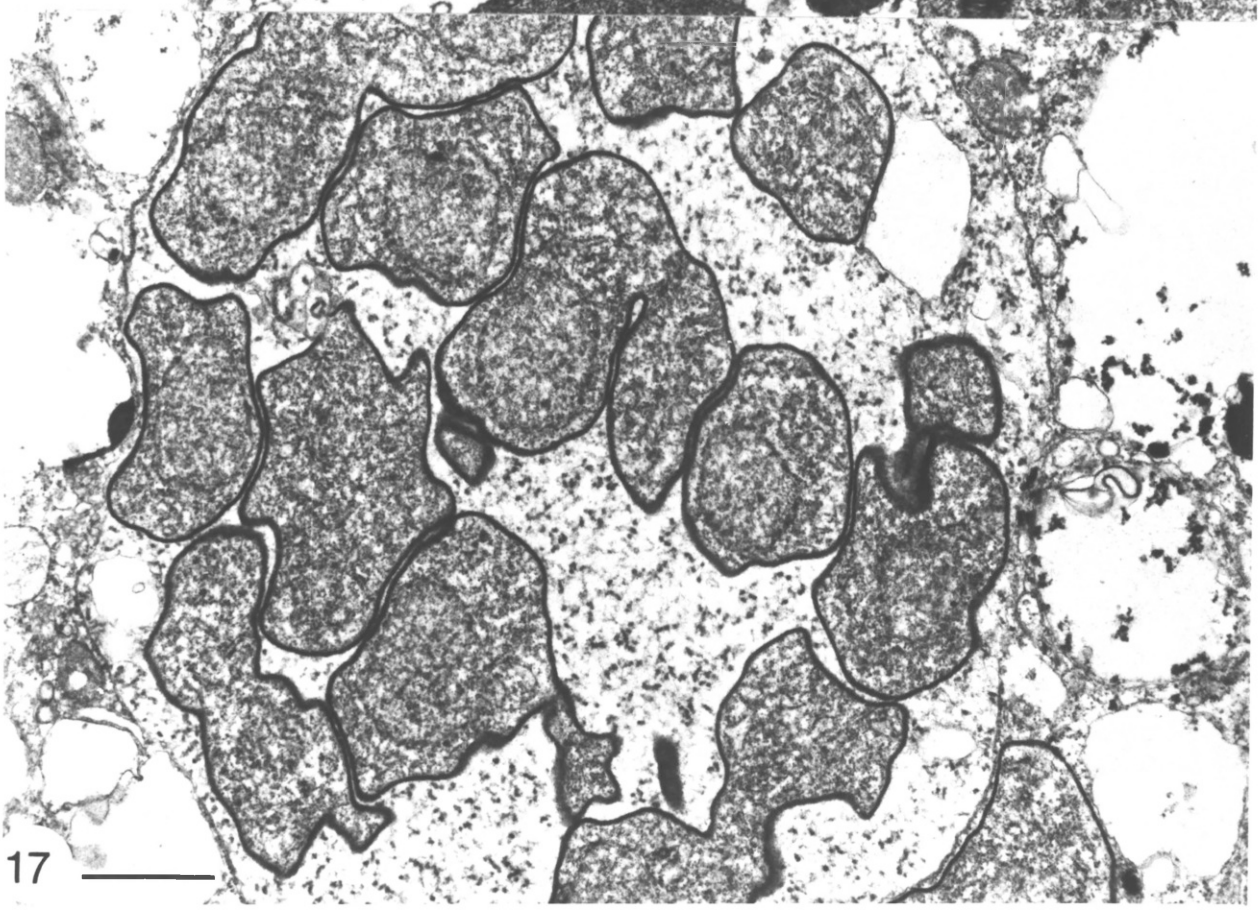

15

16. Uninucleate and binucleate sporont products within a sporophorous vesicle. The electron dense material has formed a complete covering on each segment and the ribosomal covering of the sporophorous vesicle is also almost complete.

17. Unincleate sporoblasts. Tubular profiles are now present in the sporophorous vesicle cavity.



16

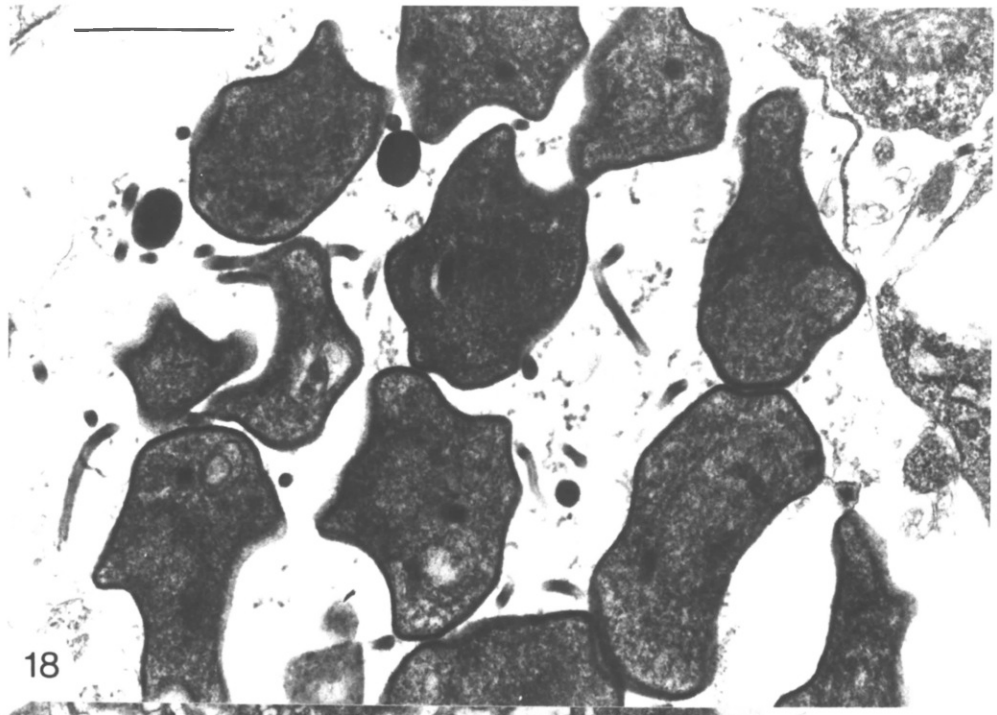


17

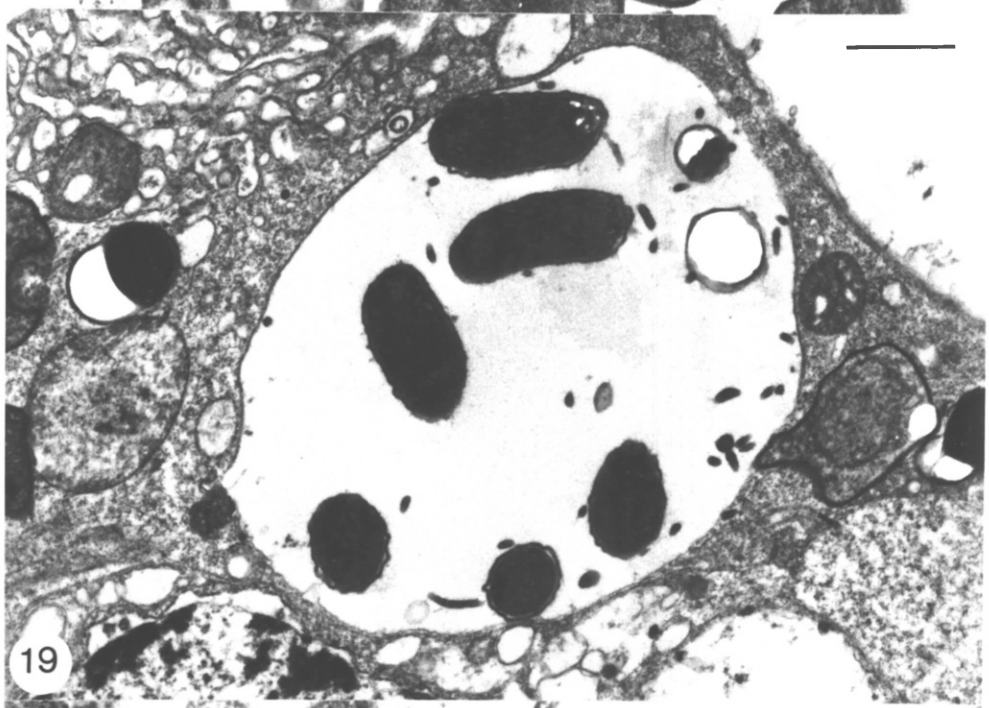
18. Mature uninucleate sporoblasts.

19. Maturing spores within the sporophorous vesicle.

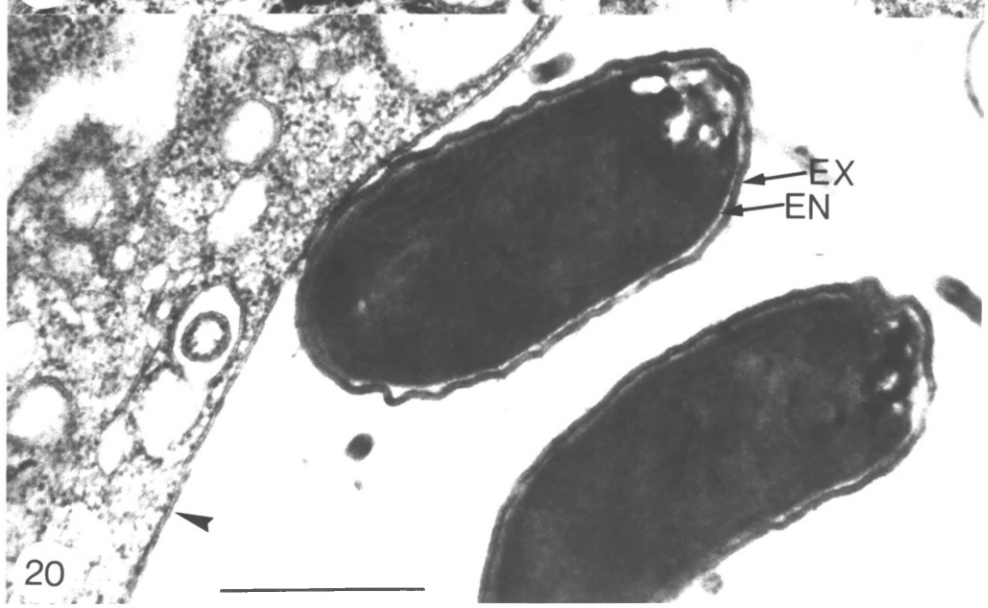
20. Detail of a maturing spore. The endospore (EN) and exospore (EX) are clearly visible and the sporophorous vesicle (indicated by an arrow) is double but no longer studded with ribosomes.



18



19



20

vesicles and these ultimately matured into spores (Figs.24), with up to 64 in each vesicle. Spores measured 2.3μm ± 0.2μm x 1.0μm ± 0.1μm (n=10) within highly variable sized vesicles depending on the number of spores within them. Vesicle diameter varied between 8.9μm and 18.4μm.

3.2 MICROSPORIDIAN INFECTIONS IN FIELD COLLECTION OF TANZANIAN MOSQUITOES

Microsporidian infections were found in 6 out of 47 larval sites and 2 out of 3 adult collection areas (Table 2). Sites where no infections were found are listed in Appendix I, Table B.

3.2.1 Prevalence of larval infections

Tanga/Pangani

At this site 8/10 Culex sp. pupae were found infected with an octosporous species. No infections were found in larvae, suggesting that heavy infections only developed at a late stage.

Kariakoo Ditch

Culex quinquifasciatus larvae were collected from this site and were returned to Britain as larvae. All were dead by the time they were examined at Silwood on the following day. The cadavers were macerated in a small volume of water and spun down to a pellet at high speed. The precipitate was then examined, revealing a very low number of spores, most of which appeared to have collapsed.

Mombo

Three 4th instar Anopheles gambiae s.l. were found with octosporous microsporidian infections (7.5%) which were identified as Parathelonania africana Hazard and Anthony (1974).

Muheza Area

In 1985 one 4th instar Mansonia africana larva out of 3 collected at Jitengeni swamp was found infected with a multisporous species of microsporidian. Also 22.6% (n=31) of Ma. africana larvae from a pond at Kisiwani were found infected with an octosporous species. In addition 9.7% (n=31) of the larvae from the pond and 12% (n=32) of adults collected around the pond

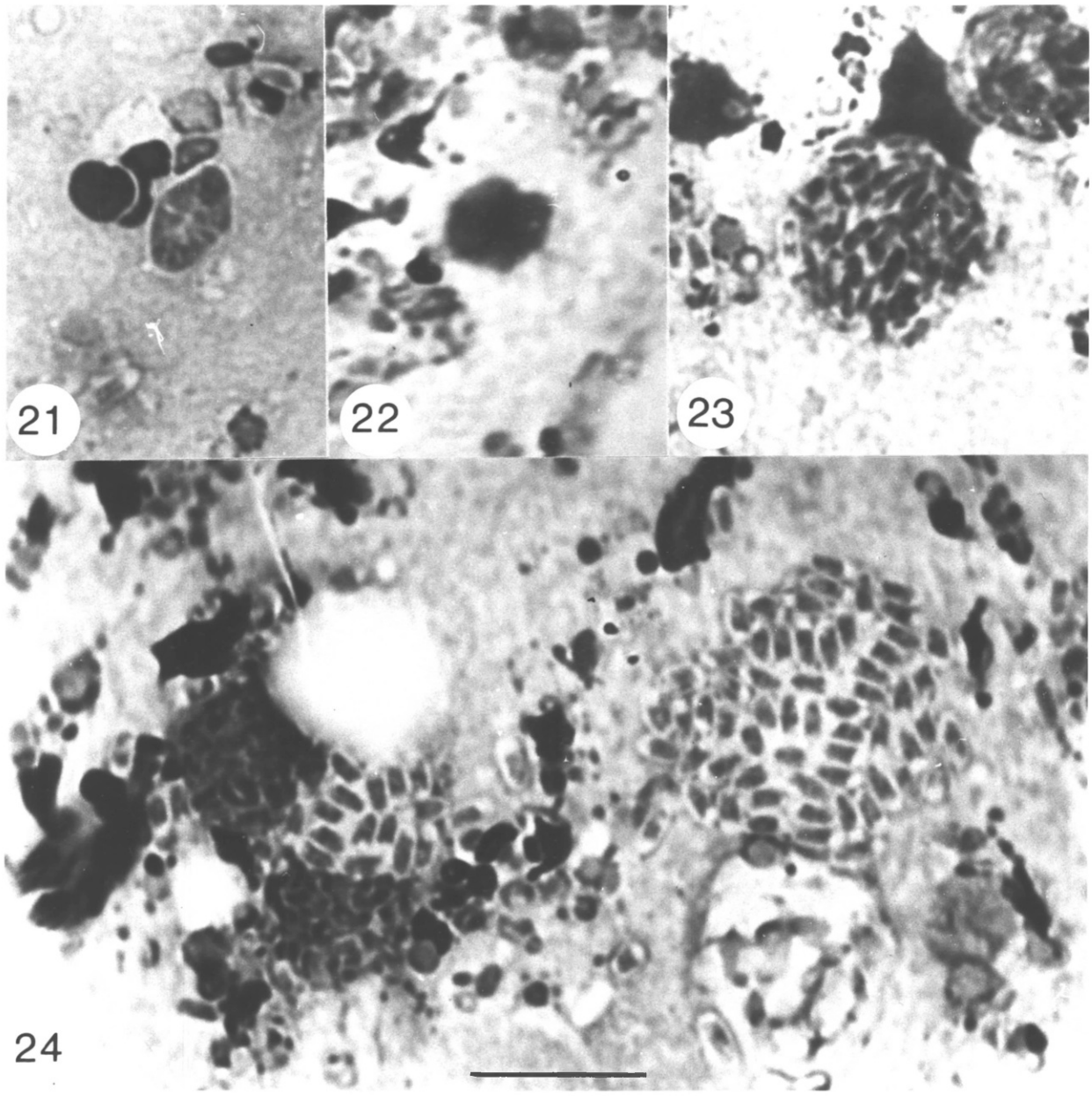
FIGS. 21-24 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF A MULTI SPOROUS INFECTION IN Culiseta annulata LARVAE (SWINLEY)
Scale bar = 10μm (Figs. 21-24)

21. Uninucleate and binucleate meronts (or early sporonts).

22. Tetranucleate sporogonic stage.

23. Multinucleate sporogonic stage.

24. Multinucleate sporogonic stage and uninucleate spores.


21
22
23
24

harboured diplokaryotic stages (Table 3, Section 3.4). In 1987 the dam containing Jitengeni swamp had been breached and the water had spilled over to form a swampy area at Muheza Sisal Estate. Collections made there, at Kisiwani and at another pond at Mamakingi, yielded octosporous, multisporous and diplokaryotic infections in larvae and diplokaryotic stages in adults. The octosporous and multisporous parasites collected at the different sites and on different years from Ma. africana were studied in detail by light and electron microscopy and were used in experimental work on transmission.

The prevalence figures for the octosporous, multisporous and diplokaryotic microsporidian infections at Kisiwani in 1985 are given in Table 3, and for these infections at Kisiwani, Muheza Sisal Estate and Mamakingi in 1987 in Tables 4, 5 and 6 respectively.

Although Coquilletidia spp. and Mansonia uniformis were collected at the same sites as the infected Ma. africana, none of the former and only one of the latter (4th instar) was found infected. The single Ma. uniformis is likely to have been a misidentification. The identification between the 2 Mansonia species depends on the number of spines on the anal segment. Ma. africana has 3 - 5 short spines and Ma. uniformis has just 2 long spines. Spines are easily broken or completely lost during transit, particularly from the moribund infected larvae, and it is considered likely that the octosporous species is restricted to Ma. africana. First instar larvae were never sampled. Pupae were collected but were never found infected.

The 1987 prevalences (June-September) for 2nd, 3rd and 4th instars at the three sites are represented in Fig.25 (octosporous species), Fig.26 (multisporous species) and Fig.27 (diplokaryotic stages).

Although the percentage of octosporous infection appears to increase with instar, when the data were transformed from percentage into proportion infected +1 and a rank sum test performed, no statistical difference was found between instars for octosporous infection, except between 2nd and 3rd instars at Mamakingi. The percentage of multisporous infection appeared highly variable between instars, although, using the same statistical analysis no significant difference was found. All figures for diplokaryotic infections were too low to allow analysis.

Figure 28 shows graphically the differences in levels of infection between sites. Using the same test for the total number of infected larvae found on each date, a significant difference at the 95% level was found

TABLE 2: Details of Tanzanian sites where microsporidian infections were found.

Date	Site	Species	Parasites/ Microsporidia	Others
Nov-Dec 1985	Roadside Pool between Pangani and Tanga	Anopheles sp. and Culex sp. larvae	8/10 Culex sp. pupae infected with an octosporous species.	0
Dec 1985	Kariakoo ditch Dar-Es Salaam	Culex quinquifasciatus larvae	Isolated spores retrieved from cadavers	0
Nov-Dec	Mombo, Irrigated Paddy Field	Anopheles gambiae s.l. and Culex sp. larvae	3/approx. 40-4th instar Anopheles gambiae larvae infected with an octosporous species.	0
Nov-Dec 1985	Jitengeni Village huts	Anopheles gambiae s.l. and Anopheles funestus adults	1/24 A. gambiae were found infected with diplokaryotic developmental stages	0
Nov-Dec 1985	Jitengeni Swamp	Mansonia africana larvae	1/3 larvae found infecteed with a multisporous species	0
Nov-Dec 1985	Kisiwani Pond	Ma. africana and Ma. uniformis larvae	*Numerous larvae infected with an octosporous species	0
June-Sept 1987	Kisiwani Pond	Ma. africana and Ma. uniformis larvae	*Numerous larvae infected with multisporous and octosporous species	0
June-Sept 1987	Muheza Sisal Estate Swamp	Ma. africana and Ma. uniformis larvae	*Numerous larvae infected with multisporous and octosporous species	0
June-Sept 1987	Mamakingi Pond	Ma. africana and Ma. uniformis larvae	*Numerous larvae infected with multispores and octospores	0
June-Sept 1987	Kumbamtoni huts	An. gambiae s.l. adults	3/39 found infected with diplokaryotic developmental stages and spores	0
		An. funestus adults	0	0

*Details in Tables 3 and 4

TABLE 3: Prevalence of microsporidian infections at Kisiwani in 1985

14.12.85	2nd instar No infected/ No collected	3rd instar No infected/ No collected	4th instar No infected/ No collected	No infected in all instars/ No collected
Octosporous	1/6	1/18	5/7	7/31
Multisporous	0/6	0/18	0/7	0/31
Diplokaryotic	0/6	3/18	0/7	3/31

TABLE 4: Prevalence of microsporidian infections at Kisiwani in 1987

TABLE 4a: Octosporous Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
13.6.87	0/ 1	1/ 3	2/ 3	3/ 7
14.6.87	0/ 1	0/ 4	1/ 3	1/ 8
15.6.87	1/ 1	0/ 3	2/ 2	3/ 6
20.8.87	0/ 7	4/30	1/ 8	5/ 45
21.8.87	1/ 8	4/38	0/10	5/ 56
1.9.87	0/ 3	2/12	2/22	4/ 37
Total number infected at all dates [%]	2/21 [9.5]	11/90 [12.2]	8/48 [16.7]	21/159 [13.2]

TABLE 4b: Multisporous Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
13.6.87	0/ 1	0/ 3	0/ 3	0/ 7
14.6.87	0/ 1	0/ 4	0/ 3	0/ 8
15.6.87	0/ 1	0/ 3	0/ 2	0/ 6
20.8.87	0/ 7	3/30	0/ 8	3/ 45
21.8.87	0/ 8	1/38	0/10	1/ 56
1.9.87	0/ 3	0/12	0/22	0/ 37
Total number infected at all dates [%]	0/21 [0]	4/90 [4.4]	0/48 [0]	4/159 [2.5]

TABLE 4c: Diplokaryotic Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
13.6.87	0/ 1	0/ 3	0/ 3	0/ 7
14.6.87	0/ 1	1/ 4	0/ 3	1/ 8
15.6.87	0/ 1	1/ 3	0/ 2	1/ 6
20.8.87	0/ 7	1/30	0/ 8	1/ 45
21.8.87	1/ 8	1/38	0/10	2/ 56
1.9.87	0/ 3	1/12	0/22	1/ 37
Total number infected at all dates [%]	1/21 [4.8]	5/90 [5.6]	0/48 [0]	6/159 [3.8]

TABLE 5: Prevalence of microsporidian infections at Muheza Sisal Estate in 1987

TABLE 5a: Octosporous Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
18.6.87	0/ 2	0/22	0/ 9	0/ 33
20.6.87	0/ 8	0/ 5	3/11	3/ 24
26.6.87	1/ 1	0/12	0/ 4	1/ 17
11.7.87	0/ 5	0/17	2/ 8	2/ 30
15.7.87	0/ 0	0/ 9	1/15	1/ 24
26.7.87	0/ 0	2/ 9	1/ 5	3/ 14
27.8.87	0/ 5	2/17	0/10	2/ 32
Total number infected at all dates [%]	1/21 [4.8]	4/91 [4.4]	7/62 [11.3]	12/174 [6.9]

TABLE 5b: Multisporous Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
18.6.87	0/ 2	1/22	2/ 9	3/ 33
20.6.87	0/ 8	0/ 5	0/11	0/ 24
26.6.87	0/ 1	0/12	0/ 4	0/ 17
11.7.87	0/ 5	0/17	0/ 8	0/ 30
15.7.87	0/ 0	0/ 9	0/15	0/ 24
26.7.87	0/ 0	0/ 9	0/ 5	0/ 14
27.8.87	2/ 5	4/17	1/10	7/ 32
Total number infected at all dates [%]	2/21 [9.5]	5/91 [5.4]	3/62 [4.8]	10/174 [5.7]

TABLE 5c: Diplokaryotic Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
18.6.87	0/ 2	0/22	0/ 9	0/ 33
20.6.87	0/ 8	0/ 5	1/11	1/ 24
26.6.87	0/ 1	0/12	0/ 4	0/ 17
11.7.87	0/ 5	0/17	0/ 8	0/ 30
15.7.87	0/ 0	0/ 9	0/15	0/ 24
26.7.87	0/ 0	0/ 9	1/ 5	1/ 14
27.8.87	0/ 5	0/17	1/10	1/ 32
Total number infected at all dates [%]	0/21 [0]	0/91 [0]	3/62 [4.8]	3/174 [11.7]

TABLE 6: Prevalence of microsporidian infection at Mamakingi in 1987

TABLE 6a: Octosporous Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
23.6.87	0/ 2	1/10	0/ 1	1/ 13
27.6.87	1/ 9	2/ 9	3/ 3	6/ 21
28.6.87	0/ 0	1/ 7	2/ 4	3/ 11
29.7.87	0/ 1	2/ 4	0/ 2	2/ 7
5.8.87	0/ 2	3/14	2/ 5	5/ 21
7.8.87	1/ 6	4/ 9	0/ 2	5/ 17
14.8.87	0/ 0	3/10	0/ 2	3/ 12
16.8.87	0/ 5	1/12	0/ 2	1/ 19
Total number infected at all dates [%]	2/25 [8.0]	17/75 [22.6]	7/21 33.3]	26/121 [21.5]

TABLE 6b: Multisporous Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
23.6.87	0/ 2	1/10	0/ 1	1/ 13
27.6.87	0/ 9	0/ 9	0/ 3	0/ 21
28.6.87	0/ 0	0/ 7	0/ 4	0/ 11
29.7.87	0/ 1	0/ 4	0/ 2	0/ 7
5.8.87	0/ 2	0/14	0/ 5	0/ 21
7.8.87	1/ 6	0/ 9	0/ 2	1/ 17
14.8.87	0/ 0	0/10	0/ 2	0/ 12
16.8.87	0/ 5	0/12	1/ 2	1/ 19
Total number infected at all dates [%]	1/25 [4.0]	1/75 [1.3]	1/21 [4.8]	3/121 [2.5]

TABLE 6c: Diplokaryotic Infection

Date	2nd Instar No infected/ No collected	3rd Instar No infected/ No collected	4th Instar No infected/ No collected	Total No Infected/ No collected all instars
23.6.87	0/ 2	0/10	0/ 1	0/ 13
27.6.87	1/ 9	0/ 9	0/ 3	1/ 21
28.7.87	0/ 0	0/ 7	0/ 4	0/ 11
29.7.87	0/ 1	0/ 4	0/ 2	0/ 7
5.8.87	0/ 2	0/14	0/ 5	0/ 21
7.8.87	0/ 6	0/ 9	0/ 2	0/ 17
14.8.87	0/ 0	0/10	0/ 2	0/ 12
16.8.87	0/ 5	0/12	0/ 2	0/ 19
Total number infected at all dates [%]	1/25 [4.0]	0/75 [0]	0/21 [0]	1/121 [0.8]

FIGURE 25: % Octosporous infection of Ma. africana larvae

June - September 1987

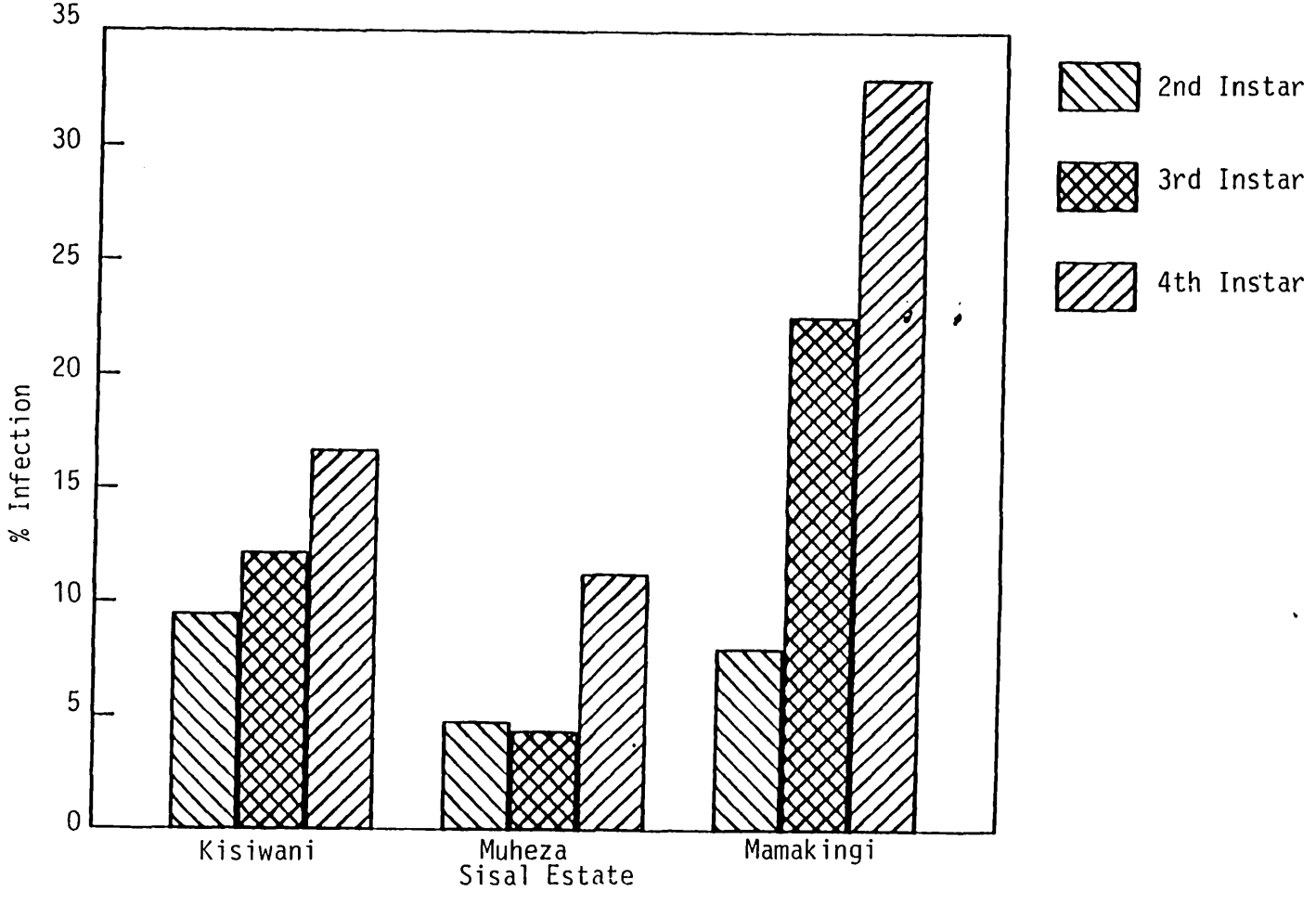


FIGURE 26: %Multisporous infection of Ma.africana larvae
June - September 1987

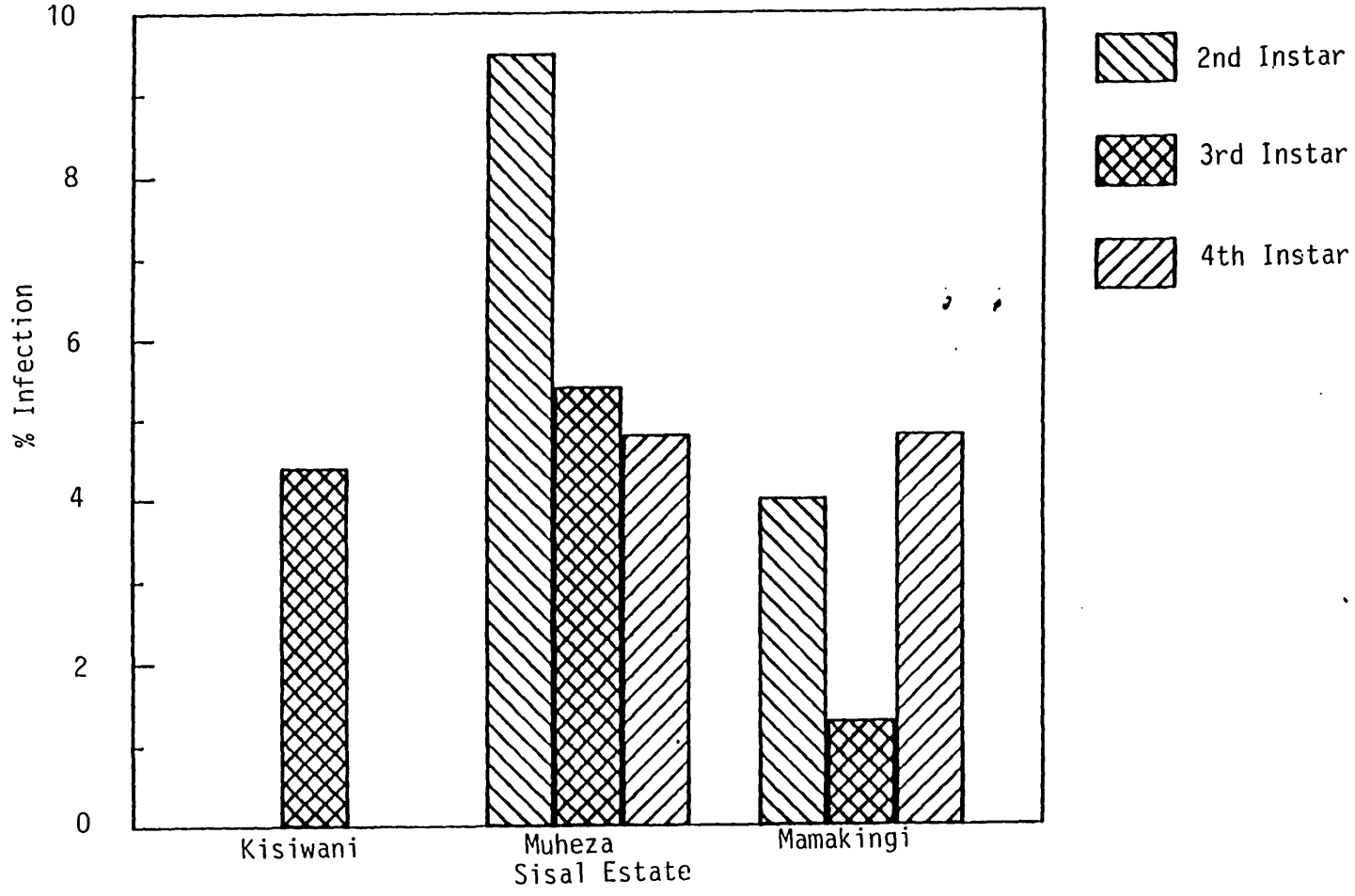
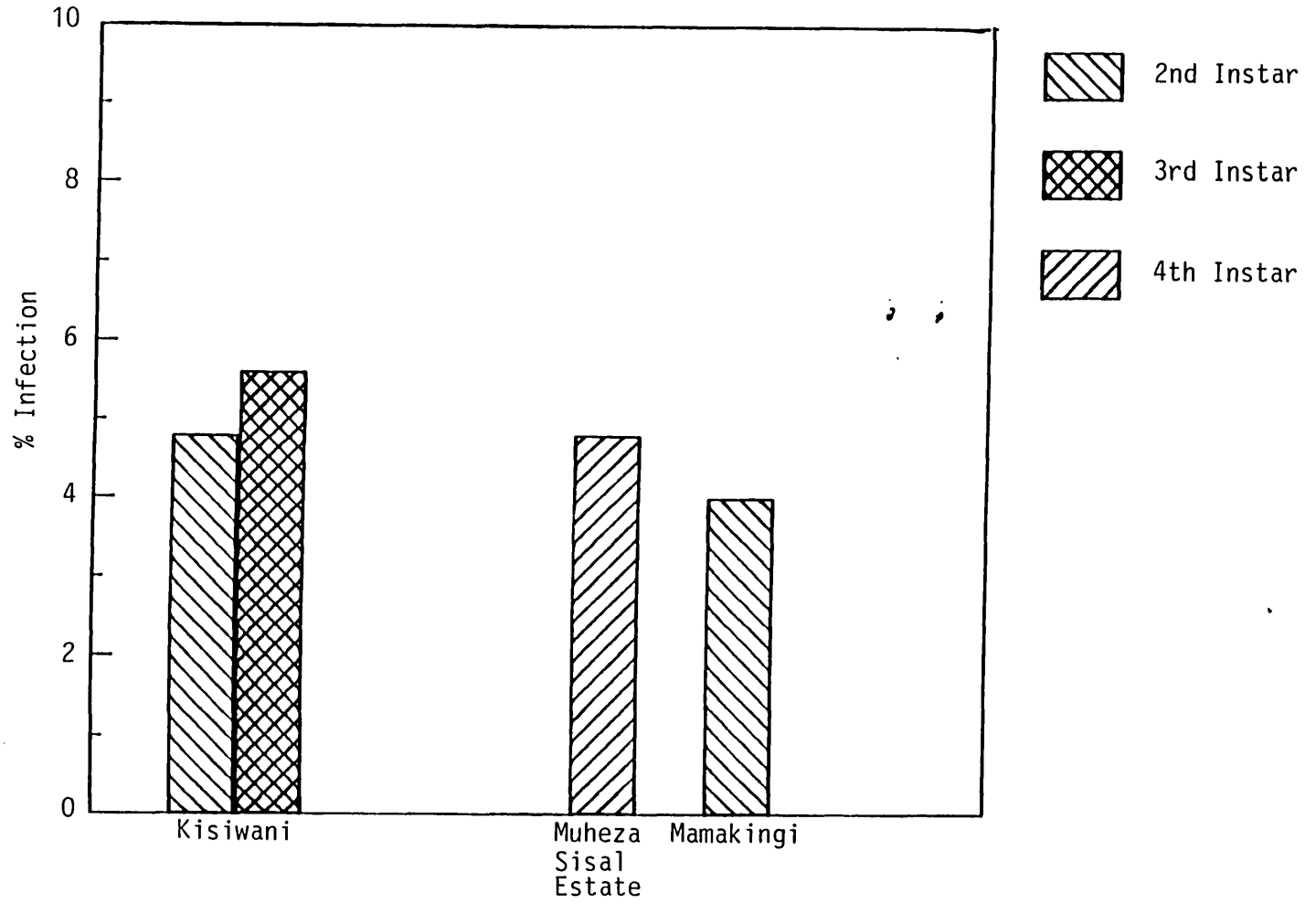


FIGURE 27: % Diplokaryotic infection of Ma.africana larvae

June - September 1987



between octosporous infections at Mamakingi and Muheza Sisal Estate. There was no significant difference between Kisiwani and Mamakingi. The difference between octosporous infections at Kisiwani and Muheza Sisal Estate fell just outside the confidence limits suggesting that although not significantly so, the levels of infection were different at these sites. There was no significant difference between sites of the multisporous infection.

The variation in infection levels with time at each of the 3 sites is represented in Figs.29,30,31. The prevalence of infection for each type (octosporous, multisporous or diplokaryotic) is represented only by the area of hatching/cross-hatching indicated on the chart e.g. at Kisiwani on June 14th 12.5% of larvae had octosporous infections and 12.5% had diplokaryotic infections. In July 1987 very few larvae were being recovered in collections from Kisiwani Pond, probably as a result of overcollection in June. In order to ensure a supply of infection for transmission experiments, uninfected 1st instar larvae which had been hatched from egg batches laid by 7 adults (subsequently examined and found free of infection) collected near the Muheza Sisal Estate site, were added to the small pond. Larvae both infected and uninfected were collected in August and September. From Fig.29 it can be seen that octosporous infections were found in all months at Kisiwani (except July when no collections were made), but at lower levels in August and September. Multisporous infections were only found in August and September. At Muheza Sisal Estate octosporous infections were found in all months, but multisporous infections were not found between mid-June and late-August (Fig.30). This was also true at Mamakingi Pond (Fig.31).

3.2.2 Morphology of larval infections

Octosporous species in Culex sp. (Pangani)

Spores only were seen, in groups of 8, within a sporophorous vesicle (Fig.32). Spores measured 3.9μm ± 0.3μm x 2.4μm ± 0.3μm (n=6) and the vesicle diameter measured 9.7μm ± 0.3μm (n=6).

Octosporous species in Anopheles gambiae s.l. (Mombo)

Material from all larvae was examined by light and electron microscopy. Measurements of spores were obtained from electron micrographs (Figs.33,34).

FIGURE 28: Overall % infections of Ma. africana larvae at Kisiwani, Muheza Sisal Estate and Mamakingi.

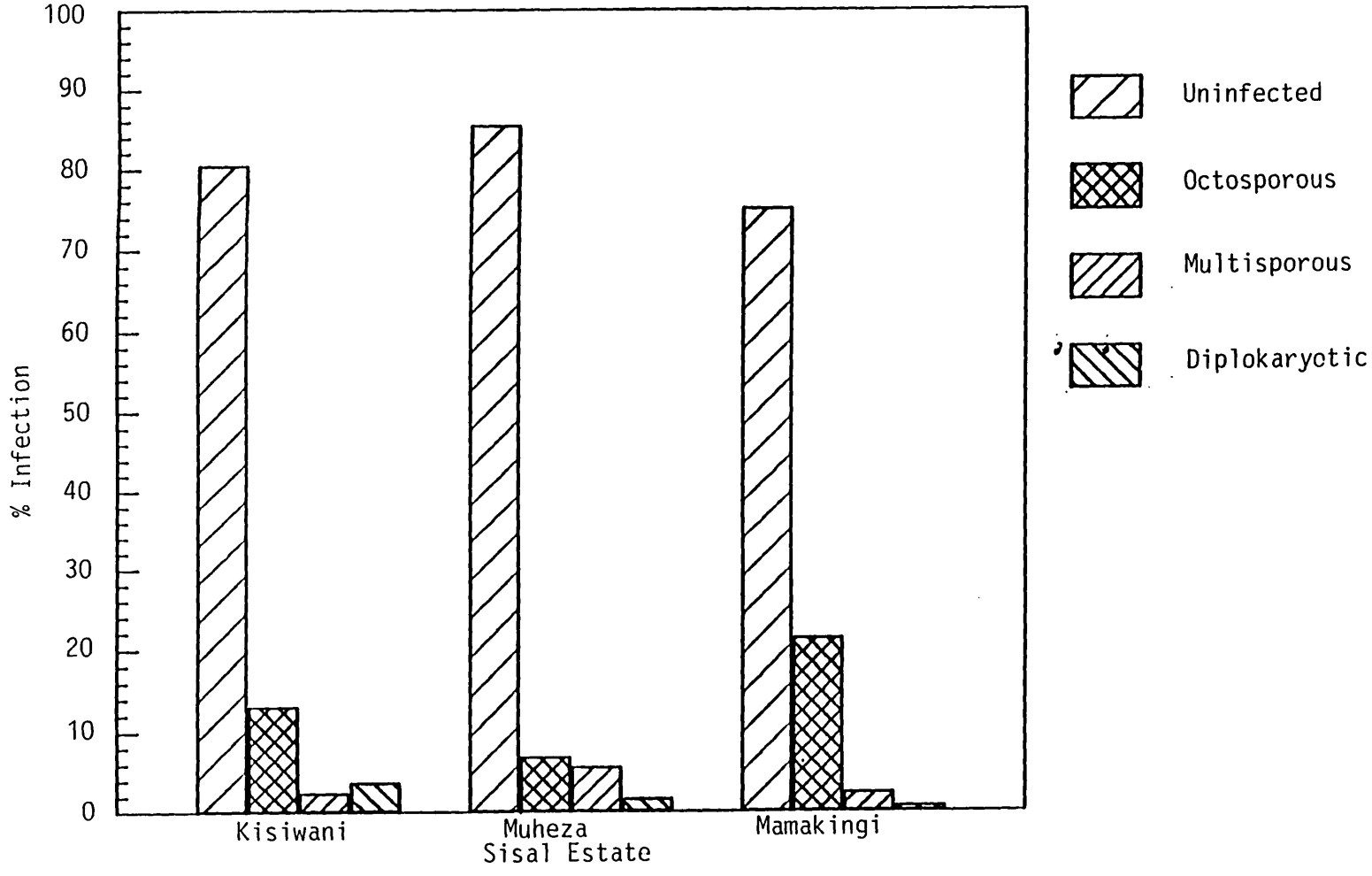


FIGURE 29: % Infection of all instars of Ma. africana

Kisiwani, 1987

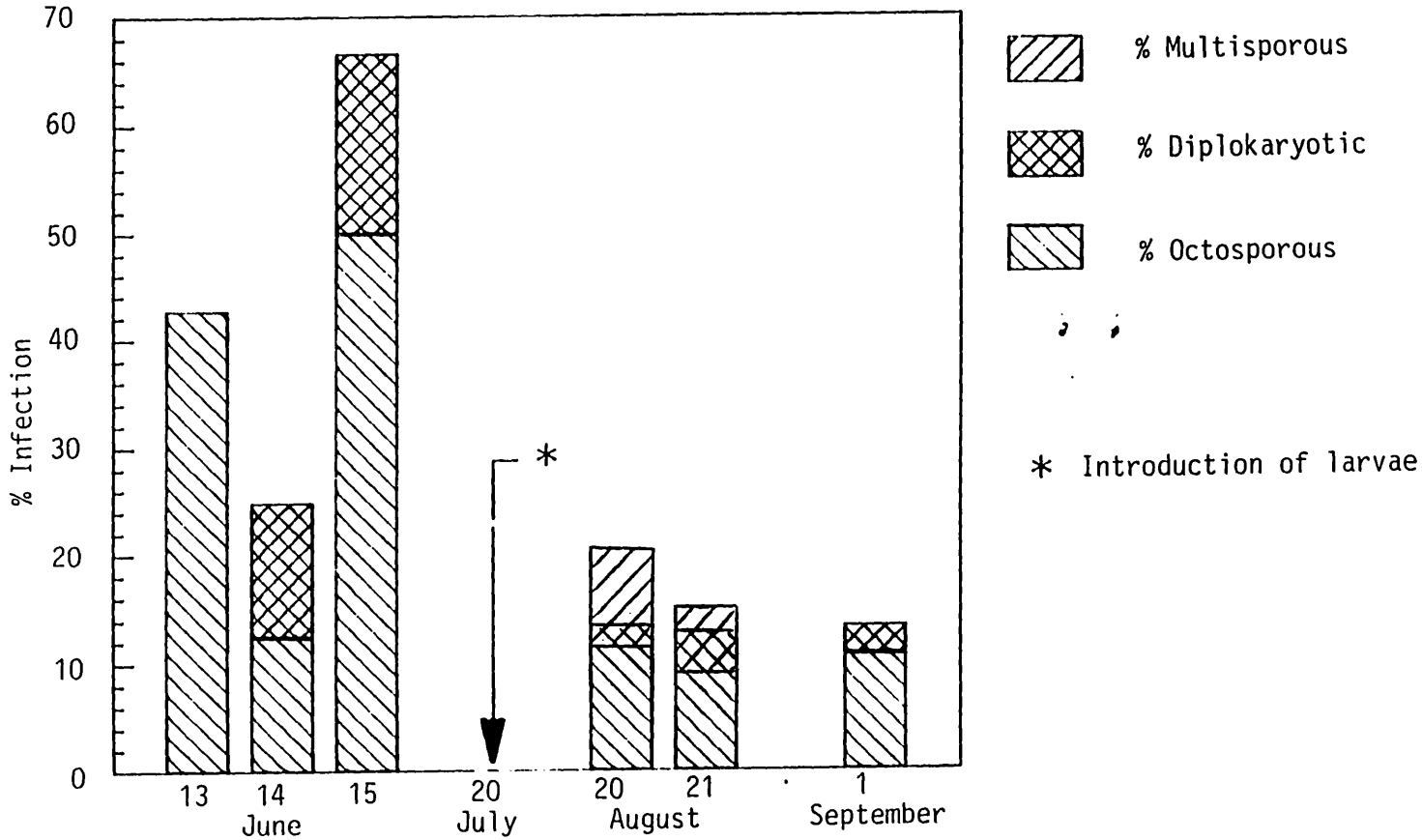


FIGURE 30: % Infection of all instars of Ma. africana
Muheza Sisal Estate, 1987

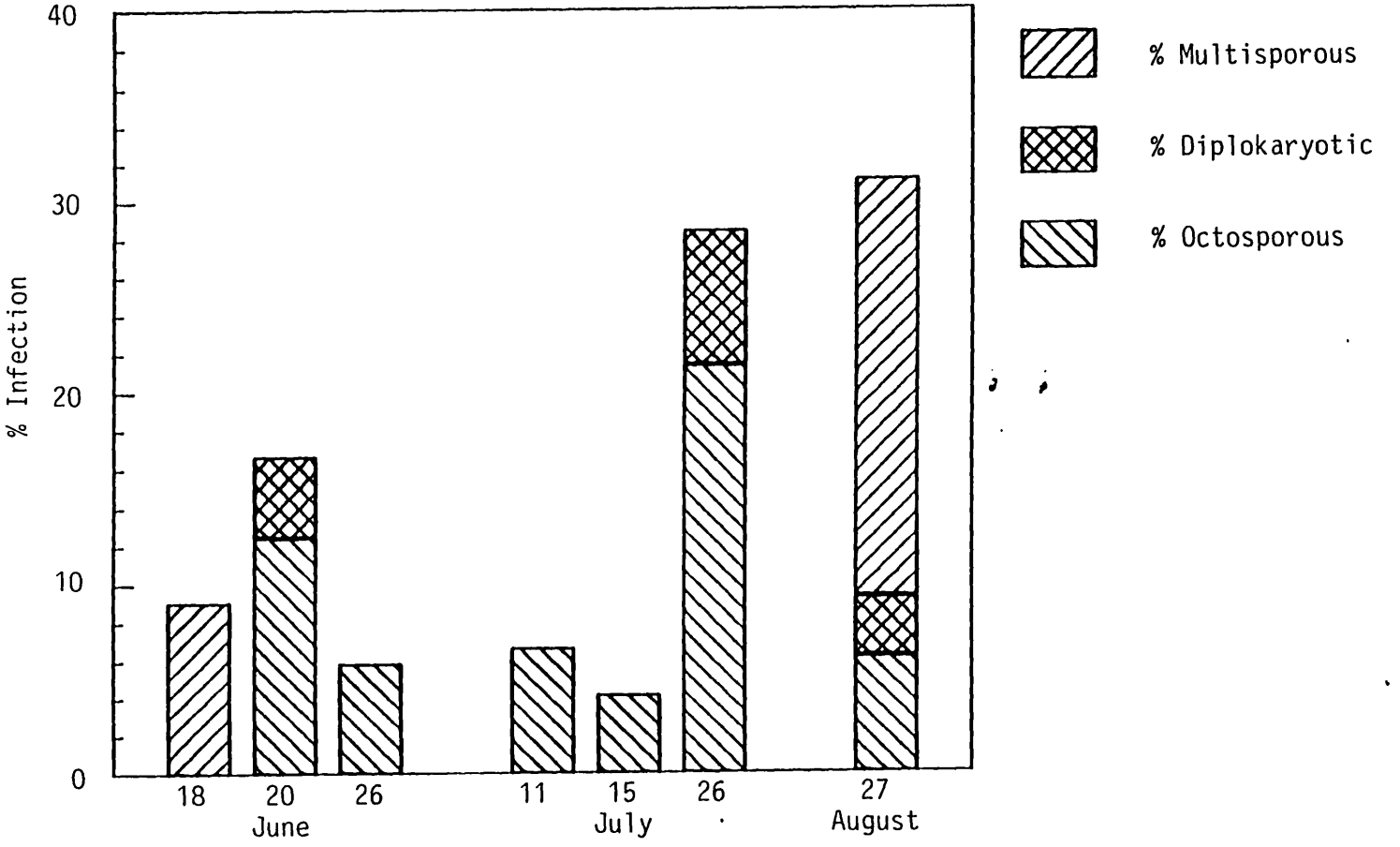


FIGURE 31; % Infection of all instars of Ma. africana

Mamakingi, 1987

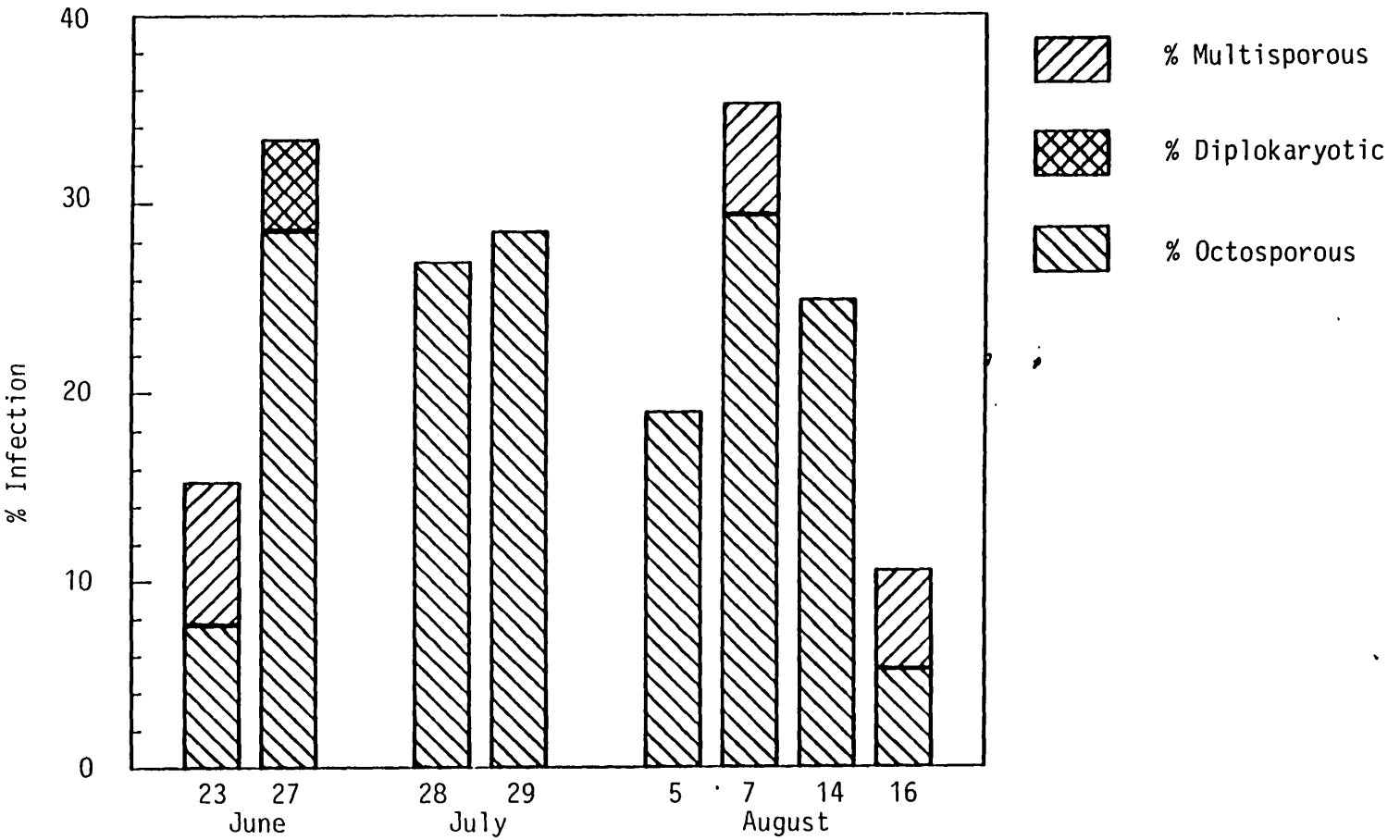


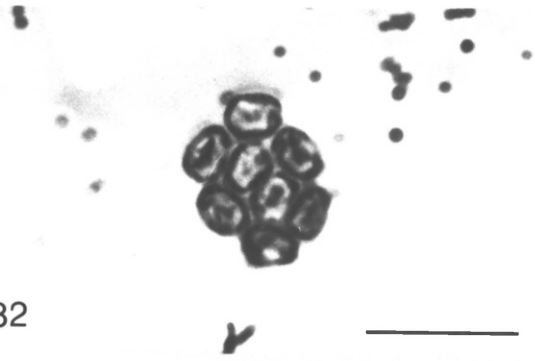
FIG.32 PHOTOMICROGRAPH OF A GIEMSA-STAINED SMEAR OF AN OCTOSPOROUS INFECTION IN *Culex* sp. LARVA (TANGA/PANGANI)
Scale bar = 10μm.

32. Spores in groups of 8.

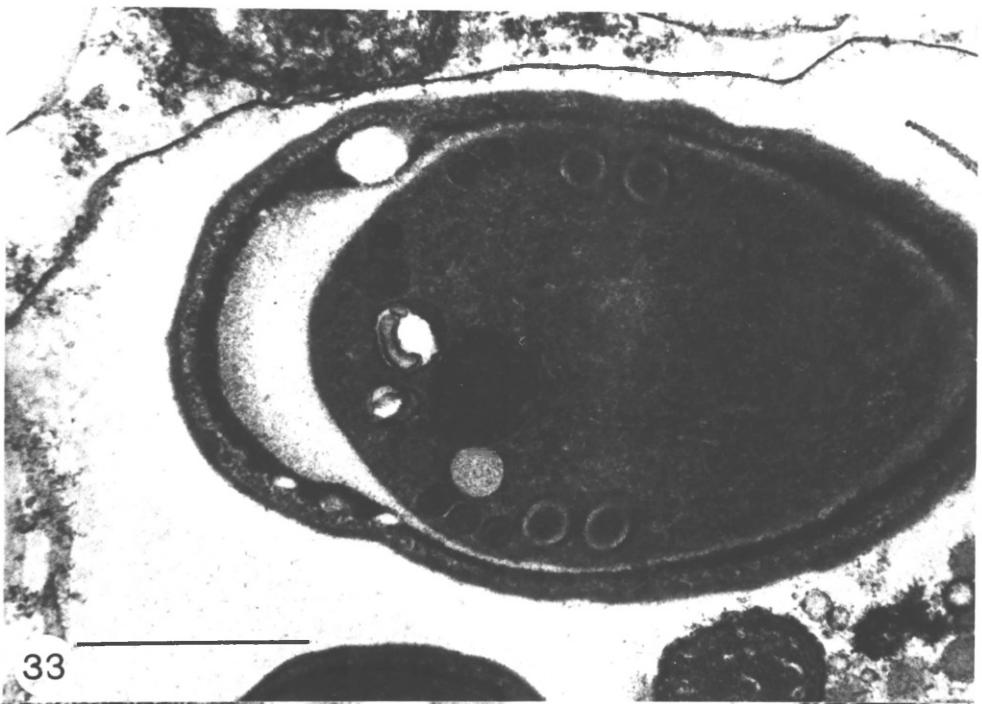
FIGS. 33-34 TRANSMISSION ELECTRON MICROGRAPHS OF AN OCTOSPOROUS INFECTION IN *Anopheles gambiae s.l.* LARVAE (MOMBO)
Scale bar = 1μm (Figs. 33-34)

33. Immature spore showing the 5 anisofilar coils of the polar filament.

34. Mature spore showing the "bottleneck" protruberance at the posterior end of the spore.



32



33



34

There were 5 coils of an anisofilar polar filament (3 small diameter coils and 2 large diameter coils measuring 0.13µm ± 0.01µm (n=6) and 0.21µm ± 0.01µm (n=4) respectively, best seen in immature spores (Fig.33). A characteristic "bottleneck" ornamentation at the posterior end was present in mature spores. The ornamentation was not as exagerated as in many Parathelohania spp., which is characteristic of Parathelohania africana. The polaroplast was lamellar. Spores measured 3.4µm ± 0.1µm x 2.4µm ± 0.1µm (n=6) (Fig.34). The measurements, structural details and host accord with the original description of P. africana Hazard and Anthony (1974).

Copepods collected at the site were identified as Mesocyclops rarus. A preliminary infection trial was carried out using 30 copepods, fed with spores from one disrupted infected larva. After 7 days no infections were seen in the 24 surviving copepods.

Octosporous species in Mansonia africana (Kisawani, Muheza Sisal Estate and Mamakingi)

Light microscopic appearance

Measurements of fresh octospores from the 1985 and 1987 collections were 3.1µm ± 0.1µm x 2.14µm ± 0.1µm (n=13) and 3.2µm ± 0.2µm x 2.4µm ± 0.3µm (n=13) respectively. They were broadly ellipsoid in shape, with one end flattened (Figs.35,36). Occasionally giant spores were seen (Fig.37), and were presumed to have resulted from incomplete division during sporogony.

The earliest stages seen in stained smears were diplokaryotic and were thought to be late meronts or early sporonts (Fig.38). After separation of the 2 nuclei of the diplokaryon and 2 nuclear division, tetranucleate and finally octonucleate sporonts were produced (Fig.39), which gave rise to eight spores (Fig.40). Sometimes the host cell nucleus and/or large accumulations of metabolic granules were present at the periphery of the sporont (Fig.39). These give the impression of supernumary parasite nuclei but were distinguished because of their different staining characteristics. The terminal flattening of the octospores was also visible after staining (Fig.40), but was not a feature of giant spores (Fig.41). Stained spores measured 3.0µm ± 0.4µm x 2.2µm ± 0.3µm (n=22).

FIGS. 35–37 PHOTOMICROGRAPHS OF FRESH SMEARS OF AN OCTOSPOROUS INFECTION IN *Mansonia africana* LARVAE (KISIWANI, MUHEZA SISAL ESTATE AND MAMAKINGI)

Scale bar = 10μm (Figs. 35–41)

35. Spores from the 1985 collections. One end of the spore is flattened (indicated by an arrow).

36. Spores from the 1987 collections. One end of the spore is flattened (indicated by an arrow).

37. "giant" spore

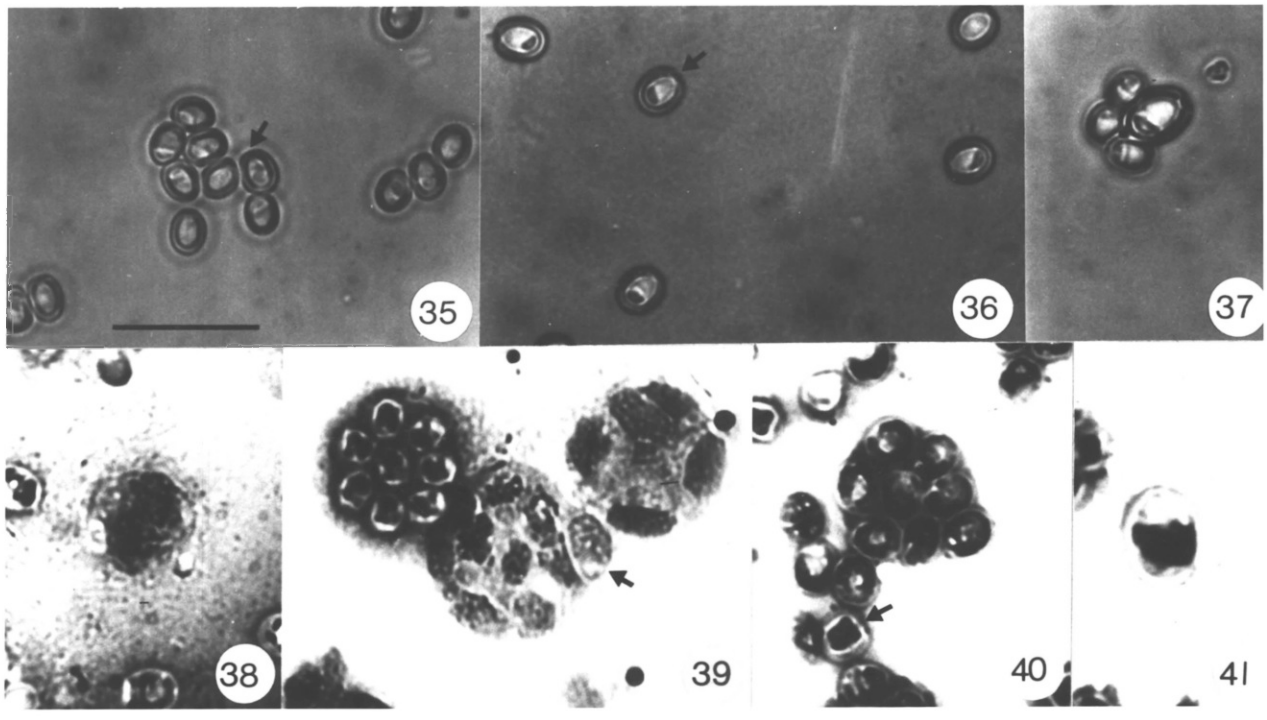
FIGS. 38–41 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF THE SAME INFECTION

38. Late meront or early sporont with diplokaryotic nucleus.

39. Two sporogonic stages, one with metabolic products (indicated by an arrow), giving the impression of supernumery nuclei. One group of 8 spores.

40. One group of 8 sporoblasts, and a number of free spores, some showing a flattened pole (indicated by an arrow).

41. "Giant" spore.


35
36
37
38
39
40
41

Ultrastructural appearance

Meronts were rounded cells with rather empty cytoplasm traversed by endoplasmic reticulum, studded with ribosomes and limited by a thin unit membrane. The large nuclei were in diplokaryotic arrangement, often with condensed chromatin and occasionally showing electron dense patches on the nuclear envelope which were thought to be spindle plaques, signifying a past or incipient karyokinesis (Fig.42).

At the onset of sporogony the 2 nuclei of the diplokaryon drew apart (Fig.43). A second membrane, later to become the sporophorous vesicle was laid down external to the plasma membrane. Deposition of clumps of electron dense material between the two membranes resulted in their separation, while some of the electron dense material spread out over the surface of the sporont, ultimately to form a complete electron dense coat (Fig.44).

Further development of the sporont involved nuclear division and separation of the cytoplasm into 8 uninucleate sporoblasts. Lobed sporonts representing incompletely separated sporoblasts and irregularly shaped stages just after separation were seen (Figs.45,46). Although sections rarely revealed more than 3 or 4 buds of a dividing sporont in one plane, the presence of octonucleate plasmodia in Giemsa stained smears showed that division is typically delayed until the octonucleate stage. Premature division of the sporont before completion of the final division (Fig.47) would result in formation of giant sporoblasts and spores as seen in light microscopy. Subsequent to division, the sporoblasts acquired first a regularly oval shape (Fig.48) and then a crenated shape, presumed to be an artifact of fixation (Fig.49) before maturing into spores.

Within the sporophorous vesicle accumulations of metabolic products gave differing appearances during the maturation of the vesicle. In the younger vesicles, they were predominantly in large aggregates which were electron dense with lighter patches (Fig.46). Other types were tubular or pale homogeneous spheres (Figs.46,48,49). As maturation proceeded the tubular and homogeneous elements increased and the large aggregates became less electron dense. In vesicles containing mature spores there was little evidence of any of the metabolic products though some structures resembling concentric lamellae persisted (Fig.52). Occasionally, a structure, resembling a host cell nucleus, was present within the boundary of the now degenerate host cell but outside the sporophorous vesicle.

FIGS. 42-52. TRANSMISSION ELECTRON-MICROGRAPHS OF AN OCTOSPOROUS INFECTION IN *Mansonia africana* LARVAE (KISIWANI, MUHEZA SISAL ESTATE AND MAMAKINGI

Scale bar = 1μm (Figs. 42-52)

42. Diplokaryotic meront with thin unit membrane. Condensed chromatin is present within the nucleus and electron dense patches on the nuclear membrane may represent centriolar plaques.

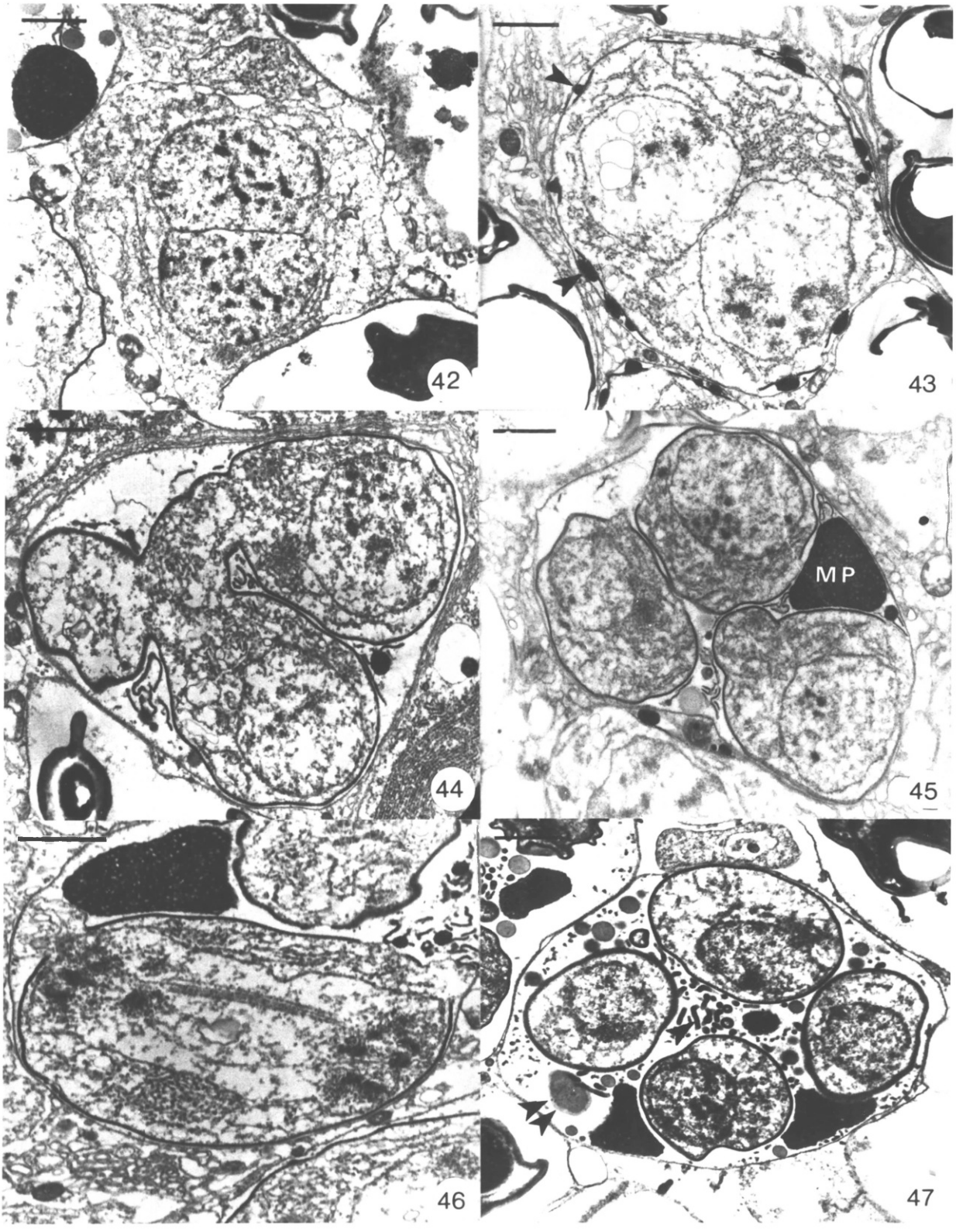
43. Early sporont in which the 2 nuclei of the diplokaryon have separated. Electron dense material (indicated by an arrow) is clumped on the surface membrane of the sporont and the sporophorous vesicle is visible as a fine membrane.

44. Sporont products: Nuclear division has occurred and the cytoplasm is constricting around each nucleus to form the sporoblasts. The surface coat is entirely thickened.

45. Uninucleate sporoblasts. Metabolic products (MP) are condensed into discreet clumps within the sporophorous vesicle.

46. Sporoblast in which nuclear division is precocious. It is possible that such a division would give rise to a giant spore.

47. Uninucleate sporoblasts with a regular oval shape. Metabolic products take the form of tubules (indicated by a single arrow) and pale homogeneous spheres (indicated by double arrows) as well as the electron dense clumps.


42
43
44
MP
45
46
47

The surface coat of the developing sporonts and sporoblasts increased in thickness and several layers were distinguished at the stage of crenation (Fig.49). The endospore was first laid down as a mottled layer of moderate electron density, but finally resolved as an inner electron lucent layer and an outer layer of moderate electron density within the fine electron dense exospore (Figs.50,52). The total thickness of the wall along the sides of the spore was 0.17µm ± 0.01µm.

At the anterior end of the spore (Fig.52) corresponding to the flattened end seen by light microscopy, the spore wall was raised into 2 processes into which all layers of the wall extended. The constant appearance of these structures as knobs whatever the angle of sectioning is consistent with the structure of an anterior rim surrounding a depression in the region of the polar cap. At the posterior end there was a single knob-like projection, also composed of all layers of the wall spores measured 3.0µm ± 0.2µm x 2.1µm ± 0.2µm. A section cutting through one of the anterior projections, the basal part of the polar filament and the posterior projection (Fig.52) demonstrated that the posterior projection was knob-like, not fin-like, as it had the same appearance from more than one angle.

Internally, the mature spore had an anterior polar cap extending outwards as fine processes beneath the endospore. The polar filament was anisofilar with 2 anterior coils of greater diameter than the 3 posterior coils. The basal region of the filament was surrounded by uniformly arranged polaroplast lamellae. The remaining area of the spore was occupied by a single nucleus and general cytoplasm containing cisternae of endoplasmic reticulum, studded with ribosomes. The latter was particularly clear in slightly immature spores (Fig.51).

Scanning electron microscopy of spores showed a more rounded shape than suggested by transmission electron microscopy. The spores were usually seen to have a slight invagination at one end, although as Fig.53 shows, some spores had slight protruberances which could have given rise to the posterior knob seen by transmission electron microscopy.

Multisporous species in Mansonia africana (Kisiwani, Muheza Sisal Estate and Mamakingi)

Light microscopic appearance

The earliest stages seen were diplokaryotic meronts with variable numbers of paired nuclei from 1 (Fig.54) to 6 and more (Fig.55), forming multi

48. Ovoid and crenated uninucleate sporoblasts. Tubular metabolic products (indicated by a single arrow) and pale homogenous spheres (indicated by a double arrow) are still present, but the large aggregates are less dense.

49. Maturing sporoblast. The developing endospore (EN) is present.

50. Immature spore. The shape of the spore has developed and the cytoplasm contains cisternae of rough endoplasmic reticulum, studded with ribosomes.

51. Mature spore. The endospore (EN) is resolved into an inner electron lucent and outer moderately electron dense layer, surrounded by the electron dense exospore (EX). The polar filament is anisofilar and the anterior end is raised into a rim into which all layers of the wall extend. At the posterior end is a single projection.

52. Mature spores. This section is cut at a different angle through the spore on the right. It cuts through the anterior rim, across the basal section of the polar filament and through the posterior section.

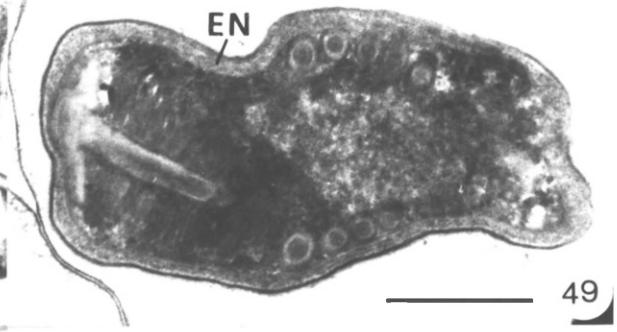
FIG. 53 SCANNING ELECTRON MICROGRAPH OF OCTOSPORES FROM Mansonia africana LARVAE (KISIWANI)

Scale bar = 1μm

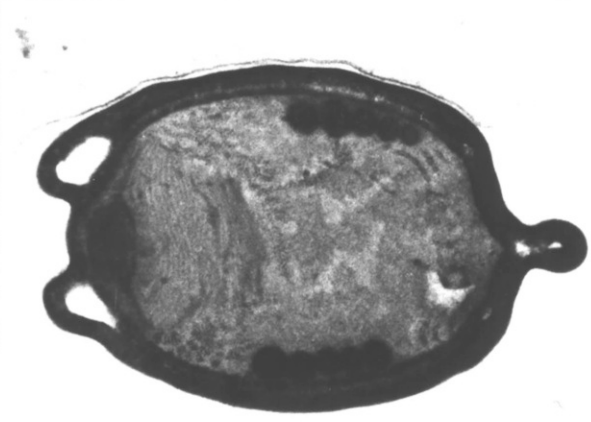
53. Octospores. Spore 1 is only flattened at one end, but spore 2 shows the rim around a central depression of the spore wall.



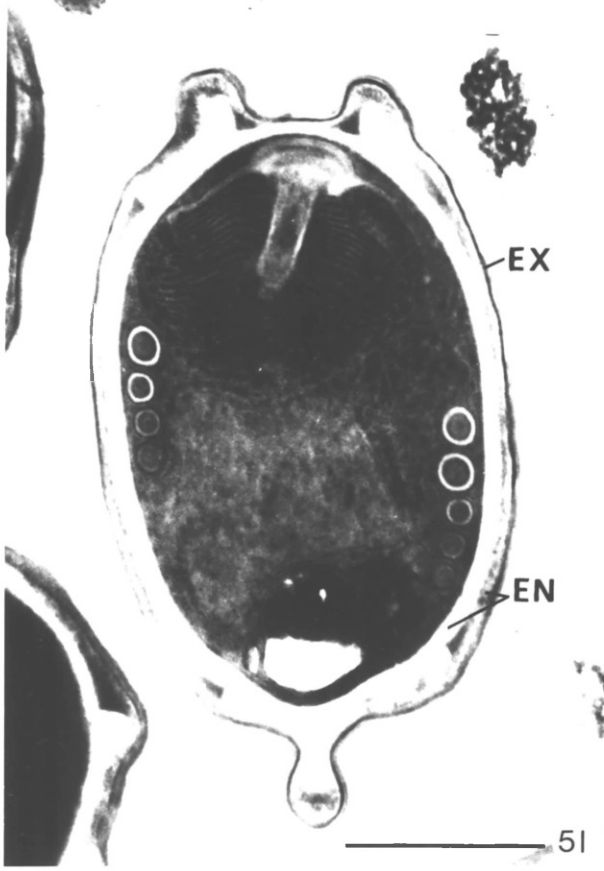
48



49



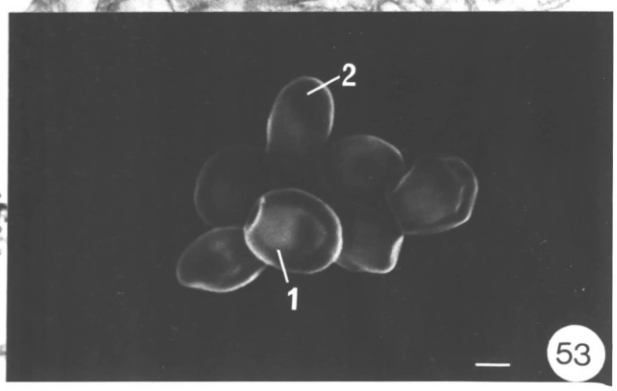
50



51



52



53

nucleate plasmodia. Long ribbon-like strings of diplokaryotic stages also formed (Fig.56).

As sporogony ensued the 2 nuclei of the diplokaryon separated then each underwent further divisions to produce plasmodia with variable numbers of nuclei (Figs.57.58.59). Cytoplasmic division gave uninucleate sporoblasts (Fig.60) and spores (Fig.61). Spores were oval and terminally, slightly flattened. They measured 2.5µm ± 0.3µm x 1.5µm ± 0.3µm (n=11). The number of spores in the sporophorous vesicle ranged from 40 - 60. The vesicle diameter varied in size from 27.5µm to 12.5µm diameter according to the number of spores contained therein.

Ultrastructural appearance

Infections were found in the tissues of the gut, often close to the microvilli (Fig.62). The earliest stages found were diplokaryotic meronts (Fig.63), limited by a single, somewhat thickened, unit membrane. Short strings of ribosomes were seen close to the meront surface (Fig.64). Repeated division of the diplokaryotic nuclei produced multinucleate plasmodia (Fig.65). The plasma membrane of the larger plasmodia was almost entirely surrounded by 2 other membranes, the outer of which was abundantly studded with ribosomes (Fig.66). These membranes were interpreted as host endoplasmic reticulum closely applied to the parasite's surface. The inner 2 membranes were so close together that they were difficult to resolve in many regions.

The surface coat of the multinucleate meronts increased in thickness and the meronts became ribbon-like prior to division (Fig.67). Division was by constriction of the cytoplasm around each diplokaryon and the encasing cisterna of host endoplasmic reticulum divided with the parasite. As a result the daughter meronts were also encased in host endoplasmic reticulum. At this stage it could be seen that sparse ribosomes were also present in the inner membrane of the endoplasmic reticulum (Fig.68).

Sporogony was signalled in multinucleate sporonts (sporogonial plasmodia) by two events: the separation of the 2 nuclei of each diplokaryon and sloughing of the electron dense coat into the space between its surface and the cisterna of endoplasmic reticulum so that the parasite surface again became a simple unit membrane (Fig.69). The effect of sloughing of the surface coat was to increase the space around the developing sporogonial plasmodium (Fig.70) which proceeded to divide sequentially into segments with fewer and

FIGS. 54-61 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF A MULTISPOROUS INFECTION IN *Mansonia africana* LARVAE (KIWISANI, MUHEZA SISAL ESTATE AND MAMAKINGI)

Scale bar = 10μm (Figs. 54-61)

54. Two meronts, 1 with a single pair of diplokaryotic nuclei and the other with 3 pairs.

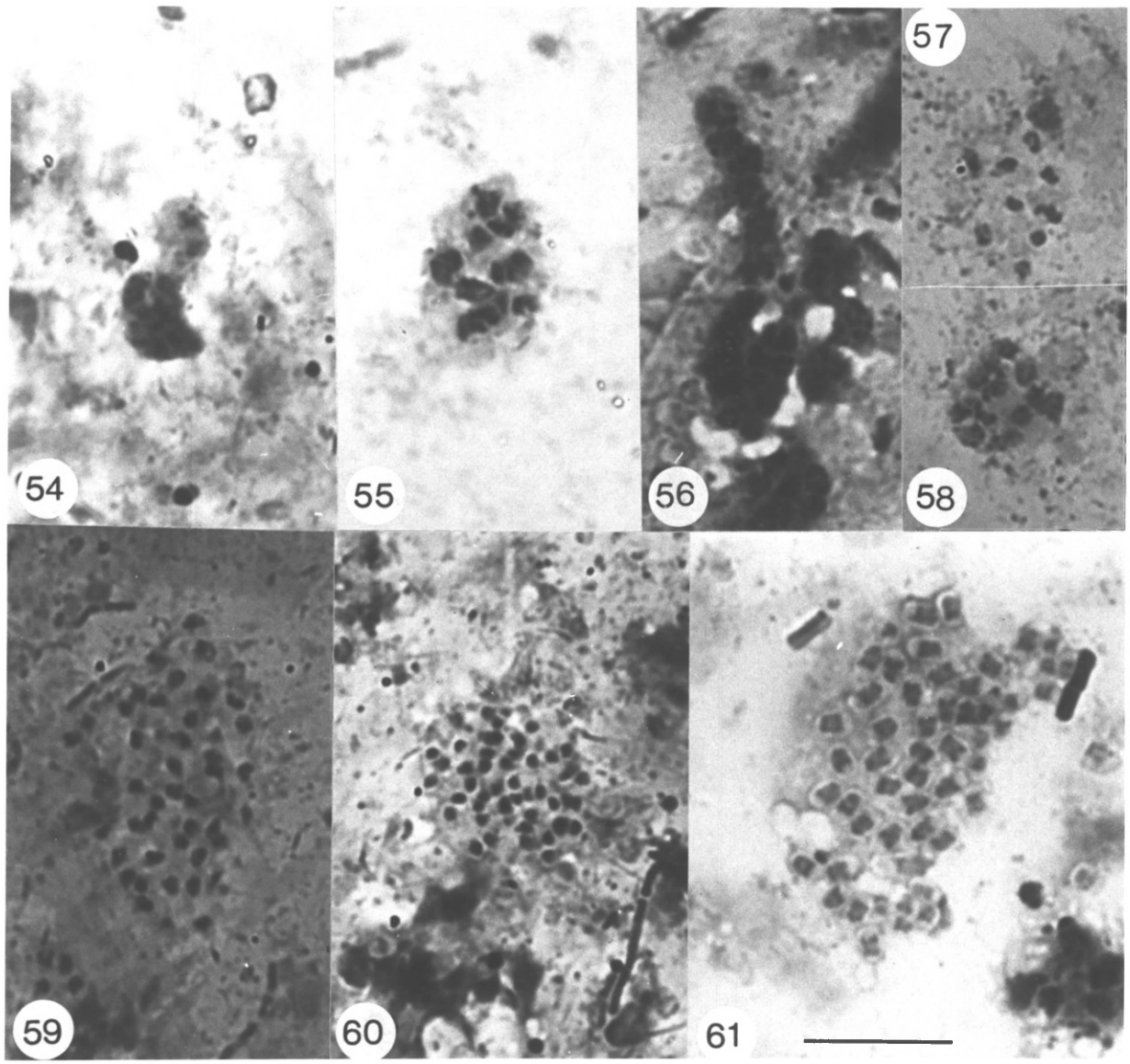
55. Multinucleate merogonic plasmodium with 6 pairs of diplokaryotic nuclei.

56. Merogonic stages formed into long ribbons with diplokaryotic nuclei.

57,58,59 - Sporogonic plasmodia with variable numbers of nuclei no longer in diplokaryotic configuration.

60. Uninucleate sporoblasts.

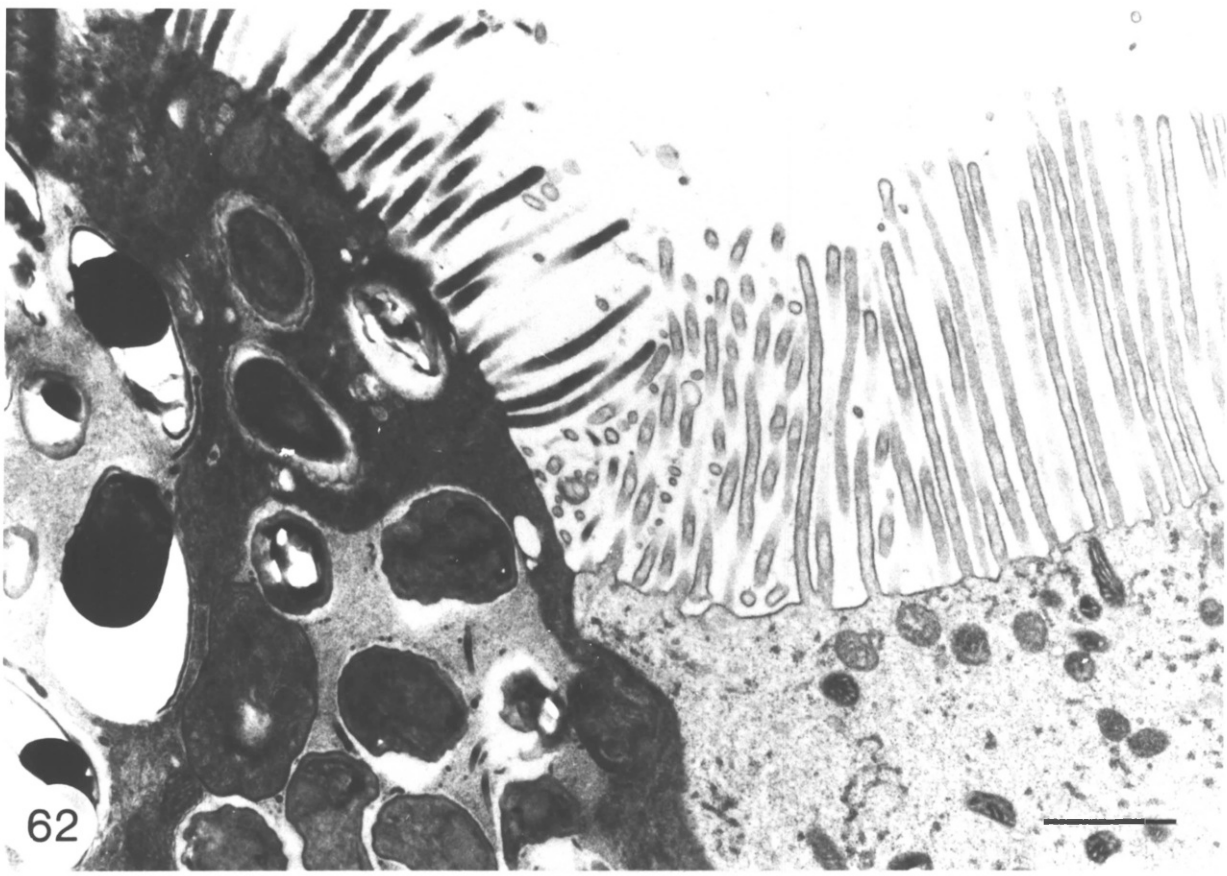
61. Uninculeate spores.


54
55
56
57
58
59
60
61

FIGS. 62-78 TRANSMISSION ELECTRON MICROGRAPHS OF A MULTISPOROUS INFECTION IN *Mansonia africana* LARVAE (KISIWANI, MUHEZA SISAL ESTATE AND MAMAKINGI)

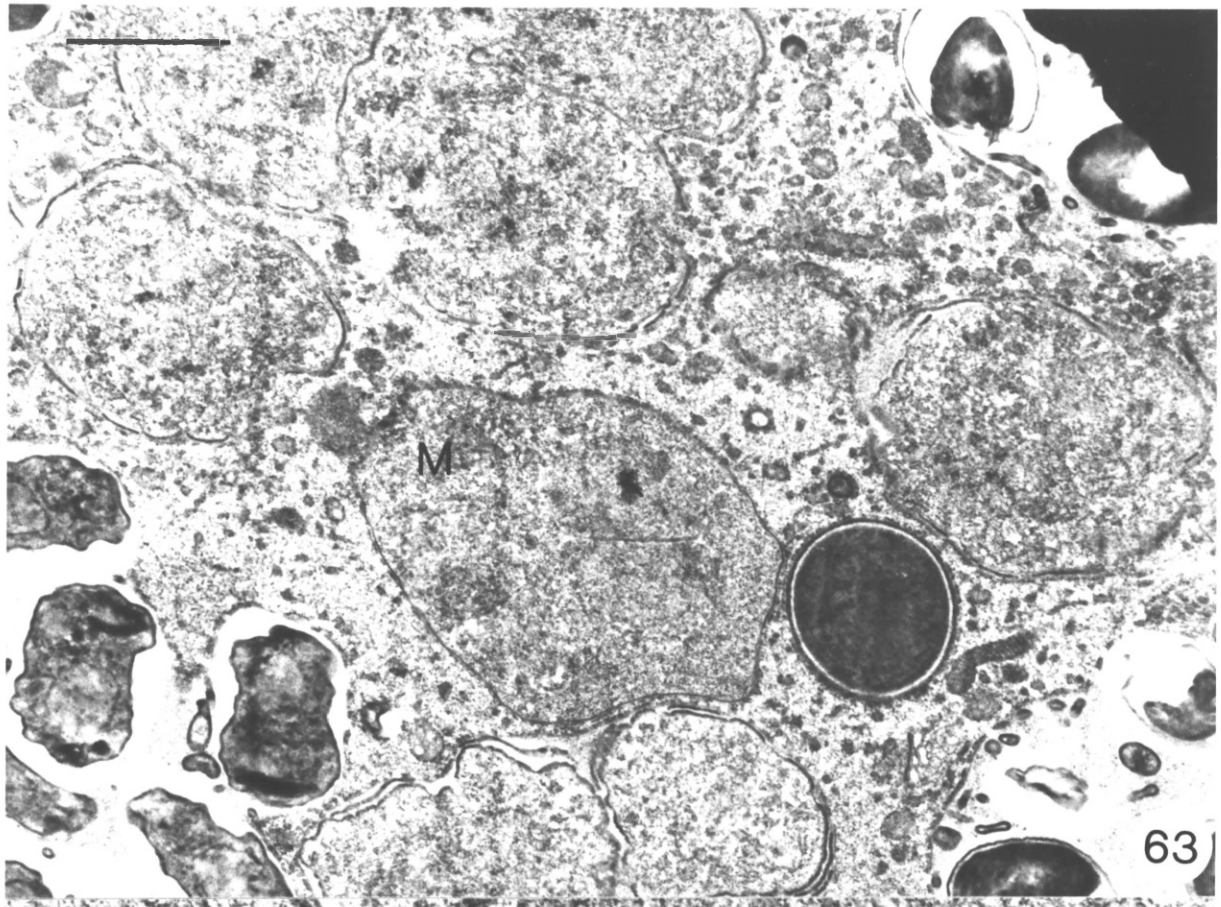
Scale bar = 1μm (Figs. 62-78)

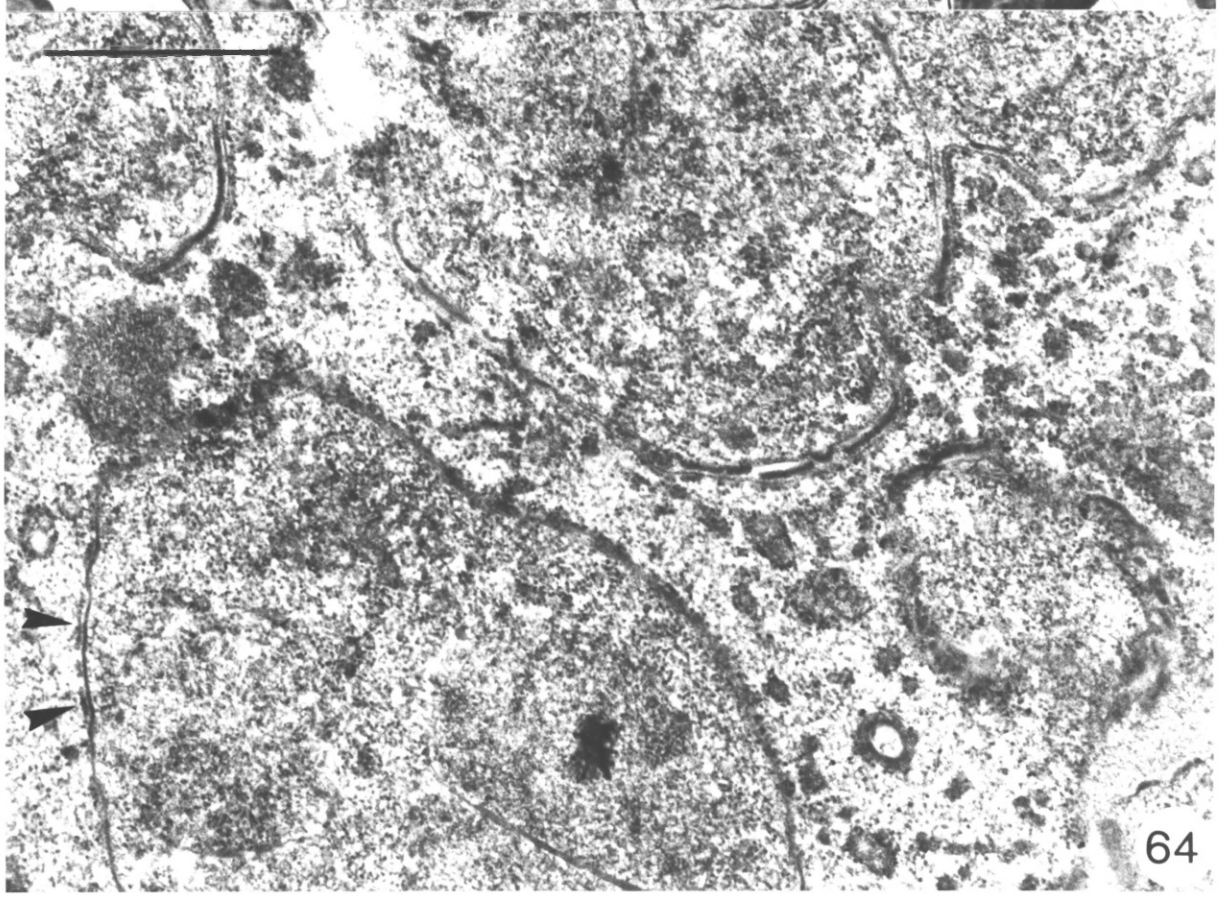
62. Various developmental stages within the epithelial cells of the gut.


62

63. Diplokaryotic meront (M) limited by a single, slightly thickened unit membrane.

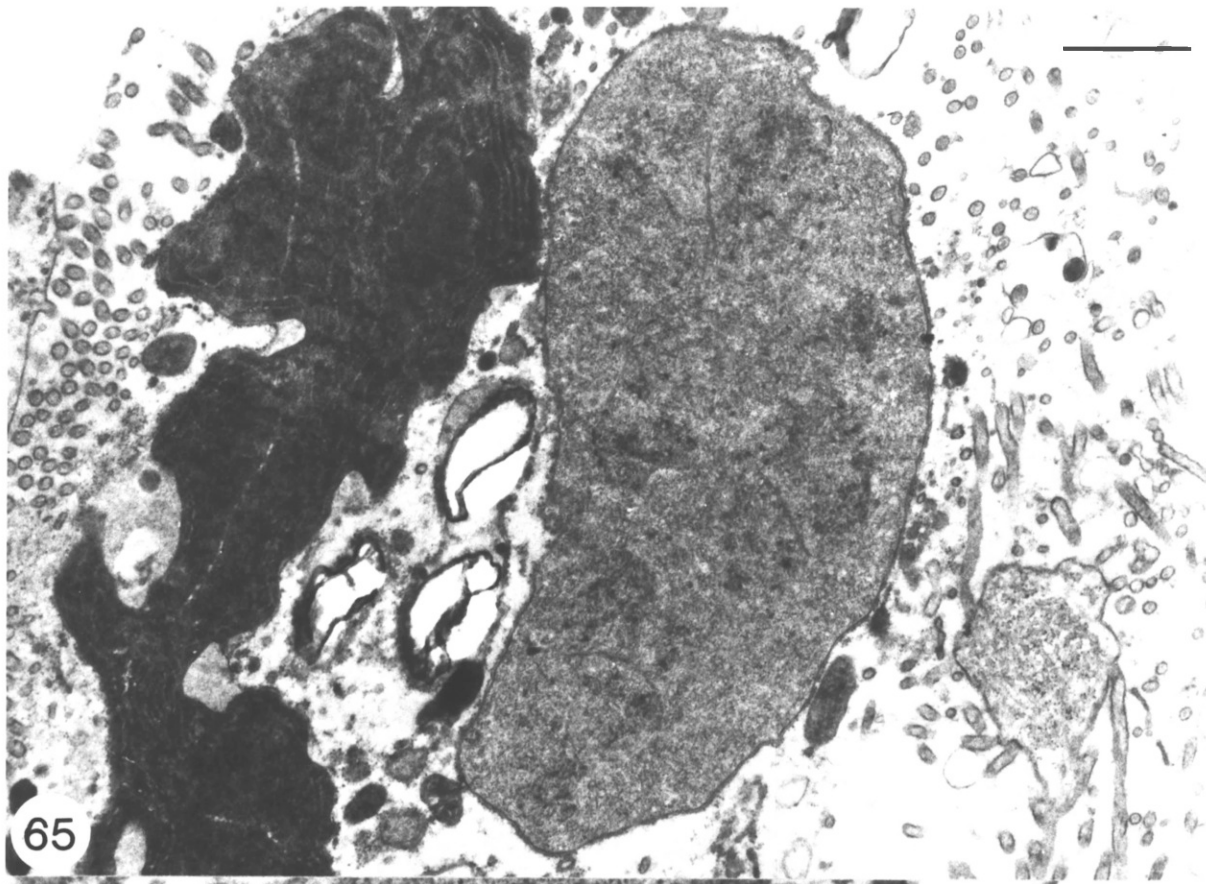
64. Detail of the meront in Fig.63, showing strings of ribosomes close to the parasite surface (indicated by arrows).


M
63

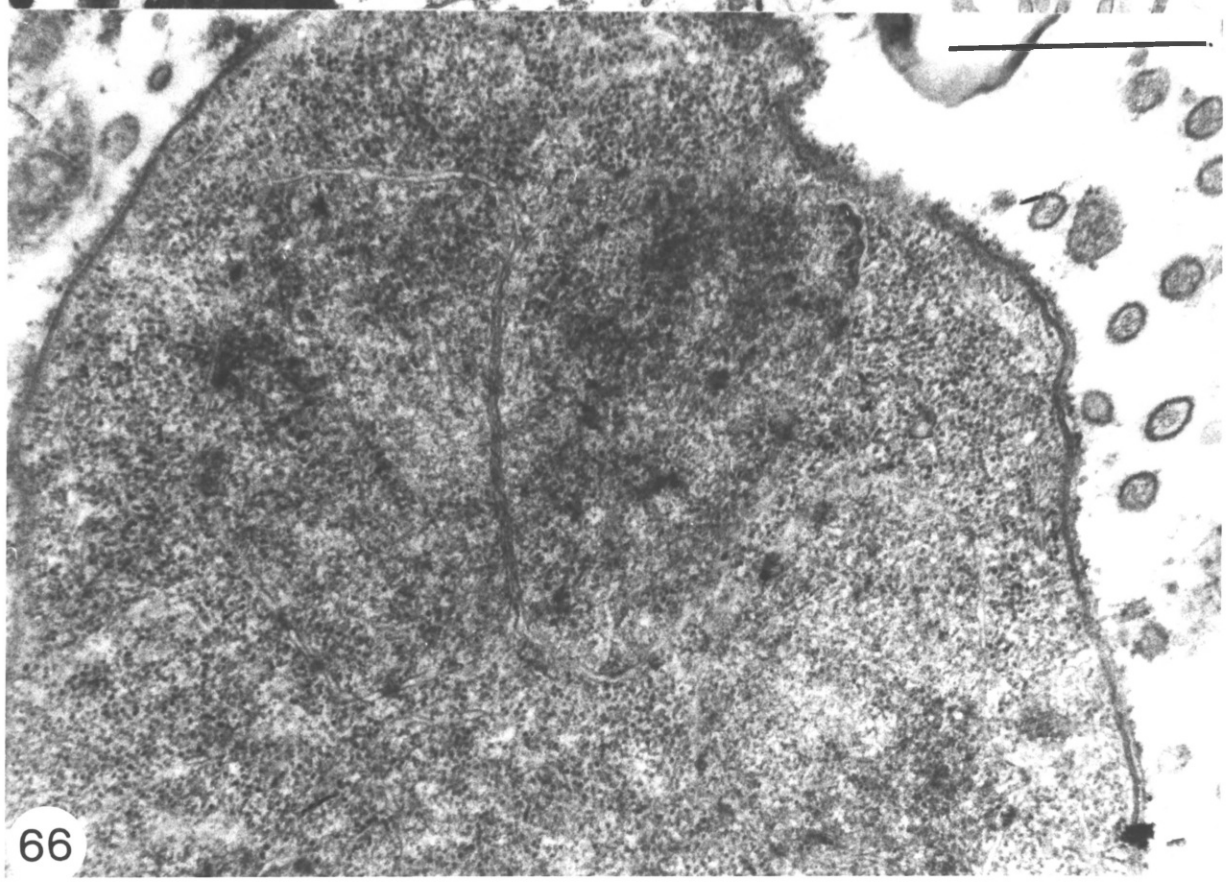

64

65. Multinucleate merogonic plasmodium

66. Surface detail of the meront in Fig.65. The plasmodia is almost entirely surrounded by 2 other membranes (thought to be host endoplasmic reticulum), the outer of which is studded with ribosomes.



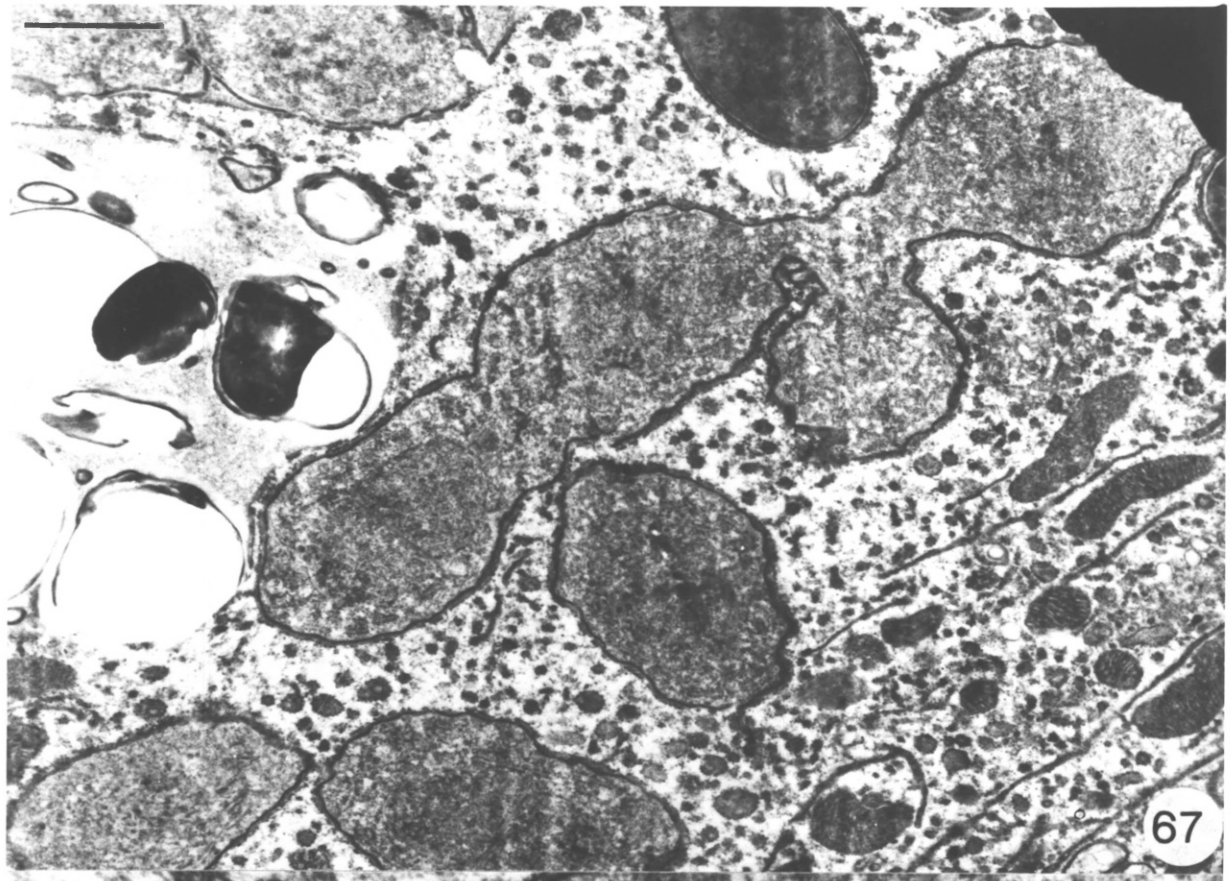
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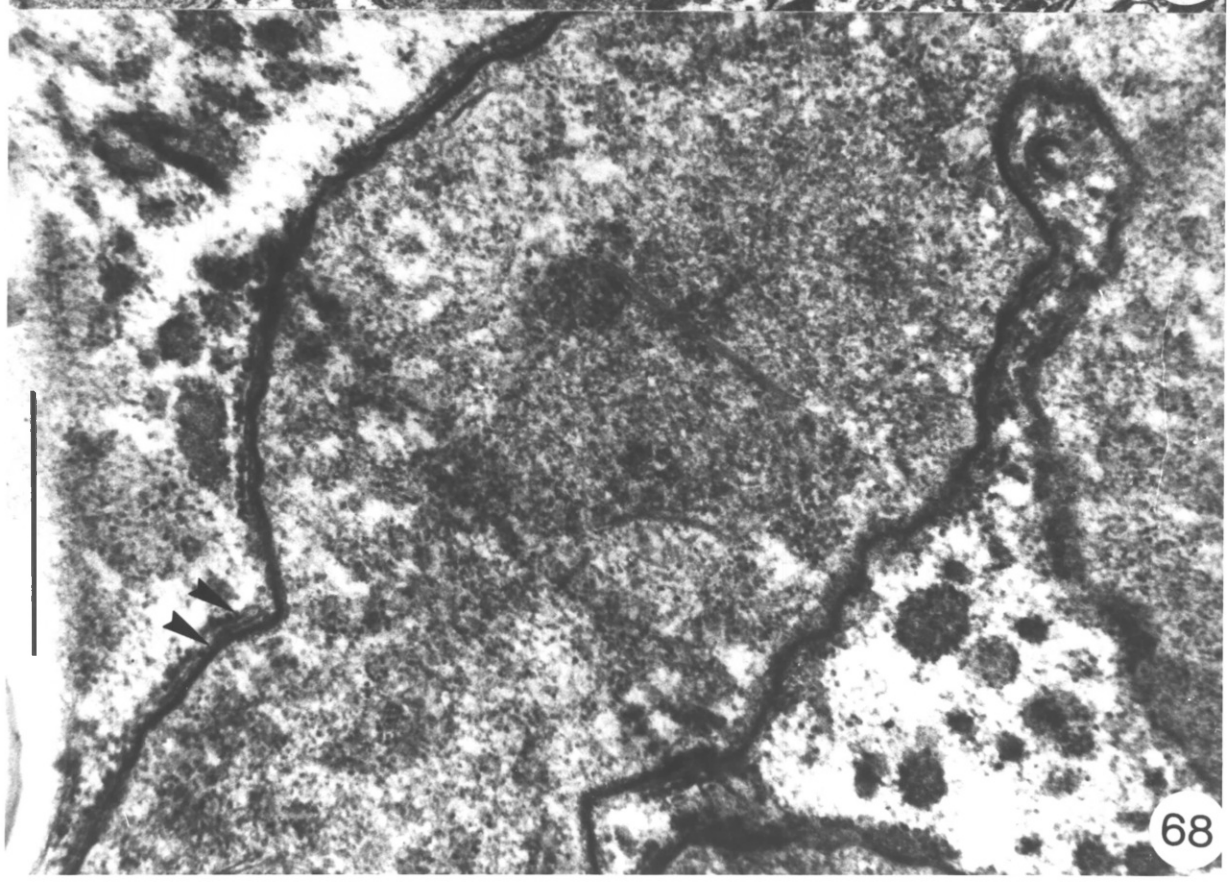


66

67. Ribbon-like meront with thickened surface coat prior to division. The host endoplasmic reticulum encasing the meronts divides with the parasite.

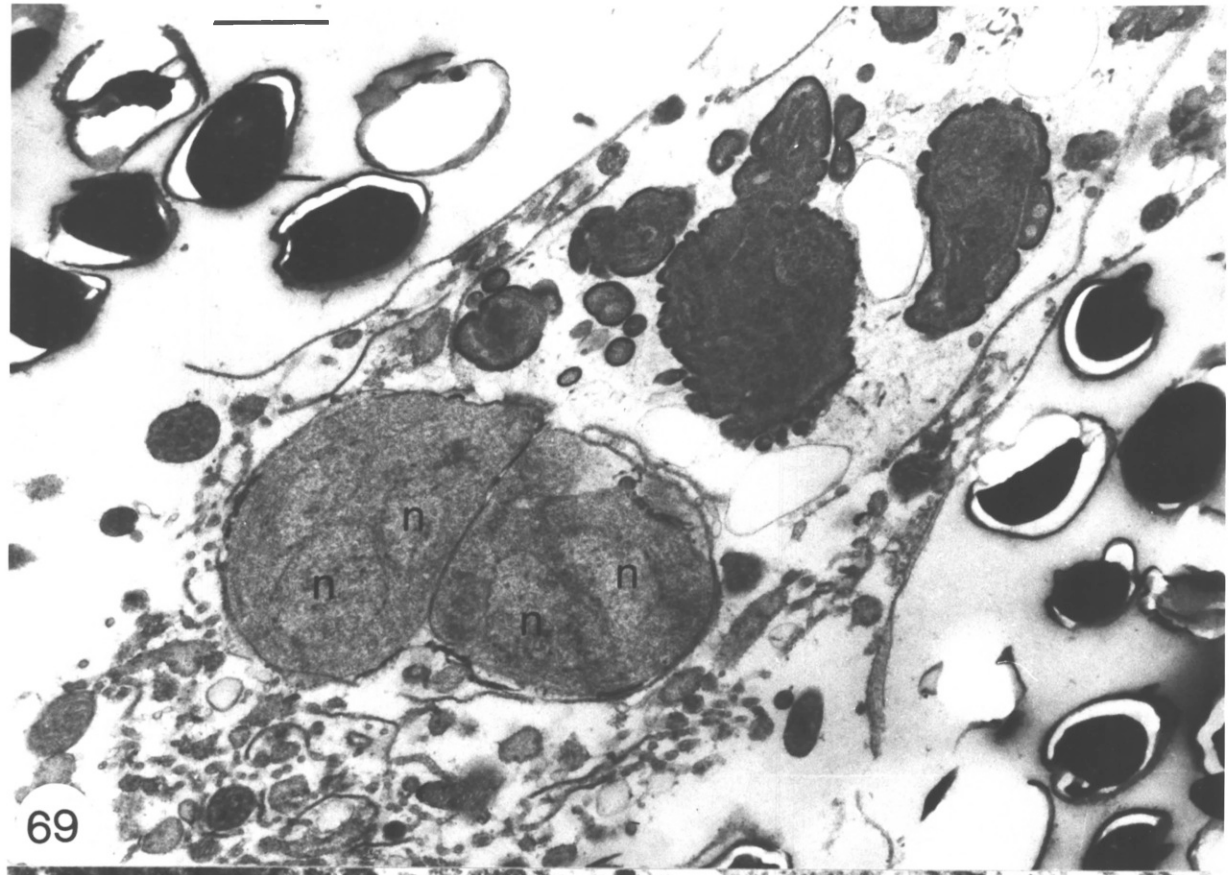
68. Detail of the dividing meront seen in Fig.67. Ribosomes are present on both the outer and inner membranes of the endoplasmic reticulum (ribosomes indicated by an arrow).


67


68

69. Early multinucleate sporont (sporogonial plasmodium). The nuclei (n) of the diplokarya are separated and the electron-dense coat present in meronts is sloughed into the space between the sporont surface and the cisterna of host endoplasmic reticulum.

70. Sporont. The sporogonial plasmodium divides sequentially. Electron-dense material continues to be sloughed off into the vesicle cavity (indicated by a single arrow), but in addition another electron-dense coat is in the process of being secreted in patches (indicated by a double arrow). The contents of the vesicle are granular.


n
n
n
n
69


70

fewer nuclei to eventually produce uninucleate sporoblasts. During this process an electron dense coat was secreted again on to the surface of the segments and the cavity bounded by the endoplasmic reticulum became full of granular metabolic products (Fig.70). The electron dense coat was secreted in patches on to the sporogonial plasmodia with unthickened regions between (Fig.70,71). The result, after fixation, was to cause blistering at the surface of the plasmodia (Fig.71). As the thickening became more extensive the bubbling reduced (Fig.69) until regularly shaped sporont products were formed (Fig.72).

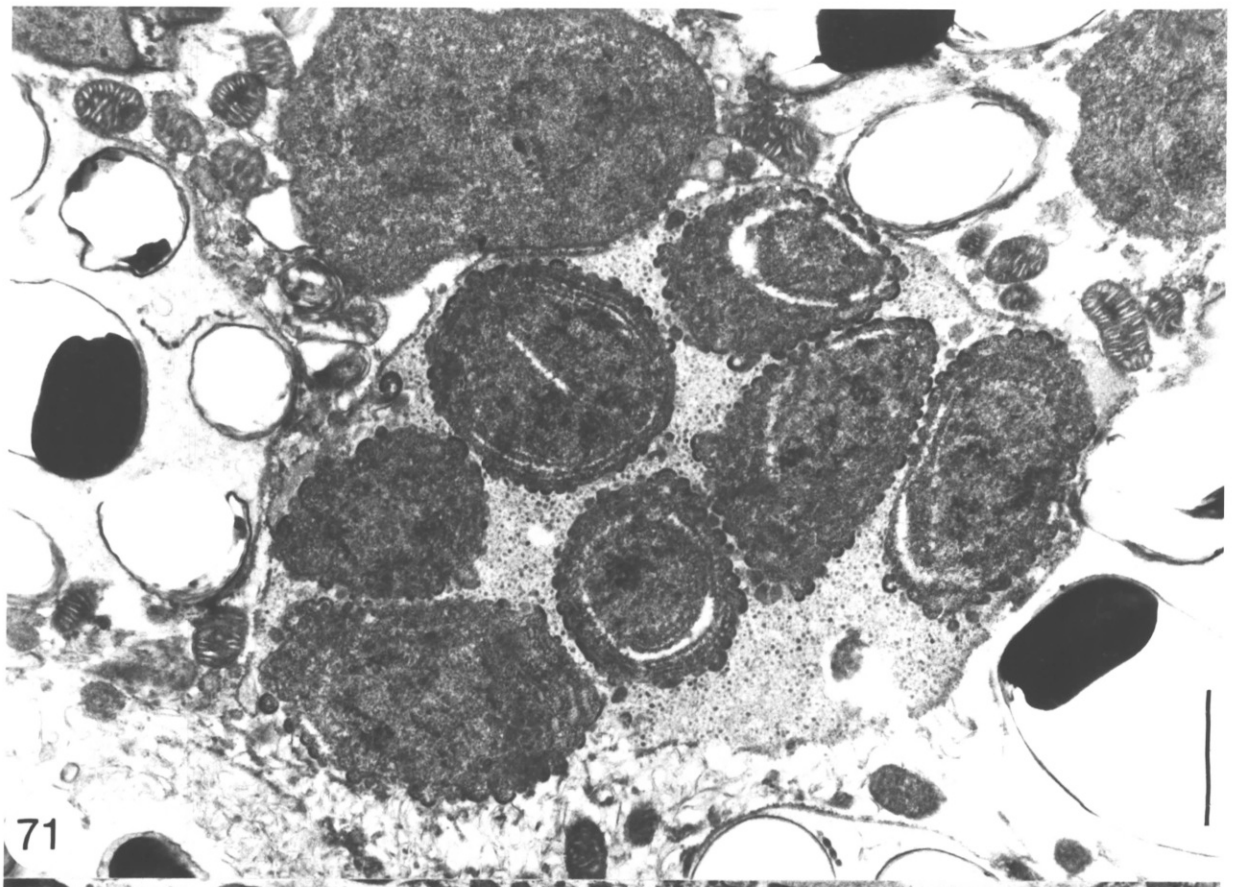
The final cytoplasmic divisions gave uninucleate sporoblasts (Fig.72) and by this stage the surface coat had been completed and the surface had become smoother, having lost the blistered appearance. Late sporoblasts, however, were crenated and showed the developing polar filaments and polaroplast (Figs.73,74,75). The surface coat was 0.045nm thick (Figs.74,75). Within the vesicle the granular homogeneous material remained, but in addition tubular profiles were present. These remained in the vesicles containing maturing spores (Fig.76), but disappeared as the spores matured. Although spores were not well preserved signs of endospore formation under the exospore were present. The polaroplast was not clear but endoplasmic reticulum, a single nucleus and one coil of the polar filament were visible (Fig.77). No more than one coil of the polar filament was observed in any spore suggesting that mature spores have a single coil (Figs.76,77). Spores were oval and measured 2.1μm $\pm$ 0.1μm x 1.0μm $\pm$ 0.1μm (n=5). Occasional large spores, each in its own cisterna of endoplasmic reticulum, were present, thought to be a result of incomplete division as there were suggestions of a double set of organelles (Fig.78).

Diplokaryotic infection in Mansonia africana larvae

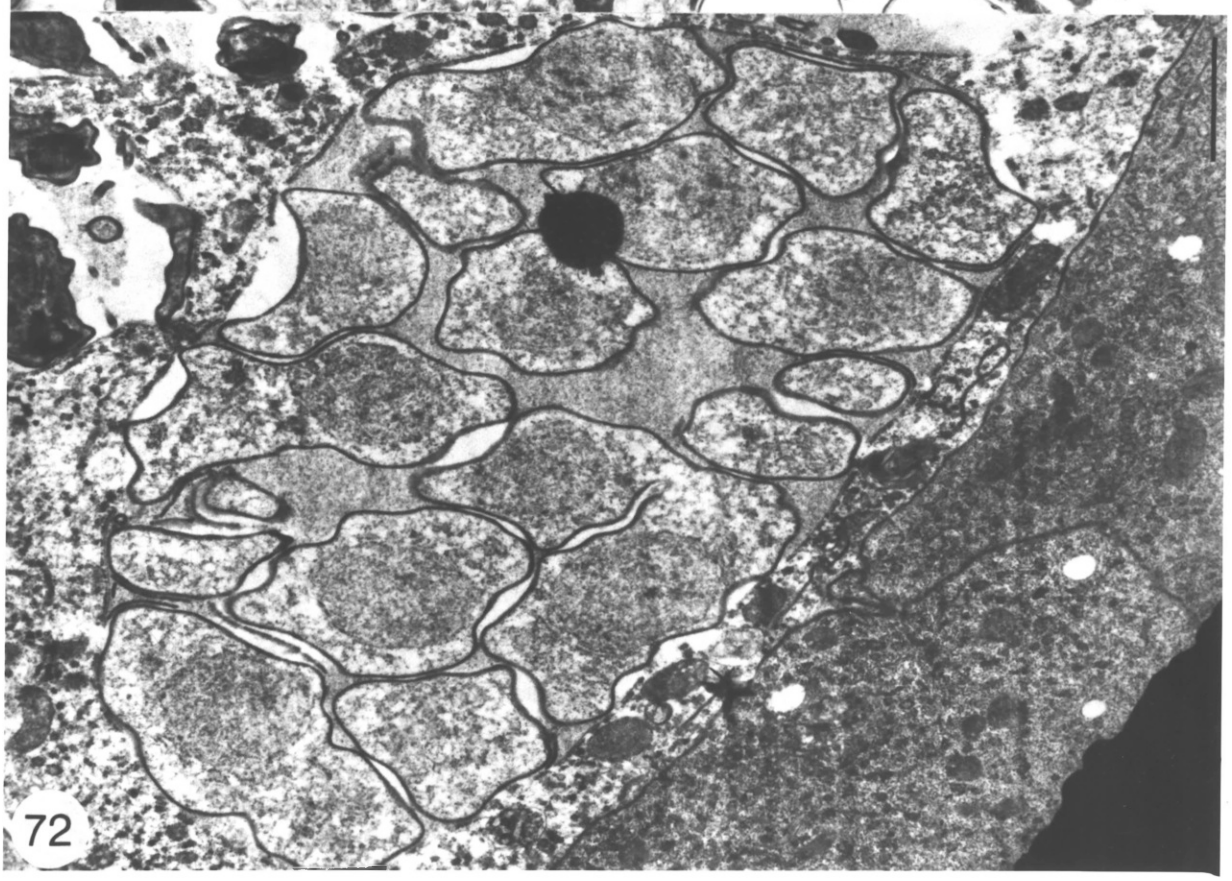
These stages are meronts, the vegetative stages, which eventually undergo sporogony in adults producing binucleate spores. The diplokarya within all infected larvae appeared similar in size, measuring 6.1μm $\pm$ 0.5μm x 3.5μm $\pm$ 0.5μm (n=8) in length and width of the diplokaryon nuclei. Figures 79 and 80 show these stages. Figure 80. shows them developing within the host cell cytoplasm.

71. Sporont products. The surface coat appears blistered as an artefact of fixation.

72. Unincleate sporoblasts.

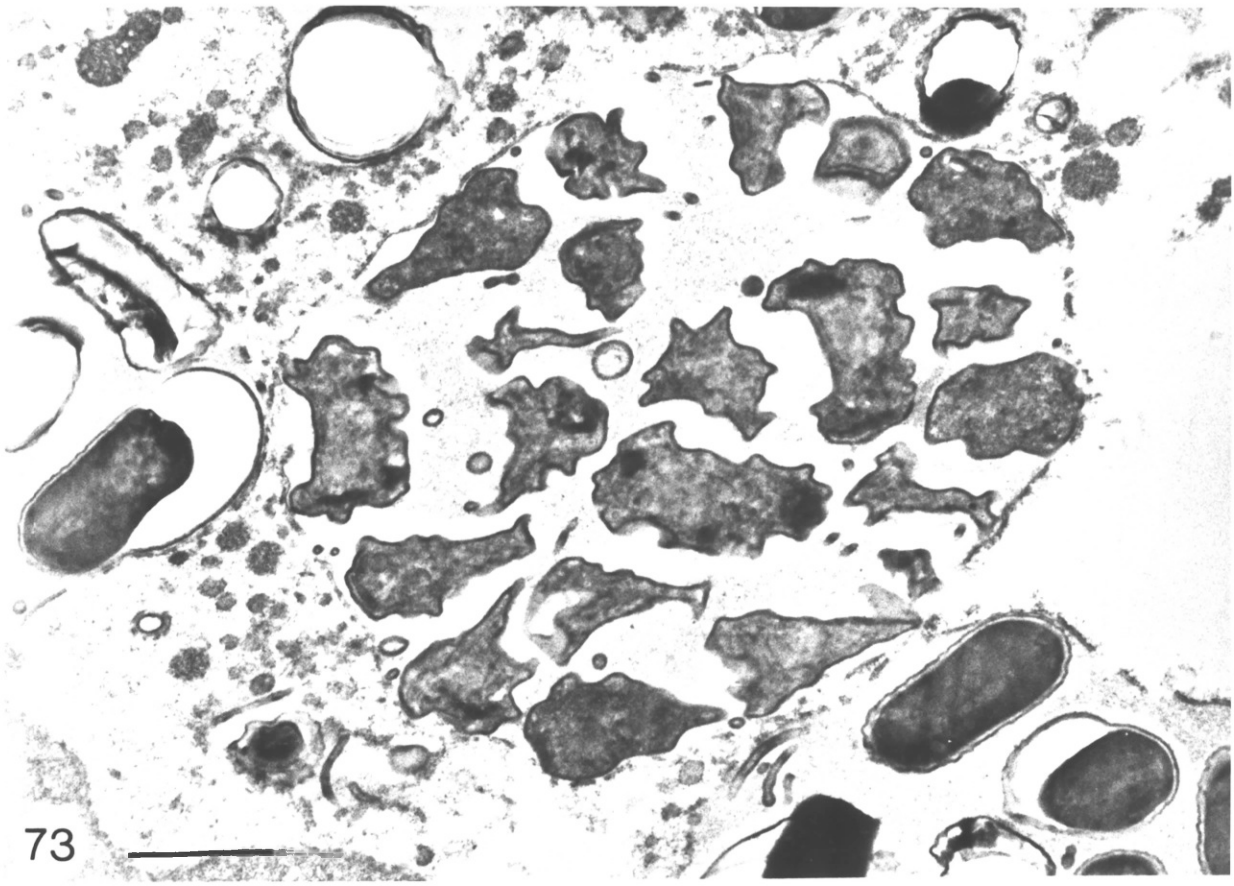


71

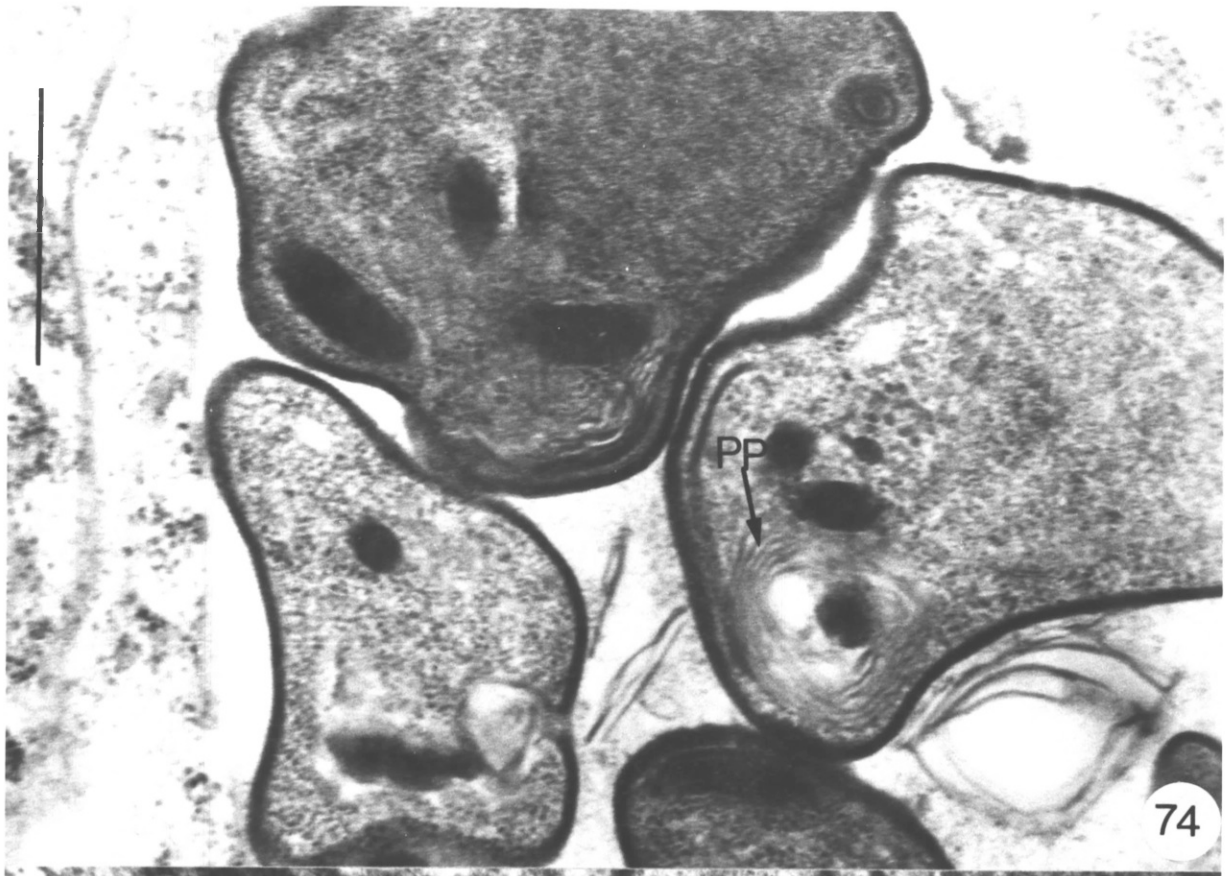


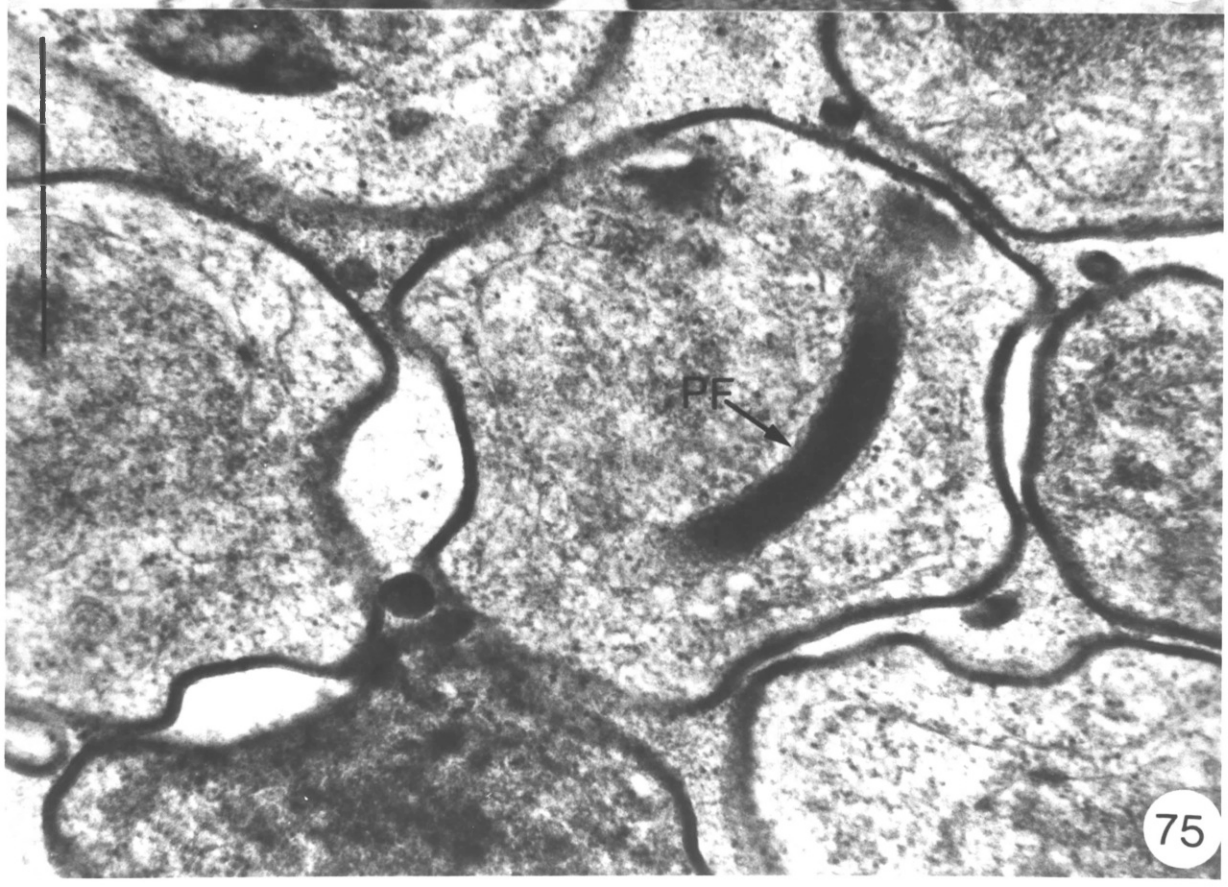
72

73. Late sporoblasts. These are crenated in shape. Metabolic products with tubular profiles can be seen in addition to the granular homogeneous material within the vesicle.


73

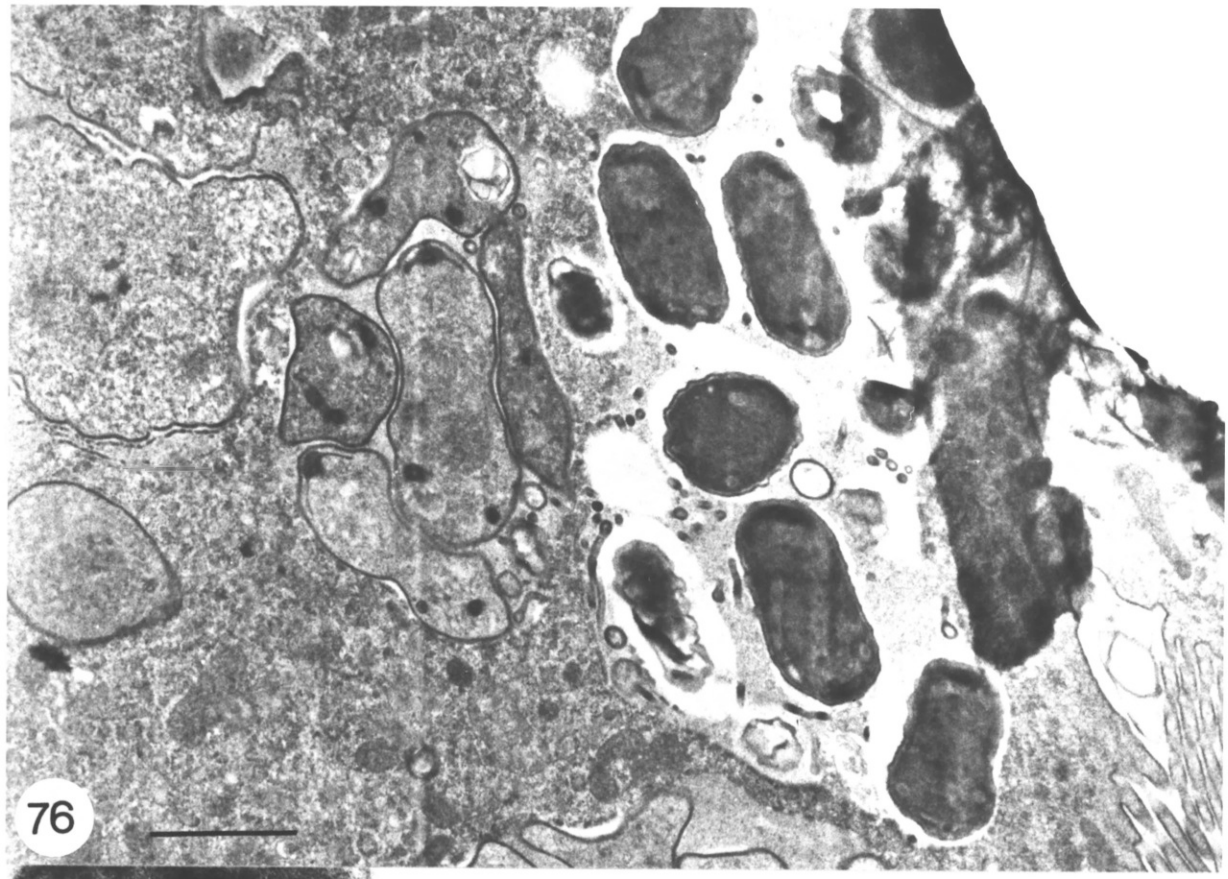
74,75. Detail of late sporoblasts. these are crenated in shape and show the developing polar filaments (PF) and polaroplast (PP).


PP
74

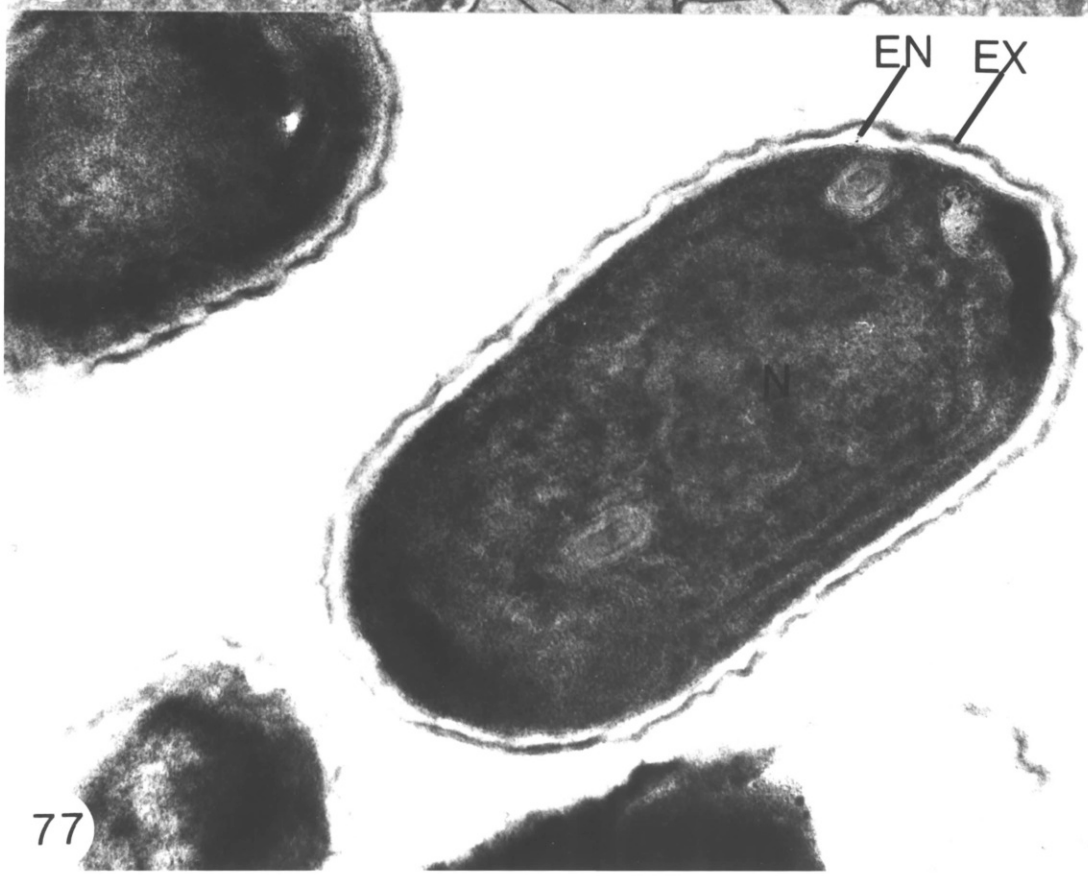

PF
75

76. Maturing spores. Tubular profiles are still present in the vesicle.

77. Maturing spores. Signs of endospore (EN) formation below the exospore (EX) are present. Spores have a single nucleus and only 1 coil of the polar filament.

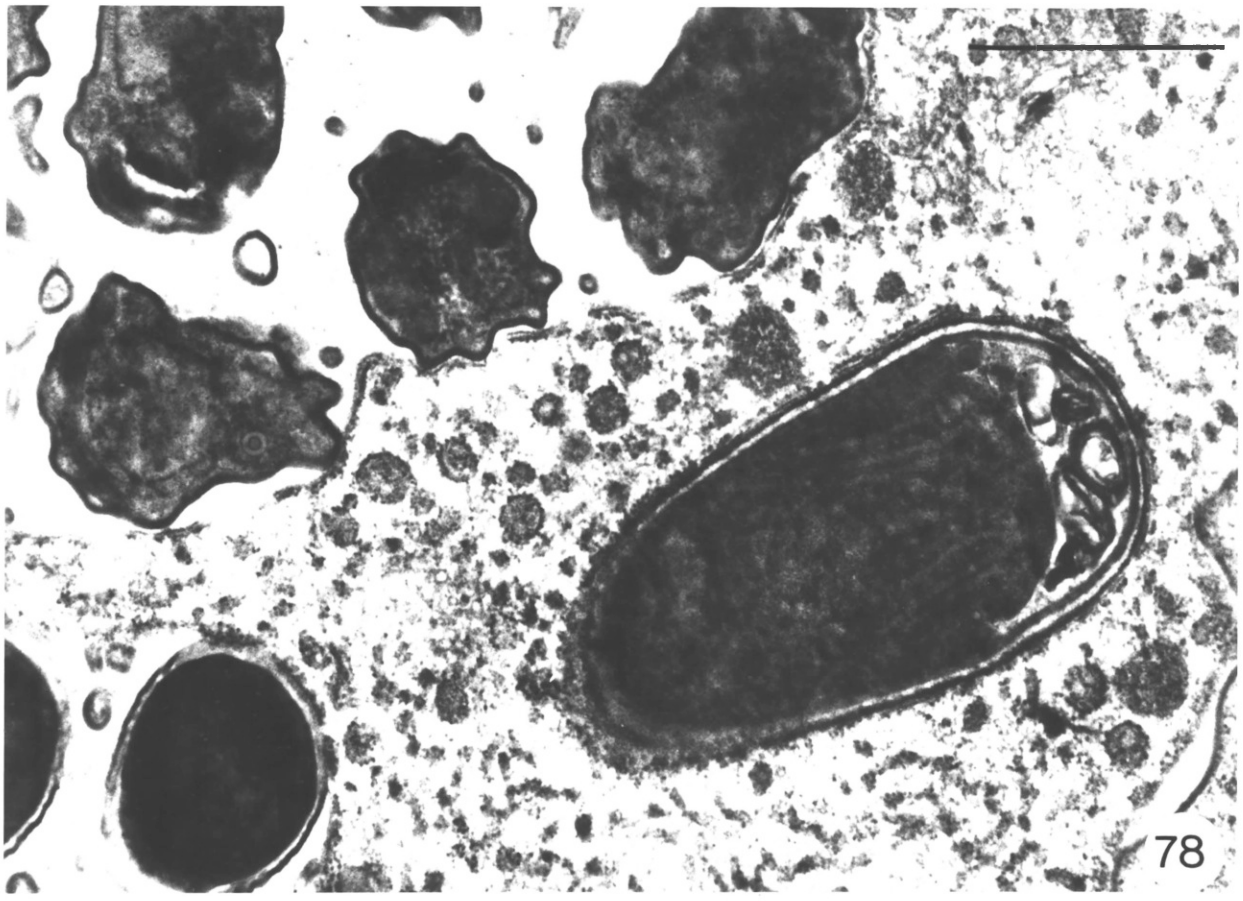


76



77

78. Large spore. This is thought to be formed as a result of incomplete division as there are suggestions of a double set of organelles.


78

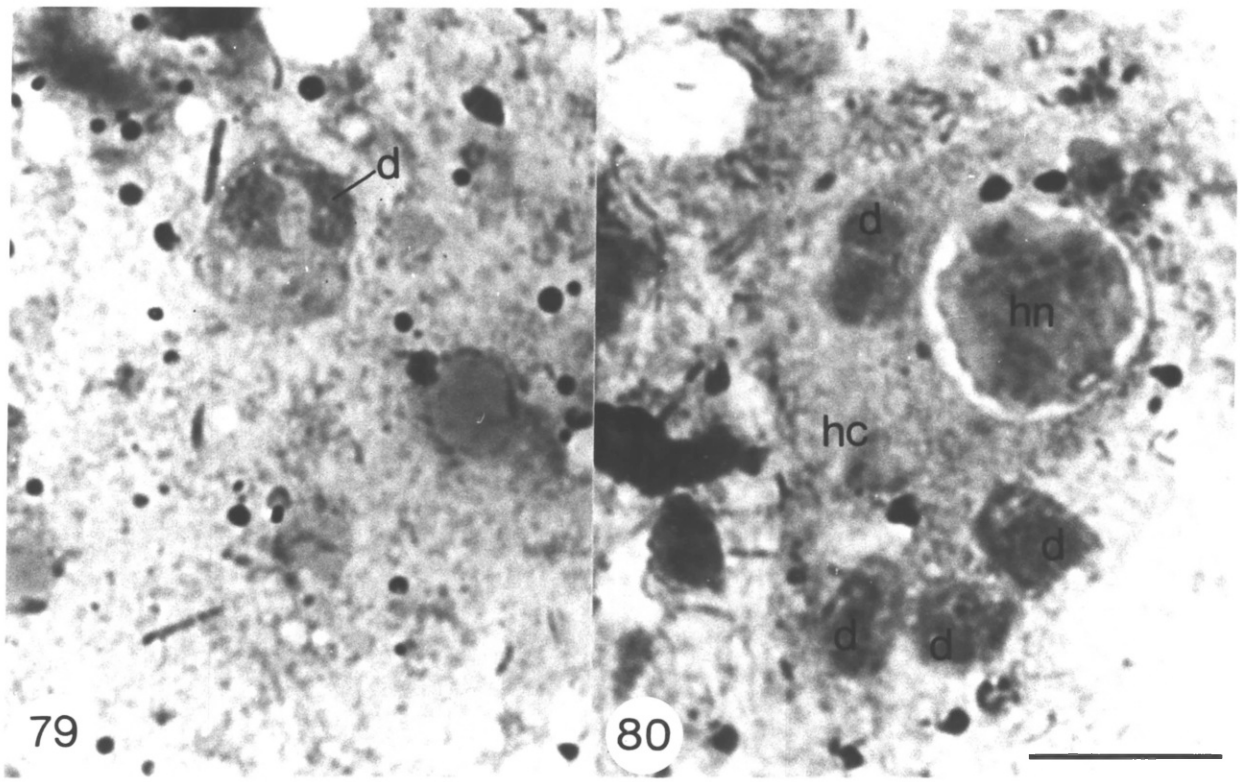
FIGS. 79-80 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF A BENIGN DIPLOKARYOTIC INFECTION IN Mansonia africana LARVAE (KISIWANI, MUHEZA SISAL ESTATE AND MAMAKINGI)

Scale bar = 10μm (Figs. 79-80)

79. Diplokaryotic meront.

80. Diplokaryotic meronts. There are 4 meronts within a host cell.

d = diplokaryon
hc = host cytoplasm
hn = host nucleus.


d
79
d
hn
hc
d
d
d
80

3.2.3 Prevalence of infections in adult mosquitoes

Jitengeni Village

In 1985, 1/24 Anopheles gambiae s.l. adults collected from huts in Jitengeni Village were found to harbour diplokaryotic microsporidian stages.

Kumbamtoni Village

In 1987 3/39 Anopheles gambiae s.l. and 0/11 An. funestus adults collected from huts were found infected with diplokaryotic stages and binucleate microsporidian spores, similar to those seen in the collection from Jitengeni in 1985.

Kisiwani and Muheza Sisal Estate

In 1985 examination of night catches of adult mosquitoes at Kisiwani pond revealed that 6/50 Ma. africana (12%) and 1/3 Ma. uniformis (33.3%) were infected with diplokaryotic developmental stages and binucleate microsporidian spores.

In 1987 no adult Mansonia were collected in night catches attempted at Kisiwani and Mamakingi. At Kisiwani it was thought that the attractive stimulus from nearby cattle was drawing adults away from the night catchers. Light traps were set up near the cattle, but again no Mansonia were caught. At Muheza Sisal Estate 5 night catches were carried out successfully. Two different types of infection were found, one with small diplokaryotic developmental stages (Species A), and the other with much larger diplokaryotic stages (Species B), (Table 7).

Before these mosquitoes were identified they had undergone a great deal of handling, resulting sometimes in loss of scales. The differentiation between Ma. africana and Ma. uniformis depends mainly on the strength of the banding colours on the legs, which are directly attributable to the presence of certain scales. Because of loss of scales some specimens could not be assigned to one or other species and were thus assigned to the group Mansonia sp.(Table 7). It is also possible that some Ma. africana were misidentified as Ma. uniformis due to loss of leg and wing scales. The infections were, therefore, not just predominantly, but possibly exclusively in Ma. africana, For this reason only figures for Ma. africana are used in subsequent graphs and comparisons.

TABLE 7: Prevalence of diplokaryotic infections in adult *Mansonia* species collected at Muheza Sisal Estate on 5 occasions in 1987

Date collected	No. collected	No infected with Species B [%]	No infected with Species A [%]	Total number infected with microsporidia [%]
24. 6.87				
Ma. africana	44	3 [6.8]	1 [2.3]	4 [9.1]
Ma. uniformis	13	0 [0]	0 [0]	0 [0]
Mansonia sp.	10	0 [0]	0 [0]	0 [0]
25. 6.87				
Ma. africana	21	0 [0]	3 [14.3]	3 [14.3]
Ma. uniformis	18	0 [0]	0 [0]	0 [0]
Mansonia sp.	9	0 [0]	0 [0]	0 [0]
13. 7.87				
Ma. africana	81	6 [7.4]	14 [17.3]	20 [24.7]
Ma. uniformis	22	2 [9.1]	0 [0]	2 [9.1]
Mansonia sp.	8	1 [12.5]	0 [0]	1 [12.5]
26. 8.87				
Ma. africana	16	1 [6.2]	0 [0]	1 [6.2]
Ma. uniformis	9	1 [11.1]	0 [0]	1 [11.9]
Mansonia sp.	5	0 [0]	0 [0]	0 [0]
4. 9.87				
Ma. africana	12	0 [0]	1 [8.3]	1 [8.3]
Ma. uniformis	10	1 [10.0]	0 [0]	1 [10.0]
Mansonia sp.	0	0 [0]	0 [0]	0 [0]

3.2.4 Morphology of infections in adult mosquitoes

Kumbamtoni Village

Developmental stages which could have been meronts or early sporonts were binucleate and tetranucleate (Figs.81,82) and finally gave rise to isolated binucleate spores (Fig.83) within the ovaries. Fixed and stained spores measured 4.4µm ± 0.3µm x 1.6µm ± 0.3µm which was in accord with the measurements of binucleate spores of Parathelohania africana from Anopheles gambiae s.l. Hazard and Anthony (1975).

Muheza Sisal Estate

Species A

The earliest stage seen was a diplokaryotic meront or early sporont (Fig.84) measuring 2.6µm ± 0.3µm (n=7) in length (diplokaryon measurement only). All subsequent stages were binucleate and never tetranucleate. Figure 85 shows a binucleate sporont which developed into a binucleate sporoblast (Fig.86) and binucleate spore (Fig.87), measuring 3.4µm ± 0.3µm x 1.8µm ± 0.3µm (n=6).

Species B

The earliest stages presumed to be merogonic were diplokaryotic with 1-4 diplokarya (Figs.88,89,90). They measured from 4.5µm ± 0.5µm to 2.6µm ± 0.5µm (n=10) in diameter. Of the sporogonic stages only binucleate sporoblasts (Figs.91,92) and spores were seen (Figs.88,93). Spores measured 6.6µm ± 0.3µm x 2.9µm ± 0.3µm (n=10) and were slightly curved into a characteristic sausage shape.

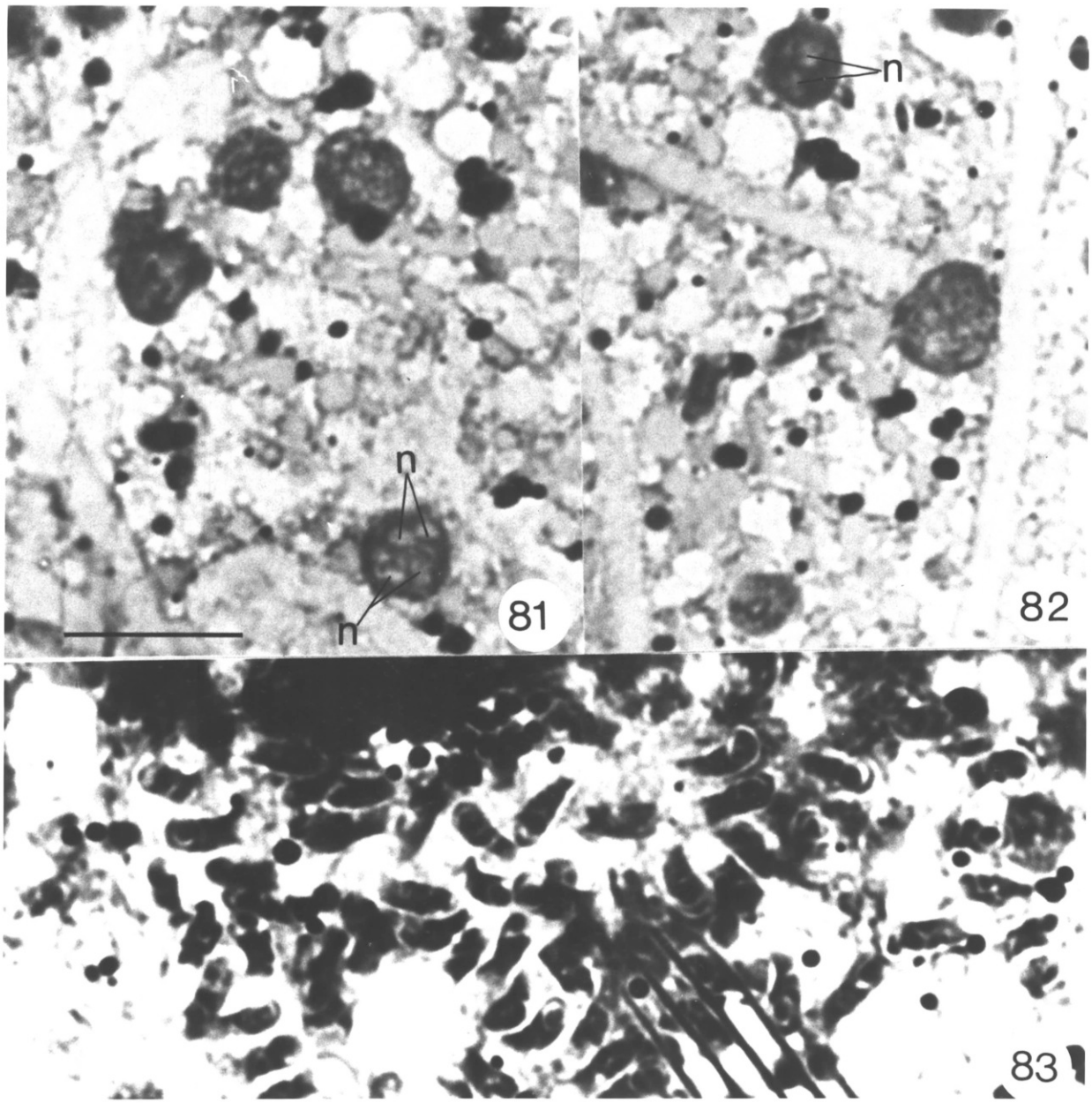
The 2 infections were not found together. The prevalence of these infections in Mansonia sp. are shown in Table 7. The microsporidian infections were predominantly in Ma. africana, although some infections of the smaller species were identified in Ma. uniformis.

FIGS.81-83. PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF A BENIGN DIPLOKARYOTIC INFECTION IN Anopheles gambiae s.l. ADULTS (KUMBAMTONI).

Scale bar = 10μm (Figs. 81-83)

81 & 82. Meronts and/or early sporonts. These stages are binucleate or tetranucleate. n = nucleus.

83. Binucleate spores.


n
n
81
n
82
83

FIGS. 84-87. PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF A DIPLOKARYOTIC INFECTION (Species A) IN Mansonia africana ADULTS (MUHEZA SISAL ESTATE)
Scale bar = 10μm (Figs. 84-87)

84. Rounded diplokaryotic meront (or early sporont).

85. Elongate binucleate sporont.

86. Binucleate sporoblast.

87. Binucleate spore.

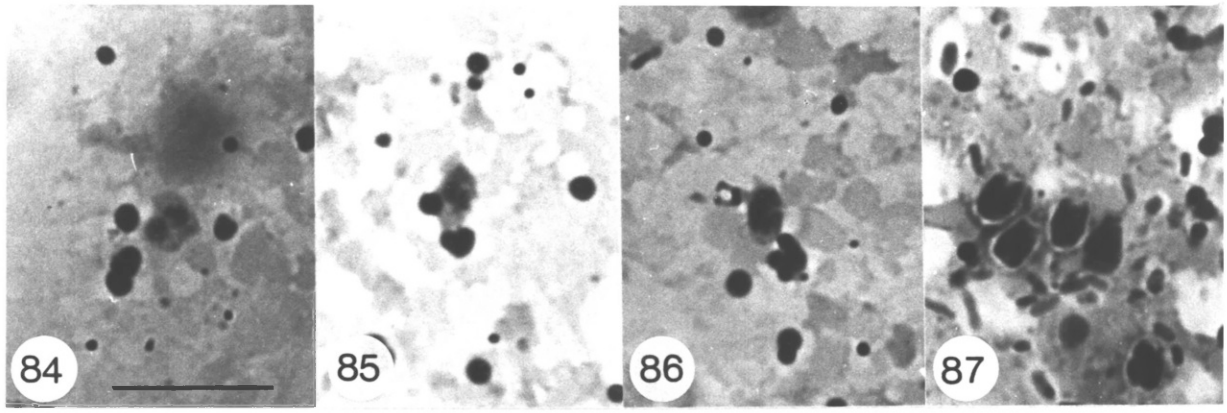
FIGS. 88-93 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF A BENIGN DIPLOKARYOTIC INFECTION (Species B) IN Mansonia africana ADULTS (MUHEZA SISAL ESTATE)

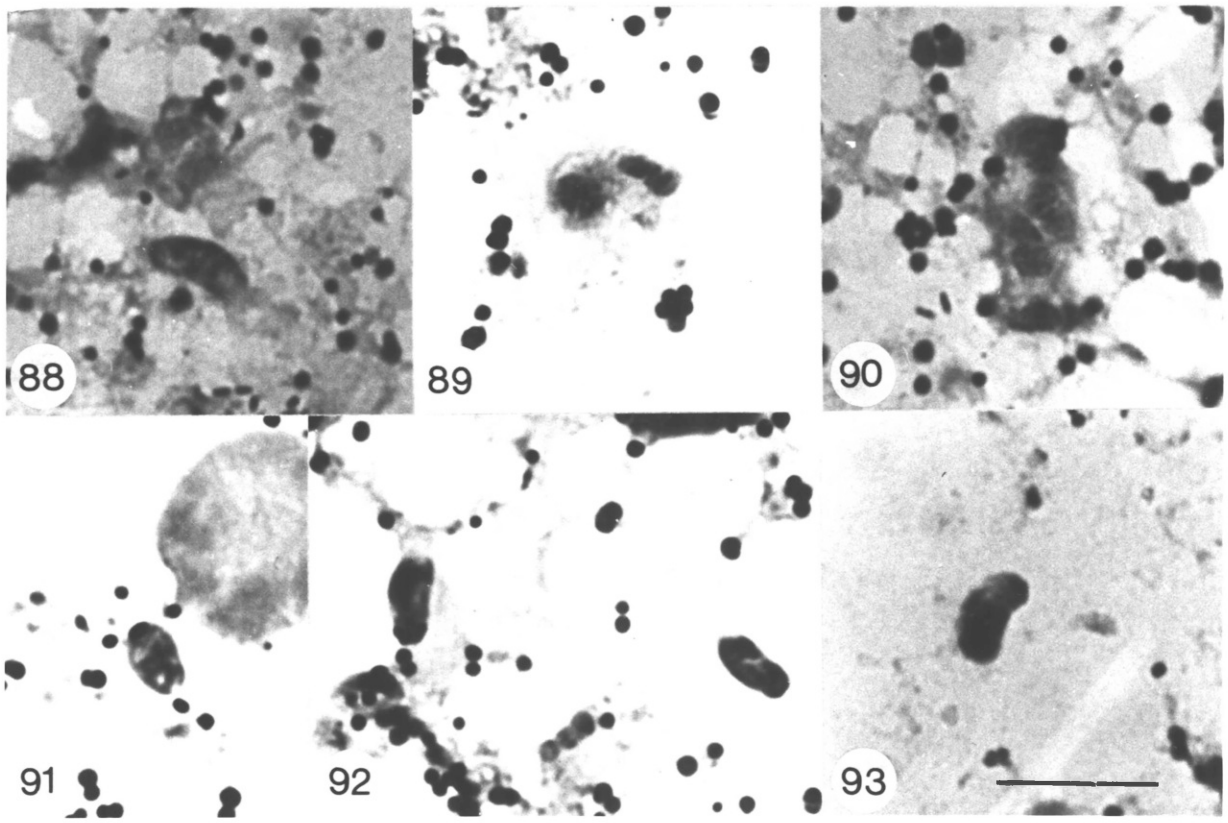
88. Meront with single diplokaryotic nucleus and a binucleate spore.

89, 90. Merogonic plasmodia with variable numbers of diplokaryotic nuclei.

91, 92. Binucleate sporoblasts

93. Binculeate spore.


84
85
86
87


88
89
90
91
92
93

3.2.5 Rearing progeny from infected female mosquitoes

Jitengeni Village

An attempt was made to rear the progeny of this female, but the larvae died after hatching and it was subsequently found that the bowl provided had previously been used in work with insecticides.

Kumbamtoni Village

The progeny of two of the infected females were reared to 4th instar and all these larvae were found to be infected with octospores (Fig.94) (17 from one egg batch and 33 from the other). Transmission electron microscopy showed that the spores were identical to those found at Mombo in 1985 and identified as Parathelohania africana Hazard and Anthony (1974). Scanning electron microscopy of the spores showed the "bottleneck" ornamentation of the spore (Fig.95). These larvae provided sufficient material for transmission experiments.

Kisiwani and Muheza Sisal Estate

From the adults caught on 13.7.87, 9 of the infected females laid eggs which hatched. These larvae were reared in the modified culture bowls, several batches per bowl because of the shortage of bowls and were smeared after 26 days. The first bowl contained 4 egg batches laid by females infected with Species A. The second bowl contained 5 egg batches, 2 from adult females infected with Species A and 3 from adult females infected with Species B. In the second bowl only 4 larvae survived, none of which showed any signs of infection. However, of the 62 larvae in the first bowl, 7 had multisporous infections (11.3%). A third bowl containing progeny from 3 uninfected adults served as controls. These larvae were uninfected. Table 8 shows the number of larvae from each instar infected.

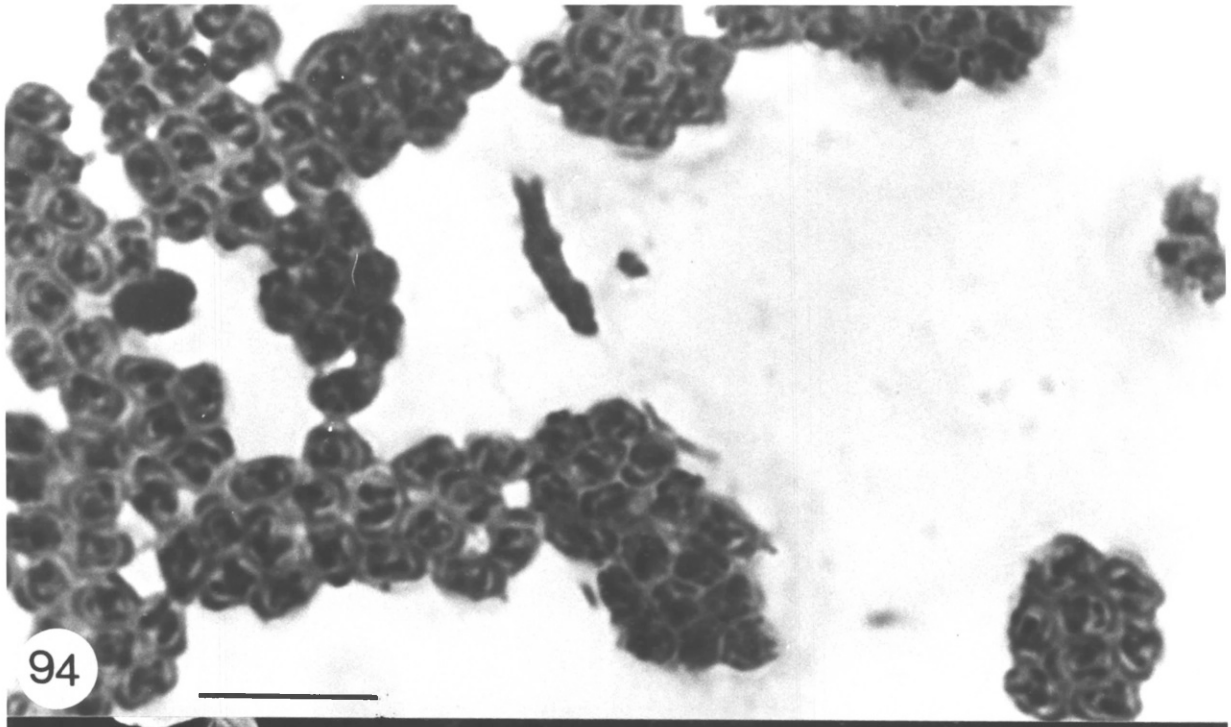
Adults brought back to Britain from Tanzania were also examined for infection after they had laid eggs. Two larval rearing bowls, each with the progeny from one female, infected with Species A were reared. A control bowl with progeny from an apparently uninfected female was also set up. The results can be seen in Table 9. In bowl 1, 15.5% of larvae were infected with a multisporous infection and in bowl 2, 18.5% carried the same multisporous species. Surprisingly, the third (control) bowl also showed 4.8% of larvae infected

FIG.94 PHOTOMICROGRAPH OF A GIEMSA-STAINED SMEAR OF AN OCTOSPOROUS INFECTION IN Anopheles gambiae s.l. LARVAE (KUMBAMTONI)
Scale bar = 10μm (Figs. 94, 95)

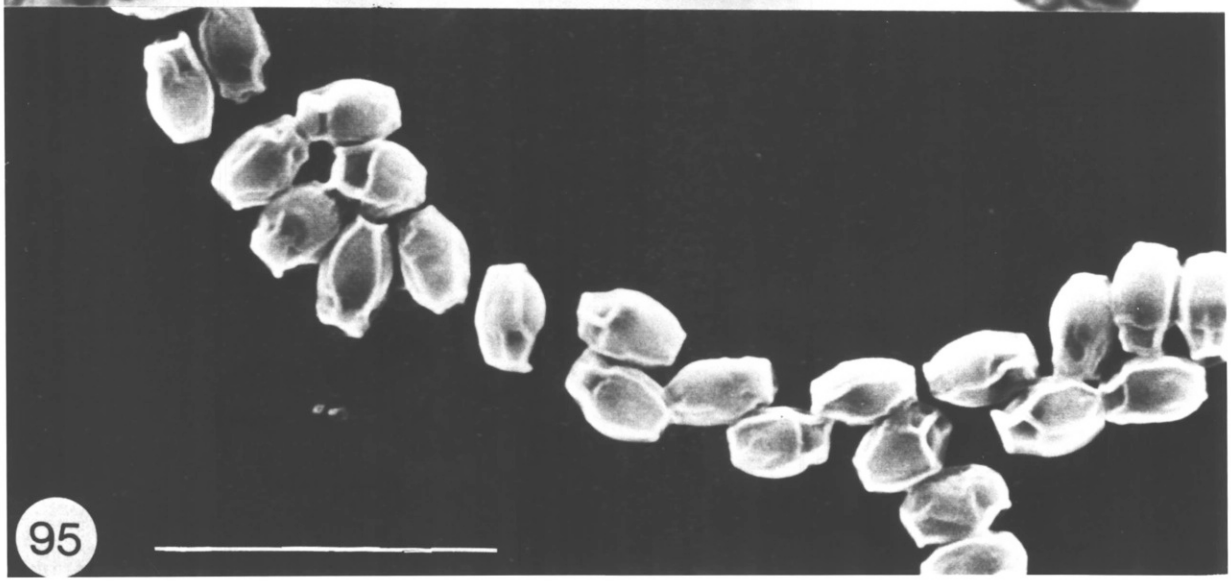
94. Octospores

FIG.95 SCANNING ELECTRON MICROGRAPH OF OCTOSPORES FROM Anopheles gambiae s.l. LARVAE (KUMBAMTONI)

95. Spores have a "bottleneck" protruberance at the posterior pole.



94



95

TABLE 8: Prevalence of multisporous infection in progeny reared from infected (Bowl 1 and 2) and uninfected adults (Bowl 3) in Tanzania

	Bowl 1	Bowl 2	Bowl 3 (Control)
Instar	No infected/No examined with multispores [%]	No infected/No examined with multispores [%]	No infected/No examined with multispores [%]
1	0/0	0/0	0/0
2	1/6	0/3	0/19
3	4/24	0/1	0/24
4	2/26	0/0	0/33
Pupae	0/6	0/0	0/4
Total	7/62	0/4	0/80
[% infected]	[11.3]		

TABLE 9: Prevalence of multisporous infection in progeny reared from infected (Bowl 1 and 2) and uninfected adults (Bowl 3) in Britain

	Bowl 1	Bowl 2	Bowl 3
Instar	No infected/No examined with multispores [%]	No infected/No examined with multispores [%]	No infected/No examined with multispores [%]
1	0/0	0/0	0/0
2	0/0	0/0	0/0
3	0/12	2/5	1/21
4	7/31	3/20	1/14
Pupae	0/1	0/2	0/7
Total	7/45	5/27	2/42
[% infected]	[15.6]	[18.5]	[4.8]

with the multisporous species. The adult which laid the eggs in the control bowl was dead when examined, and so was screened for spores rather than developmental stages. It was thought that the low level of infection arising in 2 of this female's progeny must have come from an extremely low level adult infection which was not seen in the original screening of very poor material.

Using the numbers of infections found in bowls 1 and 2 from Tables 8 and 9 (Species A) the overall number infected was 14 out of 138 (13.8%). All the adults were clearly infected when examined and yet only 13.8% of their progeny became infected transovarially. It was not possible to determine whether infections of Species B in adults were linked to the octosporous cycle in larvae as only 4 larvae survived from the progeny of 3/5 adults infected with Species B (bowls 1 and 2, Table 8).

The percentage larval infection with the multisporous species at Muheza Sisal Estate and the percentage adult infection with Species A were compared (Fig.96). It was assumed that as adults with Species A infection gave rise to progeny with multisporous infections that the levels of these parasites in the field would in some way be correlated. Although it can be seen that the level of infection increased in adults and that the prevalence in larvae was higher in August than in June, it is difficult to draw firm conclusions as there were dates when none of the larvae collected were infected with multisporous infections (20, 26 June, 11, 15, and 26 July) and dates where none of the adults collected had infections of Species A. (26 August).

The larval infection with octospores and the percentage adult infection with Species B were also compared (Fig.97) in an attempt to find if there was any correlation between them. As with the aforementioned comparison it was difficult to draw any conclusions from this, particularly as there were dates when none of the larvae collected were found with octosporous infections (18 June) and none of the adults collected were infected with the Species B infection (25 June, 4 September). The levels of infection in larvae and adults appeared to be steady between June and August at 4-10% except on July 26th. For completeness the levels of multisporous infection in larvae and Species B infection in adults were compared (Fig.98), as were levels of octosporous infection in larvae and Species A infection in adults (Fig.99). As Figures 98 and 99 show, no correlation was found.

Diplokaryotic infections in larvae were presumed to give rise to diplokaryotic infections in adults. As 2 types of infection, Species A and B

FIGURE 96: % Infection in adult and larval Ma. africana

Muheza Sisal Estate, 1987

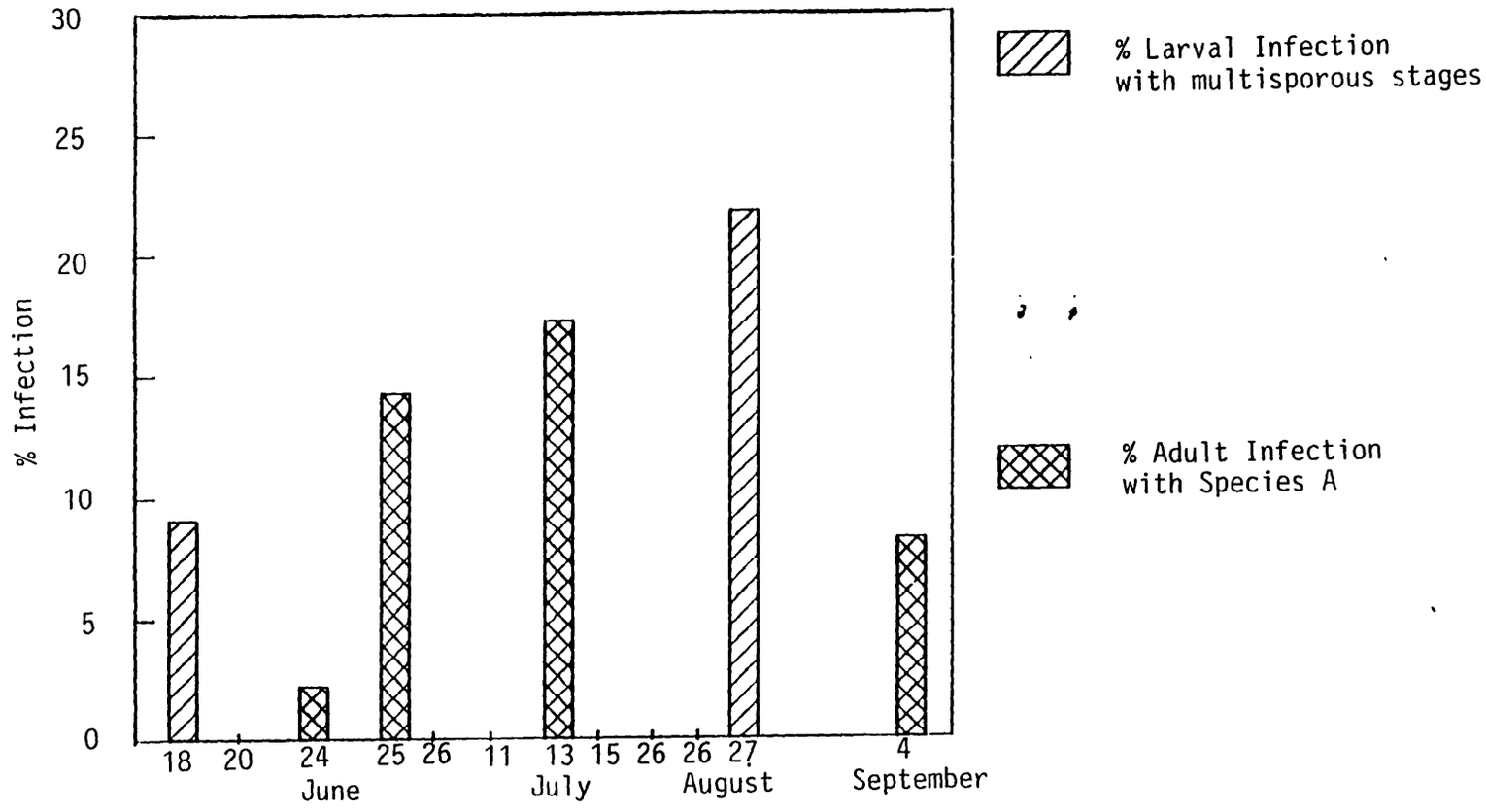


FIGURE 97: % Infection in adult and larval Ma. africana

Muheza Sisal Estate, 1987

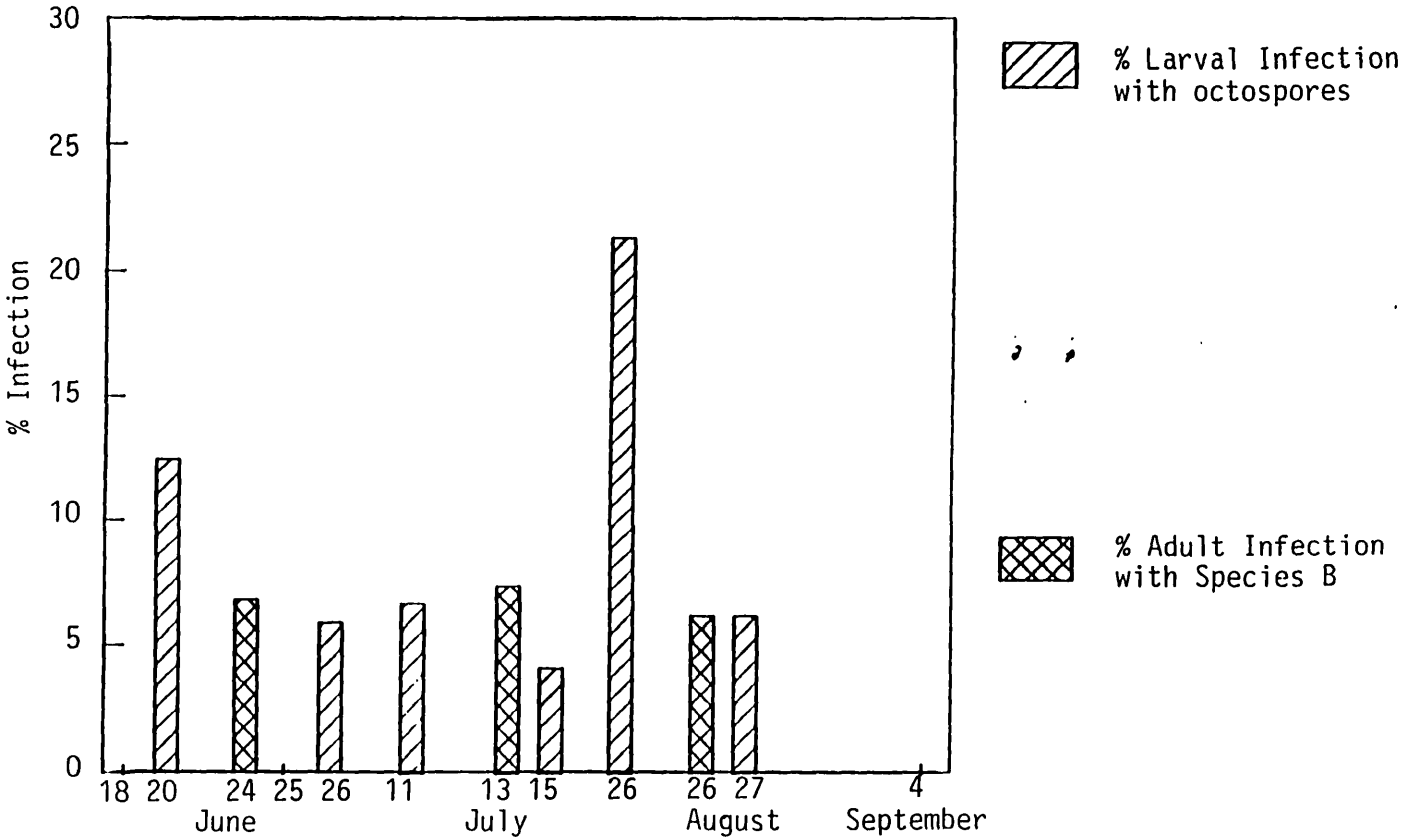


FIGURE 98: % Infection in adult and larval Ma. africana

Muheza Sisal Estate, 1987

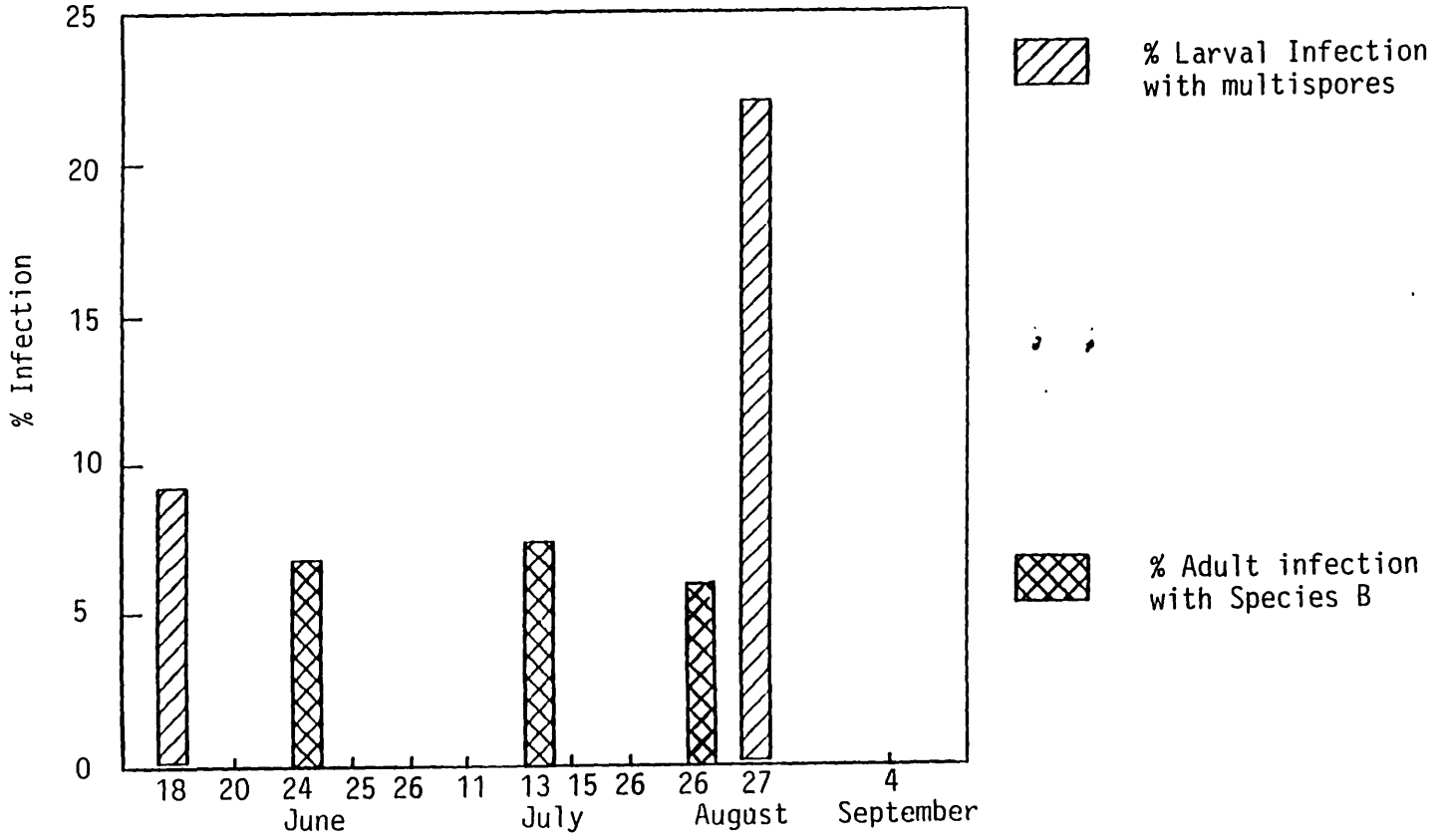
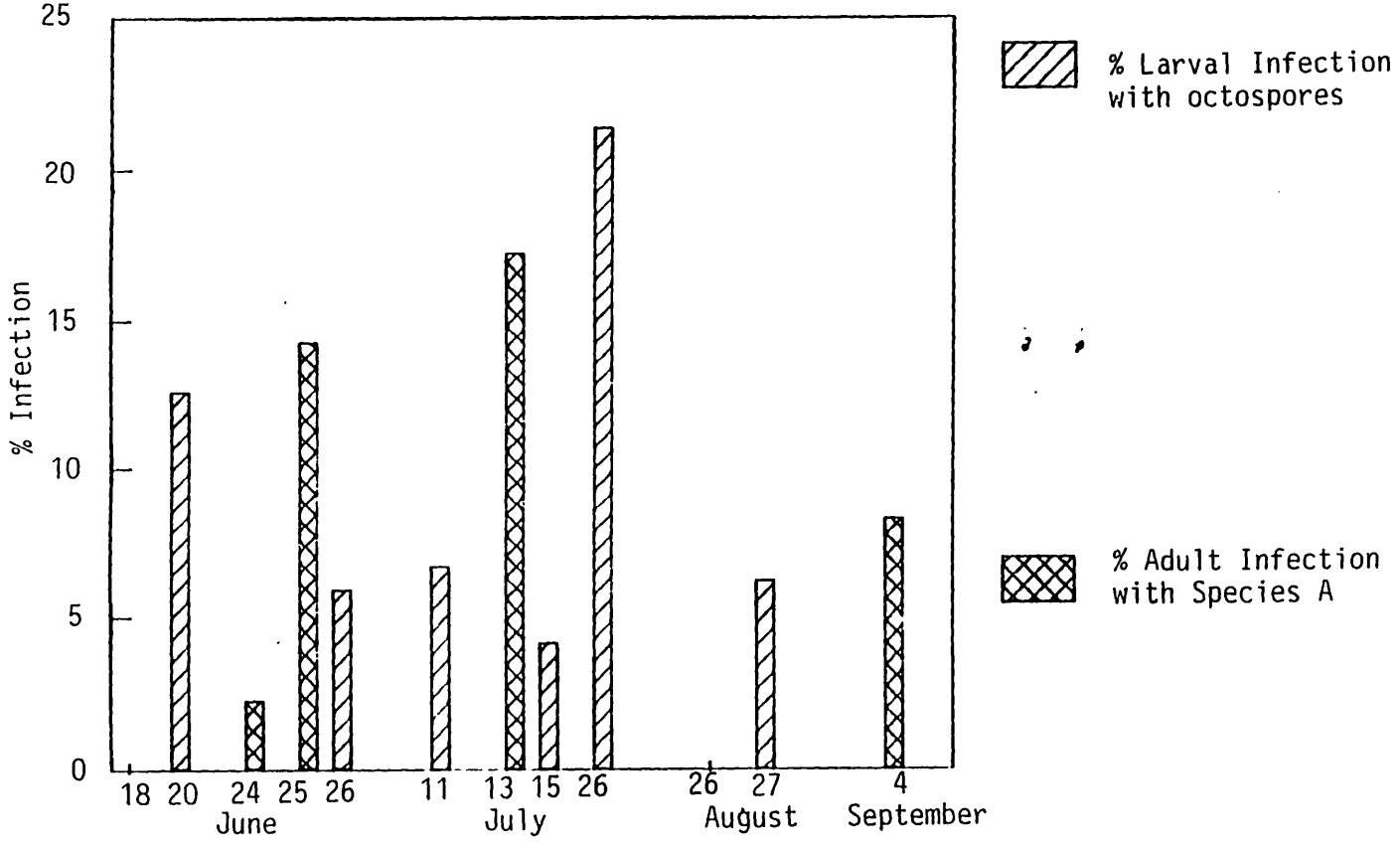


FIGURE 99: % Infection in adult and larval Ma. africana

Muheza Sisal Estate, 1987



were found in adults, it was assumed that there would be two types of larval infection. However, only large diplokaryotic stages were found, suggesting either that this represented a mixture of the two species, the merogonic stages of both species being of a similar size, or that only larvae infected with the stages corresponding to Species B were sampled.

In an attempt to clarify this, the percentage larval infection with diplokaryotic stages was first compared with the total percentage of adults infected with Species A and B together (Fig.100), and then with the percentage of adults infected solely with Species B (Fig.101).

Figures 100 and 101 show that, as with earlier comparisons, it is difficult to draw any firm conclusions, due to the fact that there are several dates when diplokaryotic larval infections were never found (18th and 26th June, 11th and 15th July) and 2 dates when no infections with Species A were found in adults (25th June and 4th September). In both comparisons there is an overall increase in infection between June and early July and a decrease between late July and September.

3.3 MICROSPORIDIAN INFECTIONS OF FIELD COLLECTIONS OF TANZANIAN COPEPODS (KISIWANI, MUHEZA SISAL ESTATE AND MAMAKINGI)

3.3.1 Prevalence of infection in Mesocyclops rarus

Because of the detailed dissection required, copepods were not identified in the field. Instead a number were collected from each site and preserved in 70% alcohol for later identification at the Natural History Museum, London. The commonest species, and the only one to be found at all three sites in abundance, was M. rarus. Occasional Ectocyclops hirsutus, Afrocyclops gibsoni and Cryptocyclops linjanti were found. However, the first 2 species were markedly smaller and the 3rd species markedly larger than M. rarus. These larger and smaller species were not found infected with microsporidia, and were always removed from collections used in infection trials.

Tables 10, 11 and 12 give the prevalences of microsporidian infection in M. rarus at the 3 sites. In some specimens only developmental stages were found and in others sporogonic stages and spores were also present. The mean percentage infections from the 3 collection sites were 0% at Kisiwani, 24.3%

FIGURE 100: % Infection in adult and larval Ma. africana

Muheza Sisal Estate, 1987

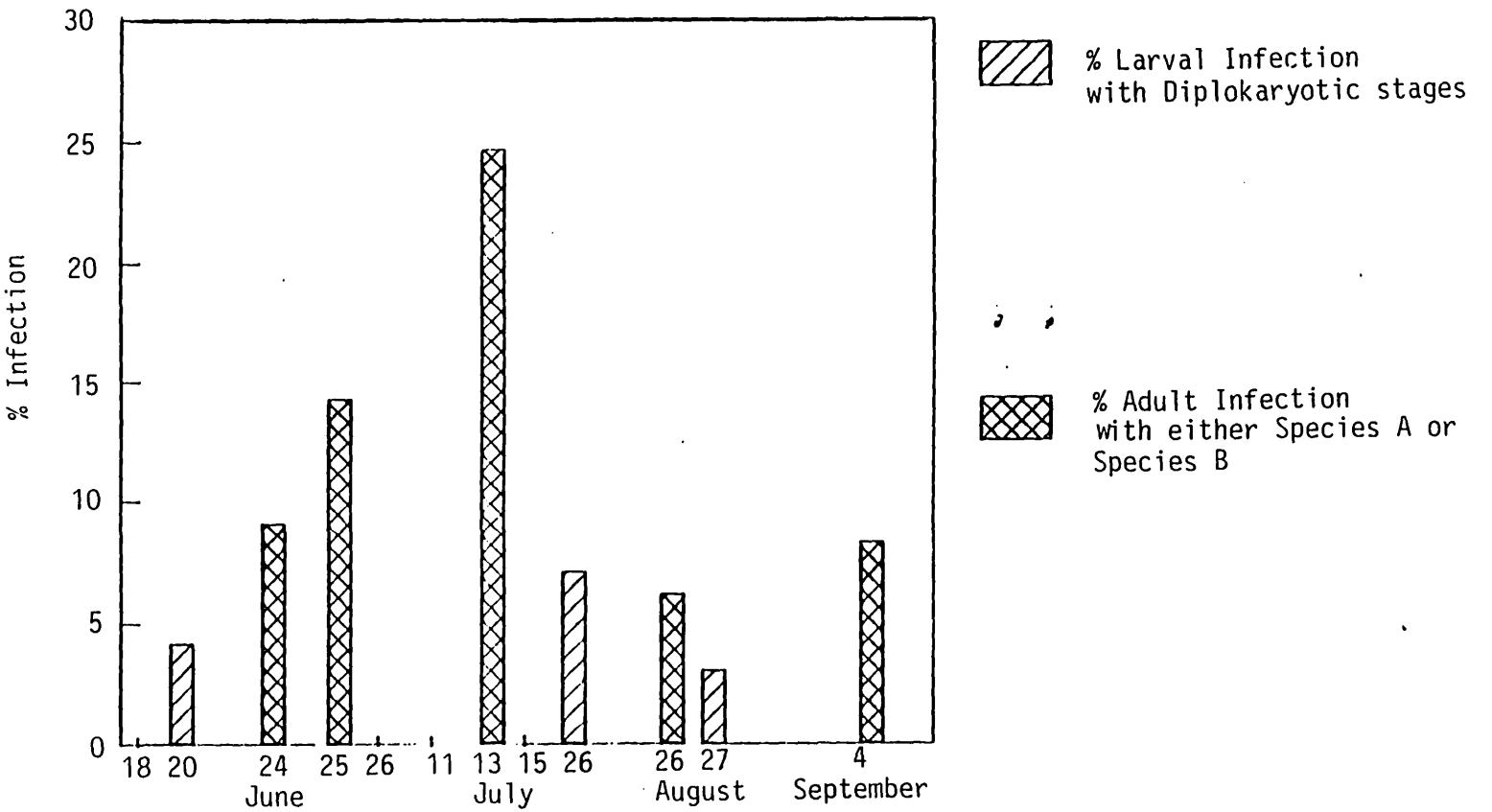


FIGURE 101: % Infection in adult and larval Ma. africana

Muheza Sisal Estate, 1987

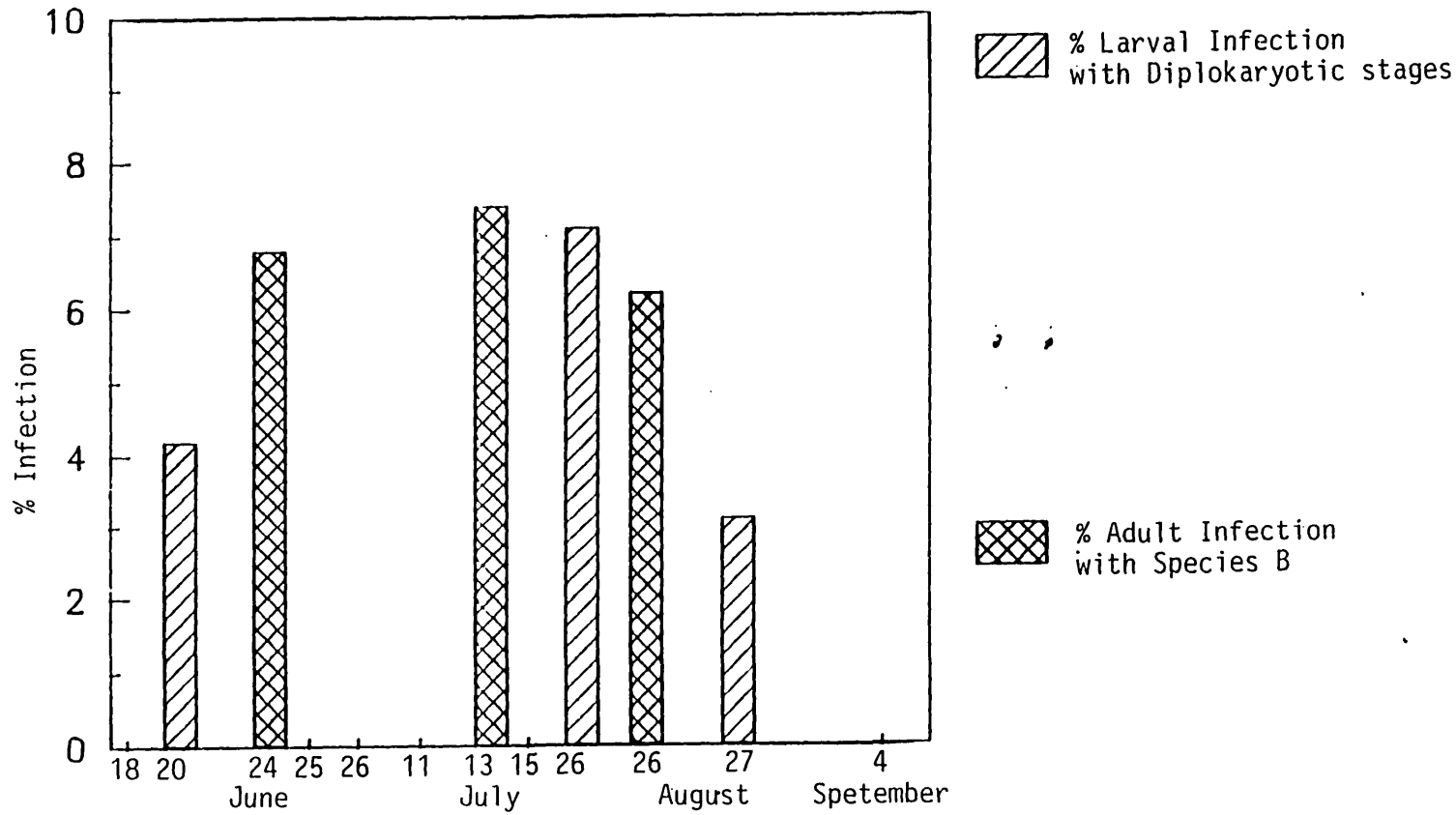


TABLE 10: Mesocyclops rarus infection at Kisiwani

Date	No collected	No infected with developmental stages and spores [%]	No infected with developmental stages only [%]
14.6.8	20	0 [0]	0 [0]
15.6.8	15	0 [0]	0 [0]
30.8.8	91	0 [0]	0 [0]

TABLE 11: Mesocyclops rarus infection at Muheza Sisal Estate

Date	No collected	No infected with developmental stages and spores [%]	No infected with developmental stages only [%]
25.7.8	35	8 [22.0]	1 [2.8]
20.9.8	72	14 [19.4]	3 [4.2]

TABLE 12: Mesocyclops rarus infection at Mamakingi Pond

Date	No collected	No infected with developmental stages and spores [%]	No infected with developmental stages only [%]
27.7.8	37	3 [8.1]	1 [2.7]
8.9.8	59	8 [13.6]	1 [1.7]

at Muheza Sisal Estate and 13.5% at Mamakingi. Infections were found in adult females which were recognised by their paired egg sacs, and in copepods which were either the same size or marginally smaller than females, but without egg sacs. These were thought to be males of the same species or females temporarily without egg sacs.

3.3.2 Morphology of infection in Mesocyclops rarus

Light microscopic appearance

Using light microscopy two separate developmental sequences were detected. These sequences were similar, only differing in the size of the parasite stages and resultant spores. Both infections were always found together and packed the entire body cavity of the copepod although the copepods did not appear overtly infected.

In the first type of infection the earliest stages seen were small rounded uninucleate and binucleate meronts (Figs.102,103). Early sporogonic stages were also uninucleate and binucleate but more oval in shape (Fig.104). After division of the nucleus, the two resulting nuclei moved towards the poles of the cytoplasm (Fig.105). The whole sporont then formed into a U-shape (Fig.106) before separating with two sporoblasts (Fig.107). Tetranucleate sporonts were also present; these became stellate during division (Fig.108). The sporoblasts became pyriform (Fig.109) and matured into small pyriform spores measuring 8.9µm ± 0.5µm x 3.9µm ± 0.3µm (n=9) (Fig.110).

In the second type of infection the earliest stages were also rounded unincleate and binucleate meronts, though rather larger, (Figs.109,110). Occasional tetranucleate meronts were also seen (Fig.112). As with the first type of infection, uninucleate sporonts (Fig.113) became binucleate and aligned themselves into a U-shape (Fig.112) Division produced crescentic sporoblasts (Fig.115) which became pyriform (Fig.111) and matured into large pyriform spores measuring 15.0µm ± 0.3µm x 6.3µm ± 0.1µm (n=11) (Fig.110). Tetranucleate, stellate forms were never seen. Even at the light microscope level, the polaroplast, nucleus, endospore and exospore of the spore wall were clearly visible in both large and small spores.

FIGS.102-115 PHOTOMICROGRAPHS OF GIEMSA-STAINED SMEARS OF AN INFECTION IN Mesocyclops rarus COPEPODS (MUHEZA SISAL ESTATE)

Scale bar = 10μm (Figs. 102-115)

102. Small uninucleate meront.

103. Small binucleate meront.

104. Early uninculeate sporont of the small staged developmental sequence.

105. Small binucleate sporont. The nuclei have migrated to the poles.

106. Small sporont. A U-shape forms before divison.

107. Early sporoblasts of the small developmental sequence.

108. Tetranucleate stellate sporont of the small developmental sequence.

109. Late, pyriform sporoblast (sb) of the small development sequence and uninucleate and binucleate meronts (m) of the larger developmental sequence. n=nucleus.

110. Pyriform spores. The spores are of 2 sizes belonging to both developmental sequences, but, within each the exospore (EX), endospore (EN), polaroplast (P) and nucleus (N) are clearly visible.

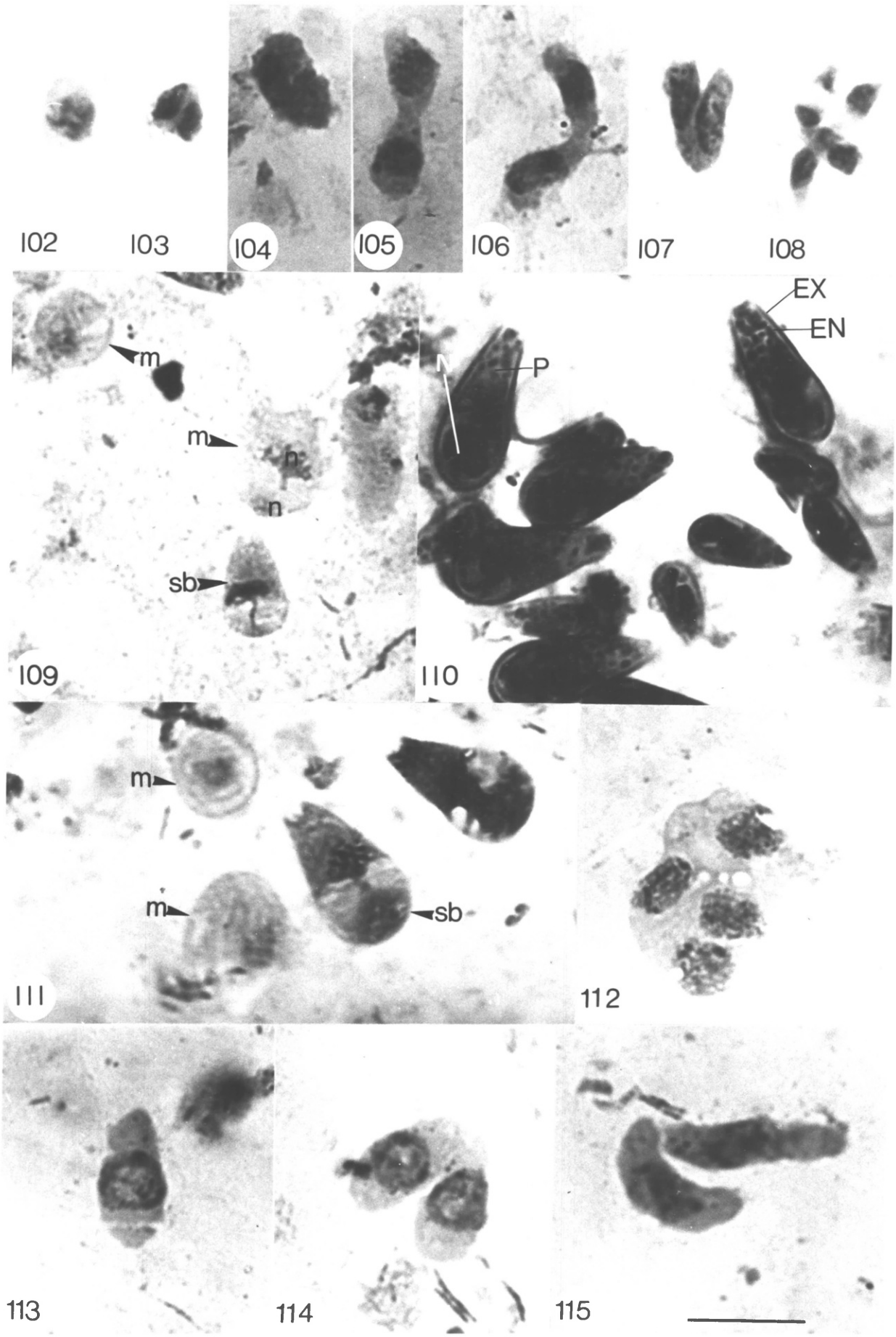
111. Large uninucleate meronts (n=nucleus) and large mature pyriform sporoblast (sb).

112. Large tetranucleate meront.

113. Large uninucleate sporont.

114. Large binucleate sporont. A U-shape forms before division.

115. Early crescentic sporoblasts of the larger developmental sequences.


102
103
104
105
106
107
108
m
m
n
n
sb
109
EX
EN
N
P
110
m
m
sb
111
112
113
114
115

Ultrastructual appearance

The earliest stages seen were meronts; which were rounded cells limited by a fine unit membrane and surrounded by host mitochondria (Fig.116).

Sporogonic stages were more elongate than mergonic stages (Fig.117). Electron dense material was laid down patchily on the plasma membrane (Figs. 118,119). At this stage large blisters were formed by the sporophorous vesicle membrane as it separated from the parasite surface (Figs.118,119). Within sporonts, membranous whorls were commonly found possibly formed from endoplasmic reticulum during polaroplast development (Fig.119).

Within the copepod there were distinct regions in which different developmental stages were found. From the cuticle inwards there was first striated muscle which was uninfected and allowed the host to remain motile. Within this, developmental stages were seen, which were both merogonic and sporogonic. The spores were located towards the centre of the copepod (Fig.120).

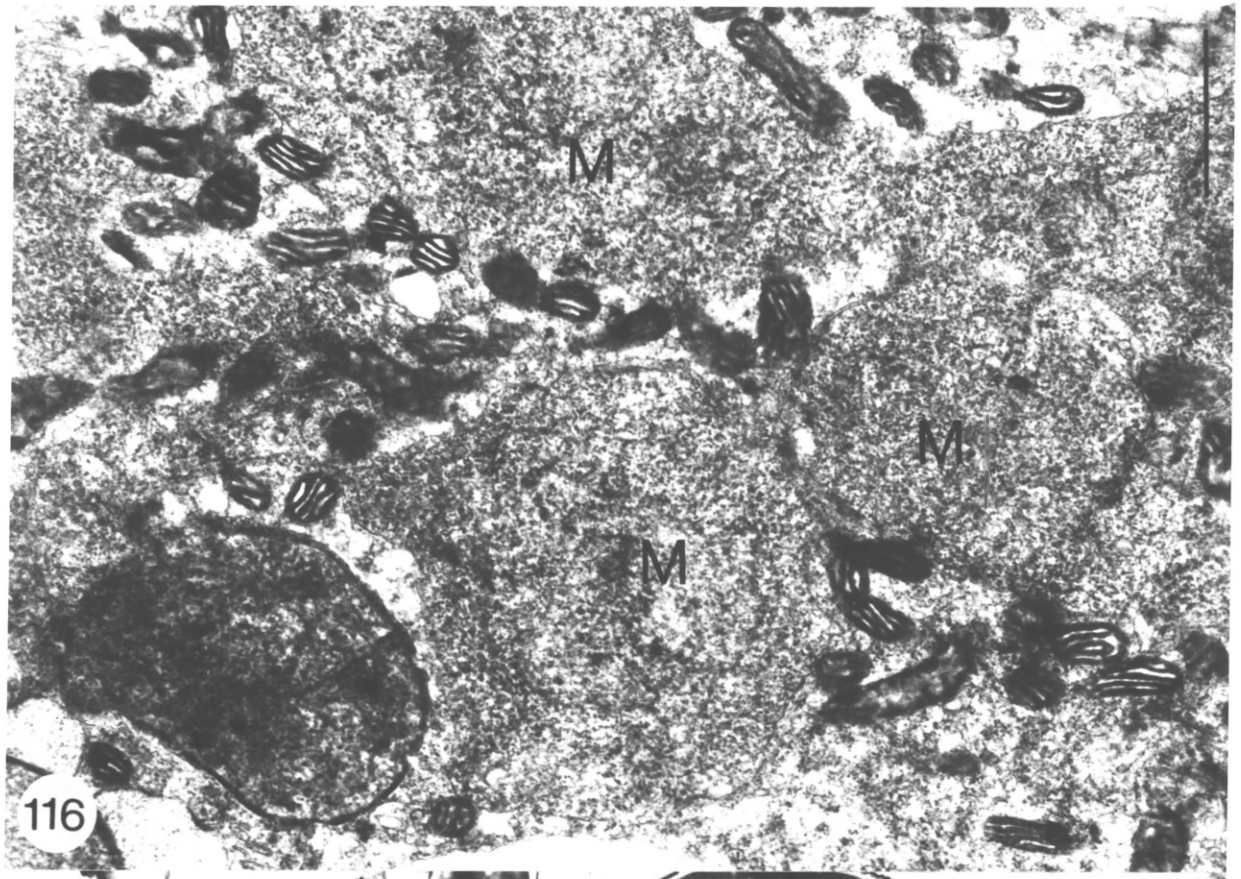
Sporoblasts, the stage before mature spores, were not seen. Pyriform spores of 2 sizes lay singly within large, empty sporophorous vesicles (Fig. 121). The small spores measured 5.6µm $\pm$ 0.05µm x 1.9µm $\pm$ 0.1µm (n=3). Unfortunately due to the great size of the larger species there were no entire cross-sections. Ultrastructurally there was little difference between the 2 types of spore. The polar filament was isofilar and the maximum number of coils was 20 (Fig.122).

The straight part of the polar filament ran through the polaroplast blocks to terminate beneath the polar cap. In cross section 5 layers could be distinguished in each polar filament. From the inside, an electron lucent core surrounded by a layer of moderate electron density, then a lucent ring another moderately dense ring and an outer sleeve of two distinct membranes (Fig.123). The polaroplast consisted of a series of blocks separated by membranes, the blocks becoming less electron dense towards the posteriad. All spores had a prominent posterior vacuole the contents of which were disrupted during fixation (Fig.122).

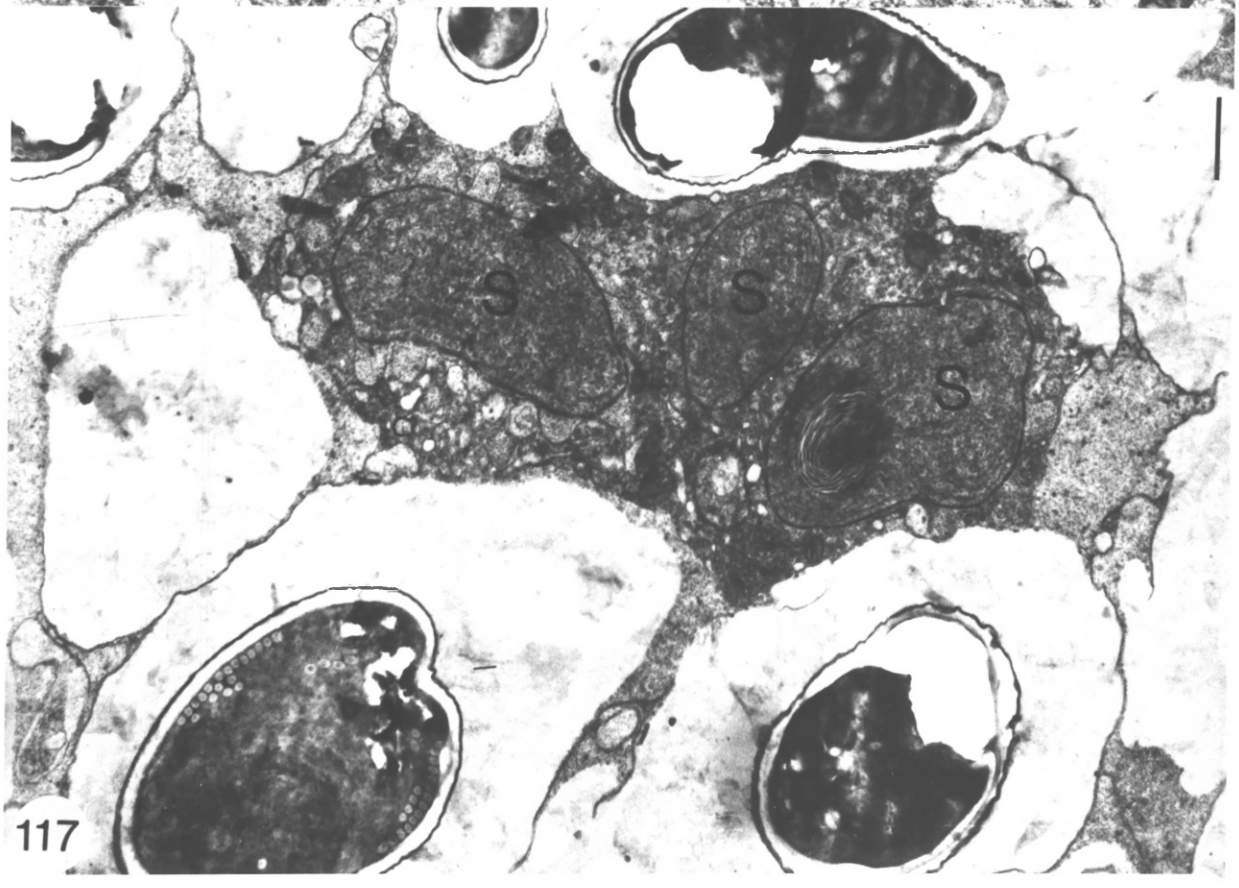
FIGS.116-123. TRANSMISSION ELECTRON MICROGRAPHS OF AN INFECTION FROM *Mesocyclops rarus* (MUHEZA SISAL ESTATE AND MAMAKINGI)
Scale bar = 1μm (Figs.116-123)

116. Uninculeate meronts (M). Rounded cells limited by single unit membranes and surrounded by host mitochondria.

117. Three elongate sporogonic stages (S)

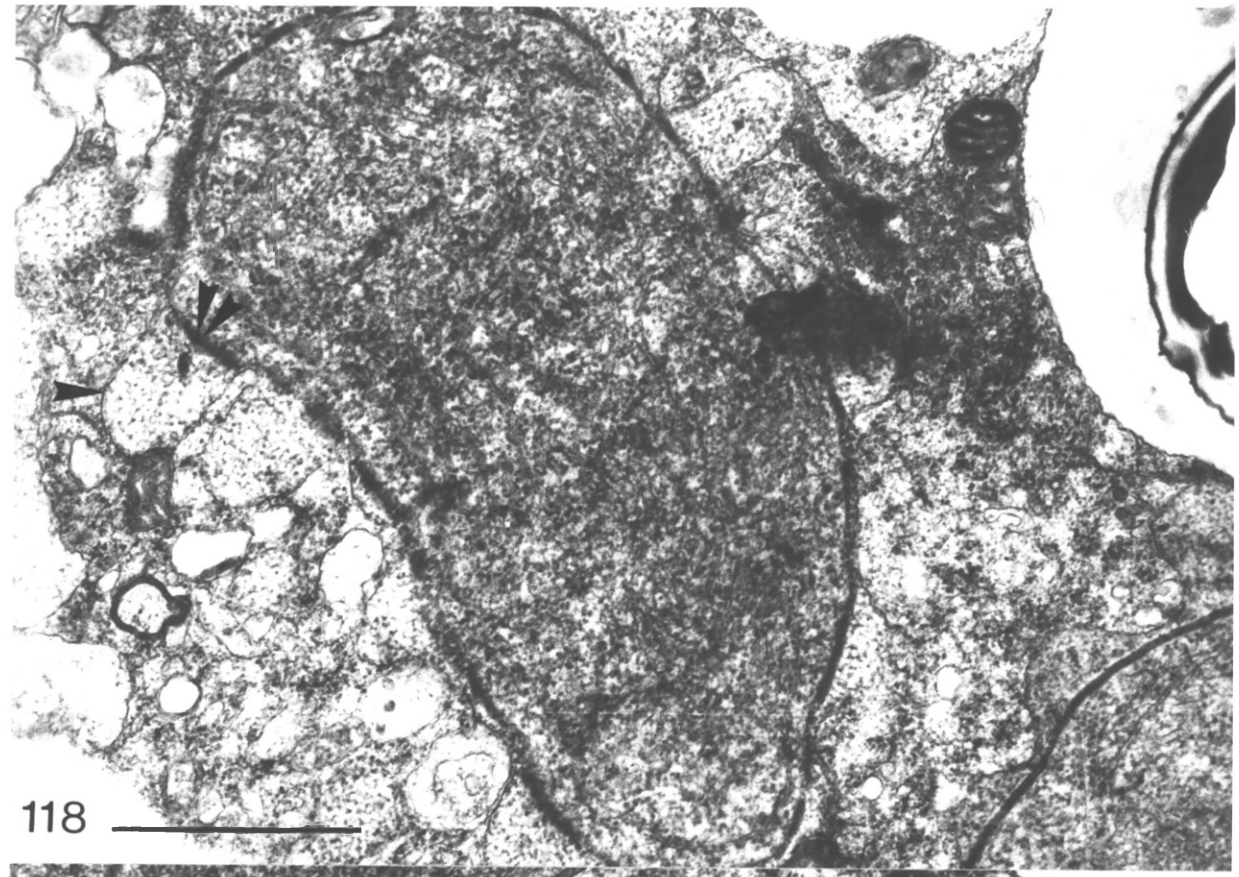


116

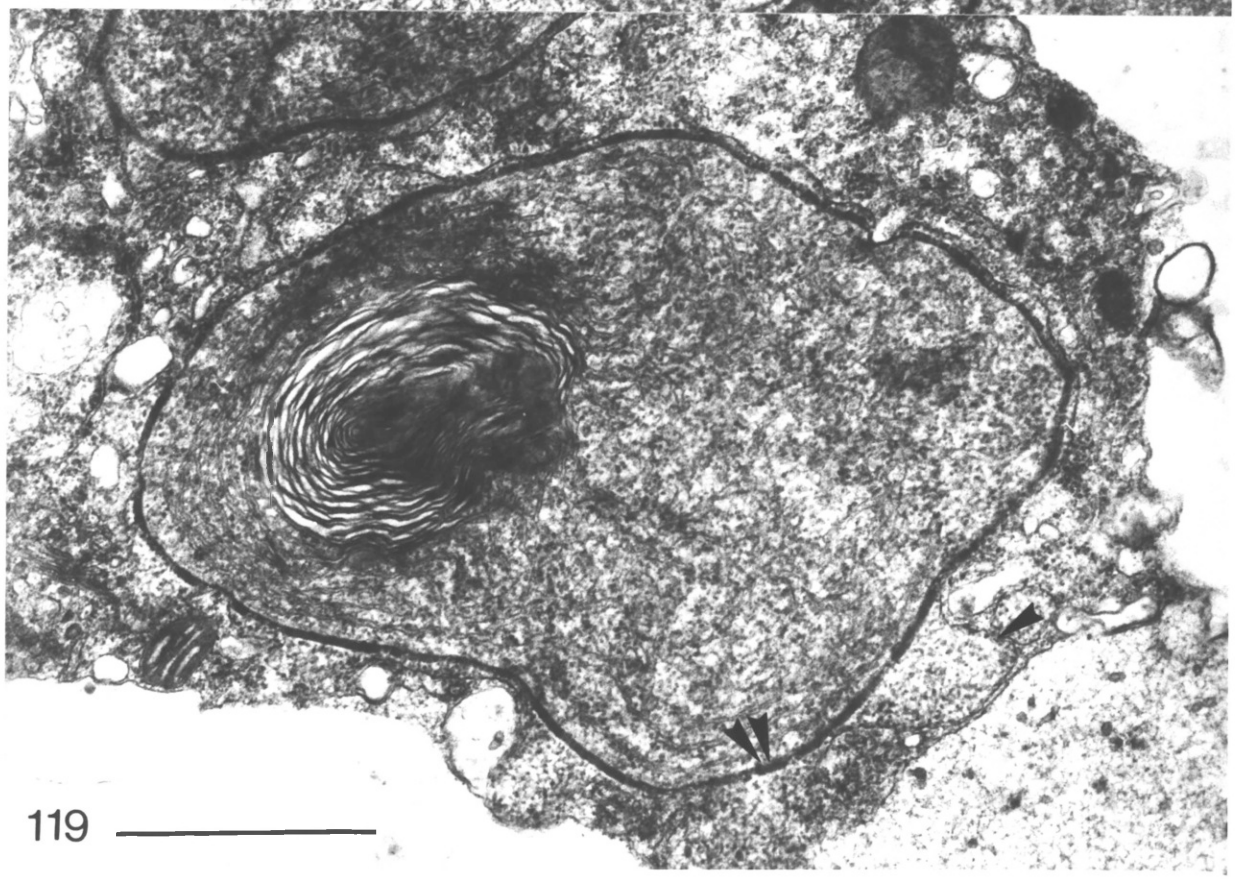


117

118, 119. Detail of 2 of the sporonts in Fig. 117. Electron-dense material is laid down patchily on the plasma membrane (indicated by double arrows). Large blisters are formed by the sporophorous veiscle membrane as it lifts away from the parasite surface (indicated by single arrows). Membranous whorls form (Fig.119).



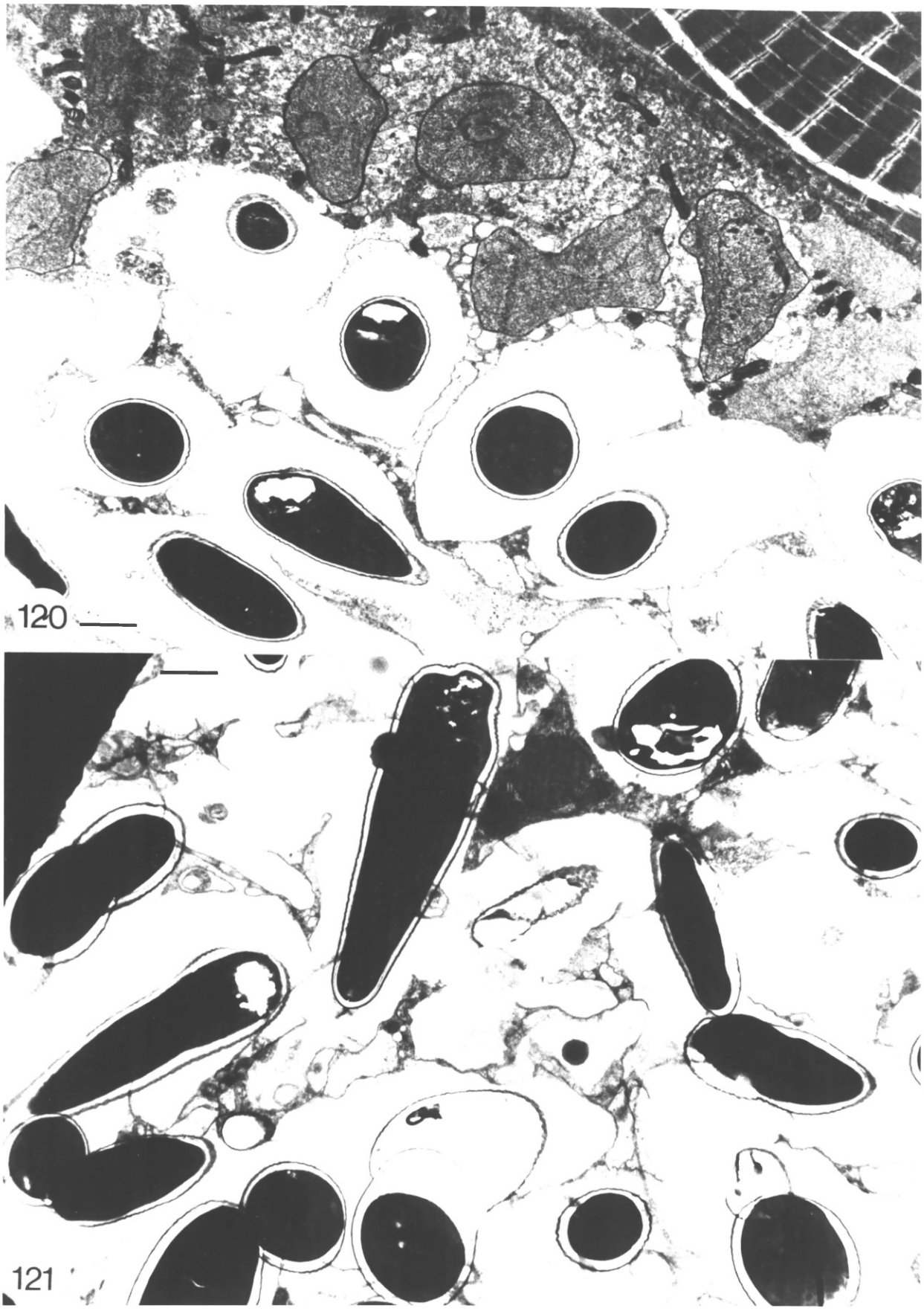
118



119

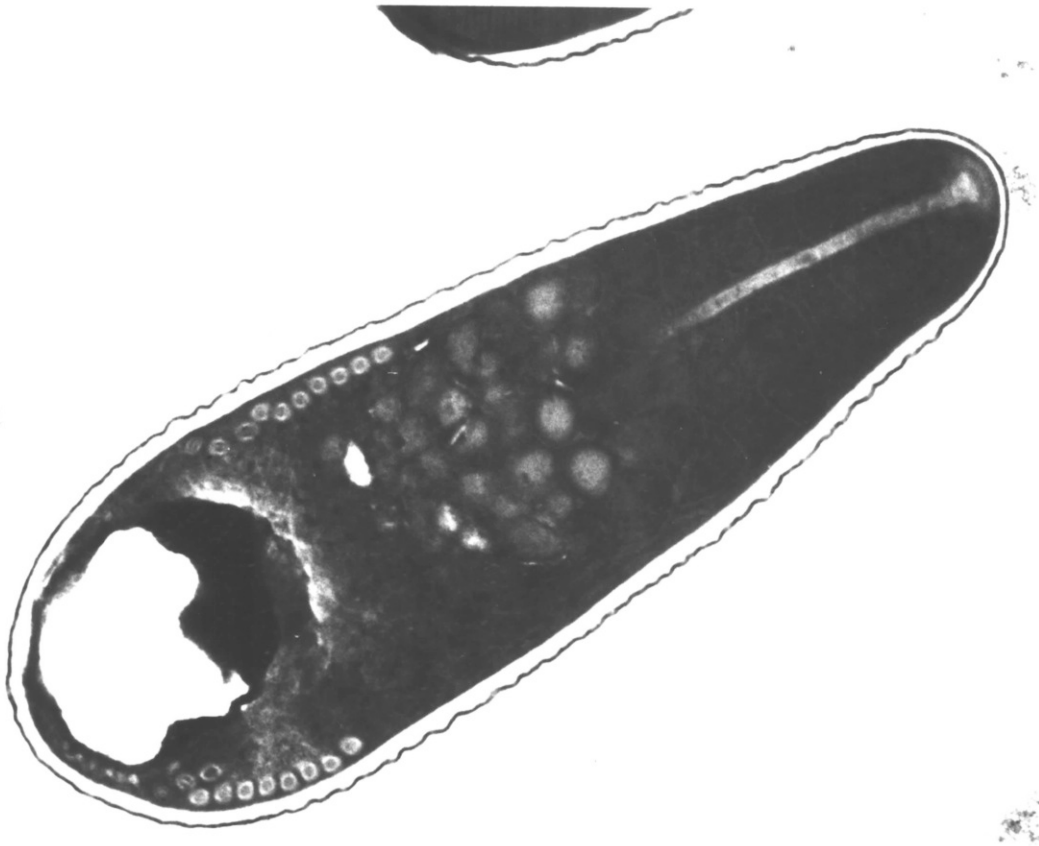
120. This section shows the relative position of the developmental stages of the parasite within the copepod. Spores are found centrally within the copepod surrounded by earlier developmental stages, and finally host muscle.

121. Pyriform spores. Each spore develops within a vesicle.

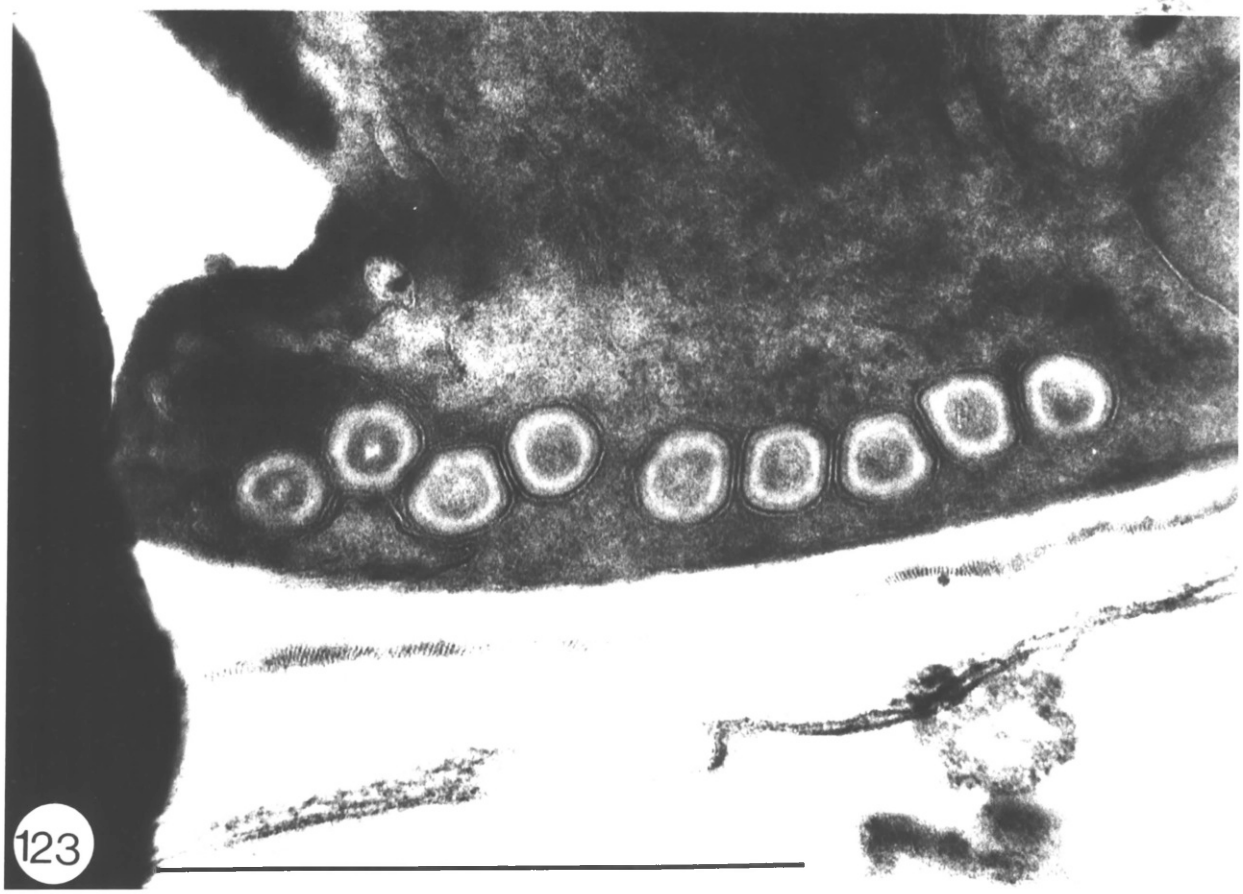

120
121

122. Mature spore. This spore is of the small type. The polar filament is isofilar with a maximum of 20 coils. The polaroplast consists of a series of blocks separated by membranes, the blocks becoming less electron-dense towards the posteriad.

123. Coils of the polar filament. The filament has 5 layers in cross-section.



122



123

3.4 ATTEMPTS TO TRANSMIT THE OCTOSPOROUS AND MULTISPOROUS MICROSPORIDIA FROM MOSQUITO LARVAE TO COPEPODS

General technique

A preliminary experiment was carried out in 1985, in which Mesocyclops rarus copepods from Kisiwani were held in 20ml of water with 1 disrupted Ma. africana larva from the same site infected with octospores. After 7 days, developmental stages of a microsporodium were found in 1 copepod out of 34 smeared. However, at that time the copepods from Kisiwani pond had not been surveyed for natural infection and no conclusions can be drawn on experimental transmission.

In subsequent experiments in 1987 Ma. africana or An. gambiae s.l. larvae infected with octosporous or multisporous infections were fed to 2 pots of copepods (1 disrupted larva per pot). A third pot was provided with an uninfected larva (also disrupted) which acted as a control.

In experiments with Ma. africana the copepods were collected from the same sites as the infected larvae. In experiments using the octospores from the progeny of adult female An. gambiae s.l. collected at Kumbamtoni, copepods from the Kwamihayo site were used. The original water-bodies where the infected adults had undergone their larval development were not known. Collections were made from 4 sites around the village but none of the larvae collected had microsporidian infections. Kumbamtoni was some distance from Muheza where the laboratories were based and was only visited once a week. For this reason it was necessary to collect copepods from a nearby site where larvae and copepods were known to be uninfected. Kwamihayo was chosen as this site. The copepods collected there were also identified as M. rarus.

The experimental regime was modified several times in an attempt to transmit the microsporidian parasites to copepods.

3.4.1 Experiment 1.

Mesocyclops rarus held in 50-75ml of tap water and provided with spores from dead infected mosquito larvae : live copepods examined every 7 days.

Tables 13, 14, 15, 16, 17 and 18 show the results of this experiment using octospores and multispores from each site. Infections were found at low levels on days 7 and 14 in infected pots and in controls. The majority of

TABLE 13: Mansonia africana octospores given to Mesocyclops rarus copepods (Kisiwani site)

No of days after experiments on 13.6.87	Pot 1 57 adults 18 copepodids No infected/ No examined	Pot 2 52 adults 20 copepodids No infected/ No examined	Pot 3 - Control 48 adults 13 copepodids No infected/ No examined
7	0/5	0/5	0/5
14	0/7	1/7	0/7
21	0/5	0/5	0/5
28	0/5	0/5	0/5
35	0/5	0/5	0/5

Those remaining were discarded

TABLE 14: Mansonia africana octospores given to Mesocyclops rarus copepods (Muheza Sisal Estate)

No of days after experiments on 7.7.87	Pot 1 86 adults 0 copepodids No infected/ No examined	Pot 2 95 adults 0 copepodids No infected/ No examined	Pot 3 - Control 55 adults 0 copepodids No infected/ No examined
7	2/5	0/5	0/5
14	0/5	0/5	0/5
21	0/6	0/5	0/5
All those remaining			
28	0/2	0/1	0/7
Total accounted for	18	16	22
% unaccounted for	79.1	83.2	60.0

TABLE 15: *Mansonia africana* multispores given to *Mesocyclops rarus* copepods (Muheza Sisal Estate)

No of days after initiation of experiment on 12.7.87	Pot 1 71 adults 0 copepodids Number infected/ Number examined	Pot 2 - Control 66 adults 0 copepodids Number infected/ Number examined
4	1/1	0/10
7	0/5	1/5
14	0/5	0/5
21	0/5	0/5
Total accounted for	16	15
% unaccounted for	77.5	77.3

TABLE 16: *Mansonia africana* octospores given to *Mesocyclops rarus* copepods (Mamakingi)

No of days after initiation of experiment on 8.7.87	Pot 1 59 adults 0 copepodids Number infected/ Number examined	Pot 2 59 adults 0 copepodids Number infected/ Number examined	Pot 3 - Control 62 adults 0 copepodids Number infected/ Number examined
7	0/5	0/5	1/5
14	1/5	0/5	0/5
21	0/6	0/6	0/5
All those remaining 28	0/0	0/0	0/16
Total accounted for	16	16	31
% unaccounted for	72.9	72.9	50.0

TABLE 17: Mansonia africana octospores given to Mesocyclops rarus copepods (Mamakingi)

No of days after initiation of experiment on 10.7.87	Pot 1 57 adults 0 copepodids Number infected/ Number examined	Pot 2 - Control 58 adults 0 copepodids Number infected/ Number examined
5	0/0	0/1
7	0/5	0/5
16	0/5	0/5
21	0/5	0/5
All those remaining 28	0/6	0/9
Total accounted for	21	23
% unaccounted for	63.2	60.3

TABLE 18: Anopheles gambiae s.l. octospores given to Mesocyclops rarus copepods (Kumbamtoni)

No of days after initiation of experiment on 12.7.87	Pot 1 71 adults 0 copepodids Number infected/ Number examined	Pot 2 - Control 66 adults 0 copepodids Number infected/ Number examined
7	0/5	0/5
14	0/5	0/5
21	0/5	0/5

Those remaining discarded

copepods in all pots disappeared during the course of the experiment. Even when copepods remaining at the conclusion of the experiment were smeared, there were still large numbers unaccounted for. In the infected pots a mean of 72% of adult copepods were lost and in control pots 59%.

As infections were only found in the first 14 days, experiment 2 was designed to sample copepods every day for a week.

3.4.2 Experiment 2.

Mesocyclops rarus from the Muheza Sisal Estate held in 50-75ml of tap water and provided with octospores from Mansonia africana larvae collected at the same site : live copepods examined daily

The results are shown in Table 19. As infections were detected in experimental and control pots at about the same levels in the first 4 days of examination, it was concluded that these infections were field derived. Again 33.6% of adult copepods in the infected pot and 14.6% of these in the control pot were not accounted for. In order to determine whether mortality was due to microsporidian infection Experiment 3 was designed.

3.4.3 Experiment 3.

Mesocyclops rarus held in 50-75ml of tap water and provided with spores from Mansonia africana and Anopheles gambiae s.l. : all dead copepods examined when found (daily)

The results are given in Tables 20, 21, 22, 23, 24 and 25. All dead copepods were examined daily. In addition occasional live copepods were sampled. The figures have been summed at 3-4 day intervals to enhance the data display.

Infections occurred mainly in the first 2 weeks of the experiments. No infections were found in experimental pots later than 24 days after initiation of the experiment and none were found in control copepods later than 18 days after initiation.

There were still large unaccountable losses of copepods in the pots, with the exception of one trial (Table 24), where more adults were examined than had been introduced originally, suggesting that copepodids had survived to adulthood.

TABLE 19: *Mansonia africana* octospores given to *Mesocyclops rarus* copepods (Muheza Sisal Estate)

No of days after initiation of experiment on 18.7.87	Pot 1 107 adults 11 copepodids Number infected/ Number examined	Pot 2 - Control 48 adults 5 copepodids Number infected/ Number examined
1	3/10	1/5
2	5/10	2/5
3	4/10	2/5
4	0/10	3/5
5	0/10	0/5
6	0/10	0/5
All those remaining 13	0/11	0/11
Total accounted for	71	41
% unaccounted for	33.6	14.56

TABLE 20: *Mansonia africana* octospores given to *Mesocyclops rarus* (Kisiwani)

No of days after initiation of experiment on 25.8.87	Pot 1 79 adults 92 copepodids Number infected/ Number examined	Pot 2 116 adults 58 copepodids Number infected/ Number examined	Pot 3 - Control 124 adults 86 copepodids Number infected/ Number examined
1-3	2/ 2	0/ 0	0/ 2
4-6	0/ 3	0/ 3	0/15
7-9	0/ 3	0/ 4	0/ 7
10-12	0/ 7	0/34	1/ 1
13-15	0/16	0/ 0	0/ 0
16-18	0/10	0/ 0	0/ 1
19-21	0/ 5	0/ 0	0/ 1
All those remaining 22-28	0/16	0/ 1	0/28
No accounted for	2/64	0/66	1/57
% unaccounted for	27.4	43.1	54.0

TABLE 21: *Mansonia africana* octospores given to *Mesocyclops rarus* (Muheza Sisal Estate)

No of days after initiation of experiment on 14.8.87	Pot 1 126 adults 25 copepodids Number infected/ Number examined	Pot 2 121 adults 24 copepodids Number infected/ Number examined	Pot 3 - Control 131 adults 29 copepodids Number infected/ Number examined
1-3	3+(1)/ 9+(5)	2/ 9+(3)	1/ 3+(5)
4-6	7/17	0/20	3/ 6
7-9	3/13	1/ 9	0/ 1
10-12	0/ 2	0/ 1	0/ 5
13-15	0/ 0	0/ 1	0/ 0
16-18	0/ 0	0/ 1	0/ 0
16-18	0/ 0	0/ 1	0/ 0
19-21	0/ 0	0/ 4	0/ 0
22-24	1/ 2	0/ 1	0/ 0
25-29	0/ 2	0/ 2	0/ 0
All those remaining 30	0+(1)/ 1+(3)	0/ 0	0/ 0+(16)
No accounted for	14+(2)/46+(8)	3/48+(3)	4/15+(21)
% unaccounted for	57.1	57.8	72.5

( )live copepods

TABLE 22: *Mansonia africana* multispores given to *Mesocyclops rarus* (Muheza Sisal Estate)

No of days after initiation of experiment on 28.8.87	Pot 1 61 adults 35 copepodids Number infected/ Number examined	Pot 2 60 adults 33 copepodids Number infected/ Number examined	Pot 3 - Control 51 adults 32 copepodids Number infected/ Number examined
1-3	1/ 3	0/ 0	0/ 1
4-6	0/ 3	0/ 0	0/ 0
7-9	0/ 0	0/ 1	0/ 0
10-12	0/ 0	0/ 1	0/ 0
13-15	0/ 0	0/ 0	0/ 1
16-18	0/ 1	0/ 0	0/ 0
All those remaining 19-29	0/ 0	0/35	0/44
No accounted for	1/ 9	0/37	0/46
% unaccounted for	86.9	38.3	9.8

TABLE 23: *Mansonia africana* octospores given to *Mesocyclops rarus* (Mamakingi)

No of days after initiation of experiment on 18.8.87	Pot 1 165 adults 55 copepodids Number infected/ Number examined	Pot 2 159 adults 59 copepodids Number infected/ Number examined	Pot 3 - Control 154 adults 47 copepodids Number infected/ Number examined
0-3	0/ 2	1/ 8	0/ 6
4-6	4/ 6	4/ 5	2/ 4
7-9	3/ 9	4/ 7	1/ 3
10-12	0/ 3	0/ 1	0/ 0
13-15	0/ 1	0/ 0	0/ 0
16-18	0/ 1	0/ 0	1/ 1
19-21	0/ 5	0/ 0	0/ 0
22-24	0/ 1	0/ 4	0/ 2
All those remaining 25-30	0/ 3	0/ 4	0/26
No accounted for	7/31	9/25	4/42
% accounted for	81.2	84.3	72.7

TABLE 24: *Mansonia africana* octospores given to *Mesocyclops rarus* (Mamakingi)

No of days after initiation of experiment on 21.8.87	Pot 1 112 adults 48 copepodids Number infected/ Number examined	Pot 2 118 adults 46 copepodids Number infected/ Number examined	Pot 3 - Control 95 adults 34 copepodids Number infected/ Number examined
0-3	7/ 8	9/13	1/ 4
4-6	5/ 7	2/29	0/ 0
7-9	7/64	4/19	2/ 3
10-12	0/ 3	0/10	0/ 2
13-15	0/ 0	0/ 0	0/ 1
16-18	0/ 0	1/ 2	0/ 8
19-21	0/ 0	0/ 0	0/ 3
All those remaining 22-28	1/18	0/49	0/40
No accounted for	18/100	16/122	1/61
% unaccounted for	10.7	-3.4	35.8

TABLE 25: *Mansonia africana* octospores given to *Mesocyclops rarus* (Mamakingi)

No of days after initiation of experiment on 9.8.87	Pot 1 90 adults 9 copepodids Number infected/ Number examined	Pot 2 88 adults 2 copepodids Number infected/ Number examined	Pot 3 - Control 71 adults 2 copepodids Number infected/ Number examined
1-3	0/ 3	0/ 1	0/ 0
4-6	0/ 2	0/ 1	0/ 0
7-9	0/ 1+(7)	0/ 0+(7)	0/ 0+(7)
10-12	0/ 2	0/ 4	0/ 0
13-15	0/ 2	0/ 3	0/ 0
16-18	0/ 4	0/ 4	0/ 0
19-21	0/ 3	0/ 2	0/ 0
22-24	0/ 2	0/ 2	0/ 2
25-27	0/ 1	0/ 0	0/ 3
All those remaining 28-30	0/23	0/ 0	0/50
No accounted for	0/43+(7)	0/17+(7)	0/55+(7)
% accounted for	44.4	72.7	12.7

( ) Live copepods

One moribund copepod was seen to be fed upon by a live copepod. Experiment 4 was designed to sample dead and live copepods to detect developing infections before the host could be ingested by other copepods. Holding each copepod separately in a small volume of water would have been the best way to inhibit cannibalism, but this was not possible under field conditions, where insufficient containers were available.

To reduce cannibalism and to minimise overcrowding effects, the holding volume was increased to 250-300ml per pot.

3.4.4 Experiment 4.

Mesocyclops rarus held in 250-300ml of tap water and provided with octospores from Mansonia africana : dead copepods examined daily when found and live copepods examined regularly

The results are given in Tables 26, 27 and 28. Again, infections were found in the first 2 weeks, none being found in any pot after day 12. There was still a large percentage of copepods that were unaccounted for, even with the increased holding volume. On return to Britain it was possible to carry out Experiment 5, using isolated copepods.

3.4.5 Experiment 5.

Mesocyclops rarus from Kisiwani site held individually in the wells of an ELISA plate and provided with octospores from Mansonia africana : individuals examined on death

M. rarus from Kisiwani were transported back to Britain. These copepods were maintained in culture for 10 weeks before the cultures died out.

The water level within each well fell slightly each day but was topped up using a dilute solution of chlorella and paramecium from cultures to provide nourishment for the copepods.

The ELISA plate with octospores (1 Pasteur pipette drop of spores produced from homogenising one infected larva in 2 ml of water per well) had 12 copepods, none of which became infected.

The control plate (1 drop of homogenised healthy larva in 2ml of water per well) had 10 copepods, none of which became infected.

TABLE 26: *Mansonia africana* octospores given to *Mesocyclops rarus* (Kisiwani)

No of days after initiation of experiment on 2.9.87	Pot 1 97 adults 51 copepodids Number infected/ Number examined	Pot 2 91 adults 57 copepodids Number infected/ Number examined	Pot 3 - Control 88 adults 50 copepodids Number infected/ Number examined
1-3	0/ 0	0/ 1	1/ 2
4-6	0/ 0	0/ 1	1/ 0
7-9	0/ 3+(5)	2/ 4+(5)	0/ 4+(5)
10-12	0/ 3+(5)	0/ 7+(5)	0/ 4+(5)
13-15	0/13	0/ 2+(5)	0/ 9+(5)
16018	0/ 0	0/ 8+(5)	0/ 0+(5)
19-21	0/ 0	0/ 5+(10)	0/ 2+(10)
All those remaining 25	0/ 0+(21)	0/ 0+(16)	0/ 0+(23)
No accounted for	0/19+(31)	2/28+(46)	2/22+(53)
% unaccounted for	48.4	19.8	14.8

( ) live copepods

TABLE 27: *Mansonia africana* octospores given to *Mesocyclops rarus* (Kisiwani)

No of days after initiation of experiment on 6.9.87	Pot 1 95 adults 26 copepodids Number infected/ Number examined	Pot 2 95 adults 26 copepodids Number infected/ Number examined	Pot 3 - Control 95 adults 26 copepodids Number infected/ Number examined
1-3	0/ 1	0/ 1	0/ 0
4-6	0/ 4	0/ 6	0/ 0
7-9	0/ 8+(5)	0/ 4+(5)	0/ 9+(5)
10-12	1+(1)/ 6+(5)	0/ 3+(5)	0/ 2+(5)
13-15	0/ 3	0/ 2+(5)	0/ 1+(5)
16-18	0/ 0	0/ 0	0/ 2
All those remaining 19	0/ 0+(6)	0/ 0+(11)	0/ 0+(24)
No accounted for	1+(1)/22+(16)	0/16+(26)	0/14+(39)
% unaccounted for	60.0	55.8	44.2

( ) live copepods

TABLE 28: Mansonia africana octospores given to Mesocyclops rarus (Mamakingi)

No of days after initiation of experiment on 4.9.87	Pot 1 95 adults 21 copepodids Number infected/ Number examined	Pot 2 94 adults 31 copepodids Number infected/ Number examined	Pot 3 - Control 96 adults 28 copepodids Number infected/ Number examined
1-3	2/ 4	0/ 6	2/ 5
4-6	5/ 9	6/19	4/ 5
7-9	0/ 4+(5)	0+(1)/ 4+(5)	1/ 3+(5)
10-12	0+(1)/ 0+(5)	1/ 4+(5)	1+(1)/ 1+(5)
13-15	0/ 1	0/ 0+(5)	0/ 2+(5)
16-18	0/ 1	0/ 3+(5)	0/ 2+(5)
All those remaining 19-21	0/ 0+(5)	0/ 0+(18)	0/ 0+(35)
No accounted for	7+(1)/19+(15)	7+(1)/36+(38)	8+(1)/18+(55)
% unaccounted for	62.2	21.3	24.0

( ) live copepods

3.4.6 Experiment 6

British copepods held in 50ml of distilled water and provided with octospores from Mansonia africana : copepods examined after 7 days

After 7 days all copepods were smeared and examined. None had become infected.

3.5 ATTEMPT TO TRANSMIT OCTOSPORES DIRECTLY BETWEEN MOSQUITOES OF THE SAME SPECIES

3.5.1 Using octospores derived from the progeny of infected adult Anopheles gambiae s.l. from Kumbamtoni

Out of 176, 4th instar larvae and pupae of An. gambiae s.l. which had been given disrupted An. gambiae s.l. larvae infected with octospores at 1st instar, no infections were detected. Control larvae fed only on uninfected disrupted larvae did not become infected. No transmission was effected.

3.5.2 Using octospores derived from Mansonia africana larvae (Muheza Sisal Estate)

Of the 100 larvae of Ma. africana held with octospores from Ma. africana for 30 min (short hold) before transfer to larval bowls, only 1 at 2nd instar, survived and this one was not infected. None of the larvae held with octospores for 12 hours (long hold) before transfer to larval bowls survived.

On return to Britain, the trial was repeated with controls, using 1st instar larvae from 2 egg batches in each bowl. In the first bowl these larvae were held for 30 min (short hold) with octospores and none out of 58 were found infected after 21 days. None out of 42 in the control bowl (held with a disrupted uninfected larva for 30 min before transfer to the larval bowl) was infected either. No transmission was effected.

3.6 ATTEMPTED TRANSMISSION OF SPORES FROM COPEPODS TO Mansonia africana LARVAE

Of the 1st instar Ma africana larvae that were held with a mixture of 50 live and 50 dead copepods for 30 min and then transferred to larval bowls to develop for 21 days, none survived. On return to Britain there were insufficient copepods to repeat the experiment. A colony was set up, but unfortunately the numbers fell and the colony finally died out.

CHAPTER 4

DISCUSSION

4.1 TAXONOMIC POSITION OF MICROSPORIDIAN INFECTION OF BRITISH MOSQUITOES USING MORPHOLOGICAL FEATURES

4.1.1 Octosporous infections of Aedes cantans/Aedes punctor and Aedes detritus

The family Thelohaniidae Hazard and Oldacre, 1975 contains 10 genera, represented by species that, after 3 sporogonic divisions give rise to 8 uninucleate spores (often referred to as octospores), enclosed in a sporophorous vesicle. Dividing sporonts secrete metabolic products which are deposited in the sporophorous vesicle and often give the superficial appearance of supernumary nuclei. Octospores usually produce gross symptoms and mortality in larvae. All species are common parasites of aquatic and semi-aquatic invertebrate animals. The infections in Aedes sp. and Ae. detritus from Englemere and Smithfield respectively developed into uninucleate octospores within sporophorous vesicles and on these characters can be assigned to the family Thelohaniidae. In the parasite from Englemere, metabolic products were seen in the sporophorous vesicles of late sporogonic stages.

Characteristics allowing new species to be assigned to genera require detailed study using electron microscopy. The Smithfield and Englemere parasites were studied by light microscopy and therefore cannot be assigned definitively to genera. Features of sporogonic division such as division by binary fission or division of an octonucleate plasmodium, morphology of spores and nature of the polar filament (isofilar or anisofilar) are all important. The genera Amblyospora Hazard and Oldacre, 1976 and Parathelohania Codreanu, 1966 are the commonest of the octosporous genera to be found in mosquitoes. Amblyospora, which has oval- or barrel-shaped spores with no surface ornamentation, is more prevalent in culicine mosquitoes. The microsporidia from Englemere and Smithfield had oval spores and it is likely that both belong to the genus Amblyospora.

Most mosquito larvae infected with octospores in the fat body show gross pathological signs, the fat body throughout the thorax and abdomen being packed with masses of spores. They appear swollen, chalky white and lethargic. This was not the case with either of the British infections. In part this may have been because the larvae had a naturally high level of pigment in their

chitin, disguising the level of infection within. Alternatively, the infections may have been limited to sites other than the fat body.

Octosporous infections have been reported, though not described in detail, from Ae. cantans by Weiser,(1961), Ae. punctor by Chapman et al. (1973) and Ae. detritus by Weiser, (1961) and Tour et al. (1971), and have been listed as Amblyospora spp. by Hazard and Oldacre, (1975). In more recent work Amblyospora opacita has been identified in Ae. cantans, Ae. punctor and Ae. detritus by Kilochitskii and Sheremet, (1978) and Dubitskii, (1978) and Parathelohania obesa has been identified in Ae. cantans by Kilochitskii and Sheremet (1978). The size range for spores of A. opacita overlap with those for the octosporous infection at Smithfield in Ae. detritus and it is possible that they are the same infection. P. obesa spores are significantly smaller than either the infection at Smithfield or Englemere (Sprague, 1977).

4.1.2 Multisporous infection of Culiseta annulata (Sandyride)

For many years microsporidia producing a variable number of sporoblasts from a sporont within a sporophorous vesicle were all included in the genus Pleistophora, Gurley 1893. Pleistophora typicalis Gurley, 1893 from the marine fish Myoxocephalus scorpius (L) was named as the type species but at that time was only known at the light microscope level. Its ultrastructure was later described by Canning and Nicholas (1980). It was the detailed studies of this species, and others, which showed that many of the species provisionally assigned to Pleistophora would be better placed in new genera. There are now 8 other genera that undergo multisporous sporogony within a sporophorous vesicle producing variable numbers of spores. These are Glugea Thelohan, 1891 redescribed by Canning et al. (1982), Pseudopleistophora Sprague, 1977, Vavraia Weiser, 1977 redescribed by Canning and Hazard (1982), Baculea Loubès and Akbarieh, 1978, Polydispyrenia Canning and Hazard, 1982, Cystosporogenes Canning et al. 1985, Ovavesicula Andreadis and Hanula, 1987 and Endoreticulatus Brooks et al. 1988.

Unfortunately merogonic stages were not found in the ultrastructural study of the parasite from Sandyride, making it difficult to assign it to a genus. Sporogonic characters can be used to exclude it from several of the defined multisporous genera. The taxonomic features of the Sandyride parasite are:

1) Sporophorous vesicle is a fine membrane, to which host ribosomes become closely applied and which ultimately becomes enclosed by a second membrane of presumed host origin.
2) Sporonts have isolated nuclei.
3) Sporoblasts are formed by repeated cytoplasmic divisions of the sporont, while nuclear divisions continue, so that multinucleate plasmodia are never formed.

Using these features it is possible to exclude this species from the genera Pleistophora and Vavraia as they have thick amorphous layers at their surface during merogony which becomes a thick tough sporophorous vesicle around the sporonts (Canning and Nicholas, 1980; Canning and Hazard, 1982). This layer was not present in the Sandyride species. Glugea can be discounted as all stages develop within a xenoma, and Pseudopleistophora because all stages of sporogony are diplokaryotic (Canning et al., 1982; Sprague, 1977). Polydispyrenia can also be excluded as early sporonts are diplokaryotic and the sporophorous vesicle is formed as a single fine layer of parasite origin (Canning and Hazard, 1982).

Ovavesicula has a thick electron dense sporophorous vesicle of parasite origin in a definite oval shape (sporophorous vesicles of other genera are spherical) within which 32 spores are regularly formed. The parasite from Sandyride did not have this form (Andreadis and Hanula, 1987). Cystosporogenes can be eliminated as, although it has a surrounding membrane at all stages, this is of enigmatic origin and is never studded with ribosomes as the parasite found at Sandyride (Canning et al., 1985).

Endoreticulatus is uninucleate throughout its development. Cytoplasmic division of the sporont involves the formation of a multinucleate plasmodium which was not seen in the Sandyride species. In addition sporonts developed entirely within vacuoles of the host endoplasmic reticulum. There was no sporophorous vesicle of parasite origin as was observed in the Sandyride species (Brooks et al., 1988). The genus Baculea can also be excluded as the spores are characteristicaly elongate, quite unlike those of the species from Sandyride (Loubès and Akbarieh, 1978).

Although the Sandyride species has similarities to several of the genera from the Pleistophora complex, it does not clearly fall into any of them.

There are also two species assigned to the collective genus Microsporidium, which undergo multisporous sporogony. These are M. itiiti Malone (1985) from the Argentine stem weevil Listronotus bonariensis and M. novacasteriensis Jones and Selman (1985) from the grey field slug Deroceras reticulatum. In M. itiiti and M. novacastriensis the sporont develops within a membrane which was interpreted as host endoplasmic reticulum. Strictly speaking this is not a sporophorous vesicle because it is not secreted by the parasite. The species in C. annulata from Sandyride also has a host component to the envelope around the sporogonic stages but this was laid down outside the fine sporophorous vesicle of parasite origin. For this reason, and because the Microsporidium spp. had ribbon-like sporonts the species in C. annulata cannot belong to the same genus.

Finally, there are 3 enigmatic Pleistophora spp. not yet placed in a genus, one from the larch sawfly Pristiphora erichsonii (Percy and Wilson, 1982), one from the mosquito Aedes sierrensis (Sanders and Poinar, 1978) and one from the lawn grass cutworm Spodoptera depravata (Iwano and Ishihara, 1984). In the first 2 the sporont divides sequentially to produce sporoblasts. However the species from Ae. sierrensis has a sporophorous vesicle of parasite origin only and the vesicle of the species from P. erichsonii is formed entirely from host endoplasmic reticulum, with no inner membrane of parasite origin. In the 3rd species the sporont also divided sequentially, but the sporophorous vesicle was of unknown origin. Clearly these 3 species cannot belong to the same genus as the Sandyride species.

The features of the species from C. annulata at Sandyride - the sporophorous vesicle of parasite origin surrounded by a second membrane presumed to be of host origin, and sequential sporogony, effectively remove it from all the genera of the Pleistophora complex. They also show that it is not closely related to the 3 enigmatic Pleistophora spp. or the 2 Microsporidium spp.. It is therefore likely that a new genus will have to be established for this species.

4.1.3 Multisporous infection of Culiseta annulata (Swinley)

This species is also a member of the Pleistophora complex but as only light microscopic details were seen it is difficult to assign it to one particular genus. On light microscopic characteristics alone it can be excluded from Ovavesicula as the sporophorous vesicles are not a regular shape

with 32 spores. Baculea can also be excluded as the spores of the Swinley species are not elongate. Details of nuclear arrangement, cytoplasmic division and origin of the sporophorous vesicle cannot be determined from light microscopy alone, making it impossible to assign it to any one of the remaining 6 genera, or to the enigmatic Pleistophora spp. (Percy and Wilson, 1982; Sanders and Poinar, 1978; Iwano and Ishihara, 1984) or Microsporidium itiiti Malone, 1984 and M. novacastriensis Jones and Selman, 1985.

4.2 LEVELS OF MICROSPORIDIAN INFECTION IN BRITISH MOSQUITOES

In natural populations of insects there are always cycles of abundance and decline and microsporidian infections contribute to these cycles (Anderson and May, 1980) though are often only present at low levels. The levels of microsporidia found at all the sites visited in this study however, were very low, possible reasons for which are discussed in this section.

Microsporidia parasitising mosquitoes are transmitted either when spores are released into water and are ingested by larvae, or by predation. If the species is transmitted directly the cadaver of a heavily infected larva sinks to the bottom where the spores are released into the water. Being relatively heavy the spore would not become resuspended in the water unless there was some turbulence. Infections are initiated when larvae ingest spores when filter feeding, or when browsing on organisms growing on detritus as some species are known to do. A mucous substance is produced on the mouthbrushes of mosquitoes (Merritt and Craig, 1987) which enables them to capture even the smallest particles including the microsporidian spores. Ingestion of spores would be enhanced in shallow water where the mouthbrushes remain near the detritus at the bottom. In deeper water larvae may only come into contact with the bottom of the pond when disturbed or during browsing.

Redistribution of spores in the water could be effected by disturbance of the sediment by fish or invertebrates or by ingestion of moribund or dead infected larvae by predators, if the spores are passed unchanged in faeces. Many spores will be lost as the sediment continually accumulates at the bottom. In field trials using Vavraia culicis against Culex quinquefasciatus (Reynolds 1972) and Nosema algerae against Anopheles albimanus (Anthony et al. 1978) high levels of infection were found initially but quickly fell away due to sedimentation of spores. Sedimentation is the main reason why spores are difficult to recycle in a population if introduced as a control agent.

The dimorphic microsporidia of the genera Amblyospora and Parathelohania have not been transmitted directly within the same species. An alternate (copepod) host has been demonstrated for 4 Amblyospora spp. parasitising mosquitoes and it is likely that alternate hosts will be found for other dimporphic species, although they may not be copepods.

Live infected larvae or cadavers may be actively ingested by detritivorous or predaceous copepods. Indeed, some species of copepod including Mesocyclops aspericornis are known to be actively predacious on mosquito larvae, having biological control implications in their own right (Riviere and Thirel, 1981). Alternatively, spores may be filtered from the water column by filter feeding species of copepod. It is believed that the necessity of another host would increase the likelihood of recycling of the microsporidian within a population especially when the principal host is not present at a site throughout the year (Andreadis, 1988c).

In Britain many mosquitoes are seasonal as an adaption to the temperate climate. They can overwinter as adults, larvae, or eggs depending on whether the breeding grounds are temporary or permanent. Culiseta annulata is one of the few species that can be found throughout the year in all its developmental stages. There would be fewer difficulties in transmission of microsporidia when larvae are present throughout the year and this may have been one of the factors which resulted in the prevalence of the multisporous species in Cs. annulata found at Sandyride being higher (17.8%) than the prevalences in other mosquito species at all the sites visited.

Larvae picking up low levels of infection may emerge as adults and produce progeny, a percentage of which may also be infected transovarially or by contamination of the eggs (Hulls, 1972). This would enable the infection to be introduced into new sites. There is no evidence for this from this study as details of transmission in this species are not known. However, any adult females entering diapause would be more likely to die if infected with microsporidia (Metspalu and Khiisaar, 1984). The site at Swinley was similar to Sandyride though the pools were of a more temporary nature perhaps accounting for the lower level of infection (2.6%).

The breeding grounds of the Ae. cantans and Ae. punctor populations at Englemere and the Ae. detritus and Ae. flavescens populations at Smithfield, where low levels of Amblyospora-type infection were found were not permanent sites. At Englemere the breeding grounds were water-filled woodland hollows

and ditches, present only during late winter and spring. In this time the eggs of both mosquito species must hatch, develop and emerge as adults, as the water level slowly decreases and finally dries up. In this short season octosporous infections developing in larvae must be transmitted to alternate hosts which in turn must transmit the infection to healthy larvae. These larvae develop to adulthood, laying infected eggs for the next season. As not all eggs hatch simultaneously there will be susceptible larvae of different instars available throughout the short season. Even so, the transmission cycle that must occur for the survival of the parasite is somewhat forshortened.

It is also necessary for the alternate host (perhaps a copepod) to pass through its development in the short season. Some copepods (Microcyclops bicolor, Mesocyclops leuckarti and Canthocamptus staphylinus) are known to encyst under drought conditions (Fryer and Smyly, 1954) and it is possible that microsporidian infections can survive drought conditions by being harboured in encysted individuals.

The microsporidian infections could only survive over the period when pools and ditches were dry as stages in mosquito eggs or encysted alternate hosts. Free spores would not survive if desiccated. Microsporidian spores are sensitive to heat and ultraviolet light, both of which reduce the infectivity of spores (Henry and Oma, 1974; Kaya, 1975; 1977; Sikorowski and Lashomb, 1977; Eggers Teetor and Kramer, 1977).

Mosquito eggs are well adapted to severe conditions but when subjected to extremes of cold and heat and to ultraviolet light especially if near the soil surface, unsheltered by leaf debris suffer mortalities. The long periods that mosquitoes in temperate countries pass as eggs delays the onset of the prolific sporogonic divisions of the microsporidia. These occur in the larvae in preparation for horizontal transmission, and the longer the microsporidia remain in the mosquito eggs, the more likely they are to be eaten by predators or suffer damage (James, 1966). The survival of infections in eggs or encysted copepods enables the parasite to remain though at very low prevalence at a site, rather than being lost altogether.

As water levels decrease mosquito larvae become crowded, and may suffer from intra- and inter-specific competition. The result of such competition is a general reduction in size of the emerging adults, the size of their bloodmeals, their fucundity and a reduction in the number of males emerging. This has been reported for both anopheline and culicine mosquitoes by (Terzian and

Stahler, 1949; Wada, 1965; Reisen, 1975; Rajagopalan et al., 1976; Siddiqui et al., 1976). Mogi (1978) showed that in Culex tritaeniorhynchus and Anopheles sinensis competition also led to cannabalism of small larvae by older instars. Infections (of whatever type) within larvae cannibalised in this way would be lost. Mosquitoes have also been known to produce their own toxic or growth retarding hydrocarbons when crowded, reducing the populations (Moore and Fisher, 1969; Moore and Whitacre, 1972; Ikeshoji and Muller, 1974a, 1974b). This might selectively act against the infected segment of the population.

At Smithfield similar effects may contibute to low levels of infection. Additionally, the water is brackish and becomes highly saline (up to 133% sea water) as water evaporates free microsporidian spores may not be able to survive although the mosquito is adapted to these conditions.

Other than ponds and lakes water remains throughout the year in tree hole sites. In England tree holes are the habitats of Aedes geniculatus and Anopheles plumbeus and it was surprising that no microsporidian infections were found in the sites examined (Appendix I Table A). This may be due to the fact that the water is often of very variable pH (Keilin, 1932) which may not be suitable for microsporidia. Acid conditions have been found to enhance transmission in the crayfish Orconectes virilis, thought to be due either to elevated rates of crayfish cannibalism, decreased host resistance when under sublethal stress or to a low pH favoured microsporidian lifecycle (France and Graham, 1985). In addition the water in tree holes can freeze over and neither eggs nor larvae can withstand freezing (Shute, 1929).

Blackflies are also common hosts of dimorphic microsporidia, but again usually at low prevalence. Mosquitoes always breed in still water, in comparison with blackflies which always breed in rivers, often in very fast flowing, turbulent water. Larvae, heavily infected with microsporidia would release spores into a vast volume of water and, as many thousands of litres of water may pass daily through a site, the spores would become increasingly diluted downstream. For transmission to occur, whether directly to another blackfly larva or to an alternate host, these spores must be ingested before they are lost to slower moving regions of water downstream.

It is unlikely that copepods will be the alternate hosts of blackfly microsporidia. Few species of copepods are ever found in fast flowing water, or even in the top layer of the river bed (the niche of benthic species). Copepods are not well adapted to flowing water, only being found in still

regions and pools at the edges of rivers (Harding and Smith, 1974). The alternate hosts are likely to be organisms adapted to fast flowing water and able to filter out and thus concentrate spores from the large volumes of water. Net-spinning caddis flies are a possibility, though they are not closely related to the Copepoda. The alternate hosts would have to occupy the same niche so the infection developing within them can subsequently be easily transmitted back to healthy blackfly larvae. Caddis fly larvae are the major predators of blackflies (Bay, 1974; Service and Elouard, 1980), and have been found to harbour microsporidian infections that resemble those found in copepods (Xie and Canning, 1986). This infection in Hydropsyche siltalai was named as Pyrotheca hydropsycheae. The spores bore the same resemblance to those in copepods, but were more elongate and found in groups of 4.

Another possibility is that the infected blackfly larva may remain attached to weeds for some time after death, during which time they may be ingested by a detritivorous organism which could act as an alternate host.

Whatever the alternate host, it is clear that there must still be large losses of spores in the flowing water, accounting for the overall low levels of infection.

In conclusion it would seem that, although both mosquitoes and microsporidia must be adapted to the harsh and seasonal conditions in Britain, many factors are involved which together result in generally low prevalences. Permanent as opposed to temporary sites seem to favour higher infection levels.

4.3 TAXONOMIC POSITION OF MICROSPORIDIAN INFECTIONS OF TANZANIAN MOSQUITOES AND COPEPODS USING MORPHOLOGICAL FEATURES

4.3.1 Octosporous infection of Culex sp. (Pangani)

For the reasons discussed in Section 4.1.1, it is likely that the infection in the Culex sp. from Pangani is a species of Amblyospora even though no stages other than octospores were found. The infected mosquitoes were all pupae, and the level of infection within each individual was low, suggesting that the pupae would survive to adulthood.

4.3.2 Octosporous infection in Anopheles gambiae s.l. (Mombo)

Features of the species found in Anopheles gambiae s.l. made it easy to place it in the genus Parathelohania. Apart from the general features of the

family Thelohaniidae, the spores were ornamented with a "bottleneck" protuberance at the posterior end. Parathelohania spp. are also almost exclusively parasites of anopheline mosquitoes. Transmission electron microscopy showed that the polar filament was anisofilar with 3 pairs of small posterior coils and 2 pairs of large anterior coils. In conjunction with the size of the spores this parasite was identified as Parathelohania africana Hazard and Anthony (1974).

4.3.3 Octosporous infection in Mansonia africana

The parasite of Ma. africana was more difficult to place. It had closest affinities with the genera Thelohania, Amblyospora and Parathelohania. The genus Thelohania is inadequately defined according to current criteria, as the type species, T. giardi Henneguy (1892), a parasite of the shrimp Crangon vulgaris was described photomicroscopically at a time when the finer ultrastructural differences separating the octosporous genera were unknown (Henneguy and Thelohan, 1892). For this reason some species attributed to the genus Thelohania may be better placed in other genera, Amblyospora and Parathelohania for example. Johnson et al. (1978), called attention to the early descriptions of T. giardi in which sporogony was reported as a series of 3 binary fissions. They themselves observed the same type of sporogony in another species, Thelohania butleri in another shrimp, Pandulus jordani, and suggested that this might be an important diagnostic character of the genus. If the genus Thelohania has no multinucleate sporogonial plasmodium, the octosporous species from Ma. africana cannot be ascribed to it. In addition, a feature of Thelohania as described by Hazard and Oldacre (1975), was the isofilar polar filament. This is also dissimilar to the species found in Ma. africana.

Amblyospora and Parathelohania are genera which are dimorphic in their mosquito hosts, one sporogonial sequence giving rise in adult females to free binucleate spores which are responsible for transovarial tranmission to the next generation of larvae. The other, an octosporous sporogony, gives rise to 8 spores in a sporophorous vesicle. The type species were designated by Hazard and Oldacre (1975), as A. californica Kellen and Lipa (1969) and P. legeri Hesse (1904). Species known up to 1977 are listed by Sprague (1977). Since then further species have been described or recorded (Larsson, 1981; Sabwa et al., 1984; Toquebaye and Marchand, 1985; Vávra et al., 1984; Weiser and Ibrahim, 1985). Only a few papers have described the sequence resulting

in binucleate spores in adult females (Hazard and Oldacre, 1976; Andreadis and Hall, 1979a,b; Hall and Washino, 1986). More recent papers have shown in 4 Amblyospora spp., that there is a further developmental sequence in an alternate copepod host (Andreadis, 1985; 1988; Sweeney et al., 1985; 1988; Becnel, 1988; Dickson, 1988), making the genus polymorphic. It is likely that alternate hosts are also required for transmission of Parathelohania spp. Work by Avery (1989) showed that spores collected from pond water and presumed to have developed in an alternate host, when ingested by Anopheles quadrimaculatus gave rise to benign diplokaryotic infections and uninculeate octospores.

Amblyospora and Parathelohania are distinguished by the structure of their octospores. In both genera the polar filament is anisofilar and the endospore layer of the spore wall has lucent and moderately electron dense layers within the exospore coat. Octospores of Amblyospora are oval, broadly rounded at their ends but appear truncate, barrel-shaped, when fixed. Those of Parathelohania are constricted and ridged at the posterior end where the lucent endospore layer is greatly thickened. At the anterior end the endospore is thinned so that the polar cap is only just separated from the exospore and the wall is raised as a rim which appears as 2 ridges in section. When viewed by phase contrast microscopy there is a characteristic internal bottle-shaped appearance.

The octospores of the microsporidium from Ma. africana more closely resemble the spores of Parathelohania than Amblyospora, in having a distinct anterior rim involving all layers of the spore wall and in having a posterior constriction. However, the posterior constriction is in the form of a simple knob, not visible by light or scanning electron microscopy, in contrast to the well developed ridged, constricted region visible by light, scanning and transmission electron microscopy in Parathelohania. For this reason and because Parathelohania is typically a parasite of anopheline mosquitoes, this parasite is thought to belong to a new genus. Other octosporous infections reported from Mansonia spp. (Hazard and Oldacre, 1975) have not been described and cannot be compared with the present species.

Although diplokaryotic meronts were found in benign infections in Ma. africana larvae and adults, it has not been possible to link these directly with octosporous infections in the next generation of larvae as all surviving progeny of adult female Ma. africana infected with diplokaryotic stages, developed multisporous, not octosporous infections.

4.3.4 Multisporous infection of Mansonia africana

As with the multisporous infection at Sandyride, this species is also one of the Pleistophora complex. Within this complex there are 9 genera. Pleistophora Gurley 1893, Glugea Thelohan 1891, Pseudopleistophora Sprague 1977. Vavraia Weiser 1977, Baculea Loubès and Akbarieh 1978, Polydispyrenia Canning and Hazard 1982, Cystosporogenes Canning et al. 1985, Ovavesicula Andreadis and Hanula 1987 and Endoreticulatus Brooks et al. 1988. In the species from Ma. africana stages of merogony were seen in addition to stages of sporogony. These meronts were diplokaryotic, often forming multinucleate plasmodia. At the surface was a plasma membrane which was thickened during development by an electron dense coat. Host ribosomes could be seen close to the parasite's surface and the larger meronts were surrounded by two additional membranes; the outer abundantly studded and the inner sparsely studded with ribosomes. This membrane interpreted as a cisterna of host endoplasmic reticulum divided with the meronts. A similar arrangement at the surface of meronts was observed in Glugea anomala (Canning et al., 1982). However, in this species, the endoplasmic reticulum and the electron dense coat on the meront plasma membrane were dispersed at the onset of sporogony and a sporophorous vesicle was newly secreted by pushing out as a new series of blisters from the surface of the sporogonial plasmodium. In the species in Ma. africana the outer layer of the endoplasmic reticulum cisterna persisted to form the vesicle (not a sporophorous vesicle) in which the sporoblasts and spores were formed. The parasite thus differs from Glugea in the origin of the vesicle membrane and because Glugea has isolated nuclei throughout development and stimulates the host to form a xenoma.

In Polydispyrenia the sporophorous vesicle is present in meronts but is fine and of parasite origin (Canning and Hazard, 1982). The species in Ma. africana cannot be placed in the genera Pleistophora or Vavraia which have a thick amorphous layer around meronts and sporonts, quite unlike the surface coat seen in this parasite (Canning and Nicholas, 1980; Canning and Hazard, 1982). Pseudopleistophora and Cystosporogenes can be discounted as the first have diplokaryotic nuclei throughout development (Sprague, 1977) and the later is uninucleate throughout development (Canning et al., 1985) as are Pleistophora and Vavraia. Also it cannot be placed in Ovavesicula in which the sporophorous vesicle, of parasite origin, is extremely thick and electron dense, has a constant shape and always contains 32 spores (Andreadis and

Hanula, 1987). Baculea can also be discounted as the spores are not elongate in the multisporous species in Ma. africana (Loubès and Akbarieh, 1978). It is possible to discount the genus Endoreticulatus as all developmental stages are uninucleate, however, the sporophorous vesicle is formed from host endoplasmic reticulum and meronts form ribbon-like strings during divison just as the multisporous species in Ma. africana (Brooks et al., 1988). Of all the genera in the Pleistophora complex this one bears the greatest similarities. There are also 2 species in the collective genus Microsporidium with multisporous sporogony. These are M. itiiti from Listronotus bonariensis and M. novacasteriensis from Deroceras reticulatum. These differ from the species from Ma. africana as they are uninucleate throughout development. However, they do have ribbon-like stages, though these stages are sporogonic and spores form within a membrane (formed initially around meronts) derived from host endoplasmic reticulum. These are clearly similarities between these 2 species and the species in Ma. africana. However, as the Microsporidium spp. are uninucleate throughout development and form ribbon-like sporonts rather than meronts the species from Ma. africana cannot be assigned to this collective genus (Malone, 1985; Jones and Selman, 1985).

Finally there are 3 enigmatic Pleistophora spp. not yet assigned to a genus. The first was from Aedes sierrensis (Sanders and Poinar, 1976). Early sporogonic stages retained the diplokaryotic nuclear arrangement which was similar to the species from Ma. africana in which separation of the diplokaryon occurred at the onset of sporogony. In contrast there were no ribbon-like forms in the microspordium from Ae. sierrensis and the sporophorous vesicle was of parasite origin. The second from Pristophora erichsonii (Percy et al., 1982) had diplokaryotic meronts and uninucleate sporonts and the sporophorous vesicle was of host origin but meronts were never ribbon-like and never had a thickened plasma membrane. The third, from Spodoptera depravata (Iwano and Ishihara, 1984) was uninucleate throughout its development and therefore not similar to the species in Ma. africana.

For the reasons discussed the multisporous species from Ma. africana, though having similarities to several species, particularly the Endoreticulatus sp. and the 2 Microsporidium spp. cannot be assigned to a specific genus. It is therefore likely that a new genus may need to be established for this parasite.

4.3.5 Microsporidian infection of Mesocyclops rarus

The microsporidian infection of M. rarus had 2 distinct developmental sequences producing 2 types of spore, which differed only in size not ultrastructure. No stages possessed diplokarya and each spore was uninucleate within a vesicle. The 2 sequences were always found together in a single host, and were therefore presumed to belong to the same species.

Using the annotated list of Sprague (1977) of microsporidia reported from Copepoda, none could be identified as the species in M. rarus. Species of Pyrotheca, Gurleya, Pleistophora and Thelohania which have been reported from copepods produce more than 1 spore per sporophorous vesicle (Maurand et al. 1972, Leblanc 1930, Moniez 1887, Lemmerman 1900, Cépède 1911, Weiser 1945 cited in Sprague, 1977). Tuzetia spp., also reported from copepods (Maurand et al. 1972, Kudo 1921 cited in Sprague, 1977) produce single spores in sporophorous vesicles but form plasmodia with many nuclei. Also the vesicles have electron dense trilaminate membranes and divide with the sporont (Larsson, 1983). The parasite of M. rarus does not, therefore, belong to the genus Tuzetia. Mrazekia cyclopis (Vávra, 1962 cited in Sprague, 1977) produces binucleate spores and the Duboscqia sp. described from several copepod species (Lom and Vávra 1963 cited in Sprague 1977) have spores covered in mucus layers. Courgourdella magna and Courgourdella pusilla from copepods (Hesse 1935 cited in Sprague, 1977), both have paired sporoblasts and lageniform spores of a different structure to the spores in M. rarus.

Amblyospora dyxenoides and Amblyospora connecticus in Mesocyclops albicans and Ancanthocyclops vernalis respectively (Sweeney et al.,1985; 1988; Andreadis,1985; 1988) bear most resemblance to the species from M. rarus in structure and development. In A. dyxenoides the sporonts divide after polarisation of the nuclei and formation of the sporont into a U-shape. Stellate forms were also seen, though more rarely. Spores were formed singly in sporophorous vesicles, and the polaroplast had the same series of blocks separated by membranes, the blocks becoming less electron dense towards the posteriad (visible by light and electron microscopy). These features were also seen in the species in M. rarus but 2 spore types were not described for A. dyxenoides or A. connecticus. However, in A. campbelli from Culiseta incidens (Dickson, 1988), 2 different spore types were seen in the copepod alternate host, Diaptomus sp.. One spore type was pyriform, very like those previously described for the copepod stages of Amblyospora spp. and the other type was

derived from an octosporous sporogony. The pyriform spores were responsible for transmission to mosquito larvae, and the octospores for transmission to other copepods. It is possible therefore that the 2 spore types in M. rarus belong to a polymorphic microsporidian species and are responsible for transmission to different hosts. Unfortunately transmissions between copepods and mosquitoes were not successful and it is not known whether the stages in M. rarus are linked with the octosporous or multisporous species in Ma. africana or with other undiscovered microsporidian infections.

4.4 VARIATION IN INFECTION LEVELS IN Mansonia africana AND Mesocyclops rarus WITH TIME AND INSTAR AT KISIWANI, MUHEZA SISAL ESTATE AND MAMAKINGI

The differences in level of microsporidian infection between sites and overall prevalence of each infection will be discussed in ecological terms in Section 4.5. In this section the fluctuations in prevalences with time and instar will be considered. First instar larvae were never collected, perhaps because when Ma. africana eggs hatch the larvae can respire at the surface for some time before attaching to roots. Only larvae on roots were sampled.

4.4.1 Octosporous infections in larvae

Octosporous infections were found in every month during the sampling period at relatively constant levels (Figs.29,30,31). There were significantly more 3rd instar larvae infected than 2nd instars at Mamakingi, and this was thought to have been caused either by the relatively low sample size for 2nd instars, or because infected 2nd instars were selectively disadvantaged.

4.4.2 Multisporous infections in larvae

At Muheza Sisal Estate and Mamakingi in 1987, infections were not found between the last week of June and the first week of August (third week of August in the case of Muheza Sisal Estate) (Figs.30,31). The overall level of infection with the multisporous species fluctuated greatly between the dates on which collection were made, but was relatively low in comparison with the octosporous infection which was found in every month. As the putative alternate host is not known, the factors affecting prevalence remain obscure and could depend largely on abundance of alternate hosts.

It was interesting that no multisporous infections were found at Kisiwani before the introduction of larvae in July (Fig.29). These larvae were the progeny of females collected in night catches at the Muheza Sisal Estate. The females had been smeared while still alive after laying their eggs and none had been found infected. It is possible that a low level infection had not been detected and that the multisporous species had been introduced accidentally. Equally the appearance of the infection at this time may have been coincidental as infected females from Mamakingi, Muheza Sisal Estate or other sites could have laid their eggs on plants at Kisiwani, as sites of infection were within the flight range of Ma. africana. The infection had not been found at Kisiwani in 1985 but the reasons for patchy distribution of infections are difficult to account for. The Kisiwani pond had become greatly reduced in size between 1985 and 1987 and may have become unattractive to Mansonia females, very few of which had oviposited there. Very few larvae were recovered in initial collections in 1987 and a high proportion of these (7/21 = 33.3%) were infected with the octosporous species. These larvae could have been derived from the eggs of a single female that oviposited there.

The putative copepod alternate hosts (Mesocyclops rarus) · although present, were not infected with microsporidia as at Mamakingi and Muheza Sisal Estate.

4.4.3 Diplokaryotic infections in adults and larvae

Infections in adult mosquitoes were of 2 distinct types, the smaller Species A and larger Species B which were never found together. Benign diplokaryotic infections in larvae were presumed to give rise to diplokaryotic infections in adults. As adult infections were of 2 types it was anticipated that larval infections would also be of two types. However, only large diplokaryotic stages were found in larvae, suggesting either that the stages in larvae were of the same size for the two species, or that only larvae infected with Species B were sampled. In order to throw light on this problem, prevalences of diplokaryotic infections in larvae were first compared with the infections in adults (Species A and B combined) and then with Species B alone (Figs.100, 101). Perhaps because the prevalence of infection in larvae was very low (often no infected larvae were collected on a particular date) or because comparisons could only be made over a 4 month period, it was difficult to draw firm conclusions on the relationship of infections in larvae and

adults. Another possible explanation for the apparent absence of Species A in larvae is that they were present in the samples but were not detected because of the techniques employed.

As diplokaryotic infections are benign, not producing patent symptoms, each larva had to be smeared and stained before infections could be detected. In order to conserve fixative for electron microscopy, infections were identified in stained smears before tissues were fixed. The larva was dissected, the thorax and a tiny piece of anterior abdomen were held on damp cotton wool at 4°C and the rest of the abdomen was smeared. When an infection was identified in the abdomen the thorax and a small piece of anterior abdomen were fixed for electron microscopy.

It is possible that diplokaryotic stages of Species B developed throughout the larval body and were therefore identified in smears of abdomen. If diplokarya of Species A were concentrated in the thorax and anterior abdomen they would not have been detected in smears. However, as all infections are ultimately concentrated around the developing ovaries there is no reason why both infections should not be found in the abdomen near the ovaries.

Slides had to be examined quickly so that the pieces of thorax could be fixed as quickly as possible. At a quick glance the larger diplokarya may have been easier to see than the smaller ones, but re-examination of smears did not reveal any of the smaller stages. It must therefore be assumed that larvae with Species A infections were not sampled, even though there was an average 16.7% infection level in adults. Unfortunately, diplokarya were never found in larvae or adults fixed for electron microscopy because infections were always at low levels.

4.4.4 Infections in *Mesocyclops rarus*

M. rarus was found infected with microsporidia at Muheza Sisal Estate and Mamakingi. The infection level at Muheza Sisal Estate (24.3%) was very nearly twice that at Mamakingi (13.5%). Copepods have been found to act as alternate hosts for two species of *Amblyospora*. The microsporidian species in *M. rarus* bore a close resemblance to *A. dyxenoides* from *M. albicans* (Sweeney *et al.*, 1985; 1988). Developmental stages including the formation of U-shaped and stellate forms was similar in both species and although the spores were of two sizes in *M. rarus* and only 1 in *M. albicans* their shape and internal structure was the same. The presence of the octosporous infection of *Ma.*

africana at all 3 sites including Kisiwani and the absence of infection in M. rarus at Kisiwani suggests that M. rarus cannot be the alternate host of the octosporous microsporidium in Ma. africana.

In contrast, the multisporous Mansonia infection was absent at Kisiwani until about 3 weeks after Ma. africana larvae had been introduced. Whether the infection was introduced artificially or naturally into the pond its absence until this time would be in accord with the absence of infection in copepods. It is possible that the M. rarus infection is part of the life cycle of the multisporous and not the octosporous infection in Ma. africana. Had sampling of copepods continued at Kisiwani from October onwards, it is possible that infections passing from the mosquitoes to the copepods would have been found. Evidence in support of the link between the microsporidia in copepods and the multisporous infection in Ma. africana comes from comparison of infection levels at the different sites. The average level of multisporous infection at Muheza Sisal Estate was very nearly double that at Mamakingi. The octosporous infection was almost 3 times higher at Mamakingi than at Muheza Sisal Estate and this also supports the link between M. rarus and the multisporous infections in Ma. africana.

4.5 LEVELS OF MICROSPORIDIAN INFECTION IN TANZANIAN MOSQUITOES

Before collections were made in Tanzania, it was assumed that levels of infection would generally be higher, as mosquitoes are not seasonal as in Britain. Most species are present in all their developmental stages throughout the year, though the time after the rainy season is more favourable as water bodies fill up, creating higher overall mosquito populations (Gillies and De Meillon, 1968).

Apart from the octosporous infection of Ma. africana the levels of infection were mostly still quite low, for instance the P. africana infection of An. gambiae s.l. (7.5%).

An. gambiae s.l. being a highly opportunistic species can often be found breeding in the most temporary water bodies, such as puddles in the road (Gillies and De Meillon, 1968). The very advantage of such sites to the mosquito is that there are no predators but for the microsporidia there are no organisms that could act as alternate hosts. Although it has not been established that an alternate host is needed in the life cycle of P. africana,

attempts to transmit it and other species of Parathelohania (Hazard and Anthony, 1975) directly have not been successful and it is likely that an alternate host is required as for Amblyospora spp.. Some temporary water bodies may remain long enough for other organisms to develop which could transmit the parasites. However, in these sites An. gambiae s.l. loses the advantage of reduced predation and population numbers drop. During the dry season there are only permanent water bodies for breeding and such sites may be the reservoirs of infection. All the adverse factors discussed for the British species would apply in these sites. Spores and infected eggs are lost in temporary sites when complete drying out occurs, as spores and anopheline eggs are only viable while they remain moist. The site at Mombo was an irrigated paddy field, however, it was still subject to drying out occasionally and in addition the majority of larvae were found in pools and ditches, which commonly dried out, around the paddy fields. The effect of site drying would also apply to the Culex sp. at Pangani, breeding in a shallow ditch, that would eventually dry out. However, the infection here was high (80%) showing that discussion of prevalence levels without knowledge of the transmission cycles is highly speculative.

Culex quinquefasciatus larvae from a polluted ditch in Dar-Es-Salaam were found infected with an unidentified microsporidian. This mosquito was commonly found in Tanzania, breeding in pit latrines where they fed on suspended protein (Van Handel, 1986). Like An. gambiae s.l. they gain an advantage by breedinig in a site unsuitable for many other competing species. However, these sites are often so foul that few organisms survive other than the mosquitoes. No copepods, the putative alternate hosts for dimorphic microsporidia, were ever found. It is also quite common for latrines to dry out, with subsequent loss of spores. For this reason it is unlikely that transmission of microsporidia to alternate hosts would occur in pit latrines. Even the spores of directly transmissible species may not survive in the harsh conditions of a latrine. Transmission is more likely to occur in sites such as the polluted Kariakoo ditch which are also favoured by Cx. quinquefasciatus and where other organisms which could act as alternate hosts, can survive.

Mansonia africana is a non-seasonal mosquito, breeding in permanent sites. Of all the microsporidian infections found, the octosporous species in Ma. africana was at the highest prevalence (13.2%, 6.9%, 21.5% at Kisiwani, Muheza Sisal Estate and Mamakingi in 1987 respectively). Within each infection

site two different infections in mosquitoes and one in copepods (possibly linked to the mosquito infections) were found. Clearly, these sites were favourable for transmission, especially as the multisporous infection, though at a lower prevalence was also present.

The permanence of the site seems an important factor in increasing the overall level of infection. If water is always present at a site, spores free in the water column or in the mud have an extended period of time during which they can be transmitted. Although spores will be removed from the feeding area by settling as previously discussed they still have a chance of being brought into the water column by turbulence caused by fish etc.. In addition, the larvae are attached to plant roots which are often in close proximity to the mud and dead, possibly infected organisms at the bottom.

The permanence of the site also means that all hosts required for the life cycle are present together. Predators may also be present, but may not necessarily reduce the rate of transmission as Mansonia sp. being less motile when attached to plant roots are less readily eaten by predators (Van den Assem, 1958). Even if spores within a larva or copepod are ingested by a predator other than an alternate host, they may pass unharmed through its gut and remain infective.

Mamakingi Pond had a significantly higher level of infection than Muheza Sisal Estate. It is possible that this is due to the overall size of the two sites. Mamakingi was only a small pond with a small surface area, but quite deep. The water surface was completely covered with Pistia stratiotes plants. In comparison the Muheza Sisal Estate site was a far more extensive, but shallower site, not entirely covered with plants.

The plant covering at Mamakingi reduced evaporation from the water surface. The rate of evaporation was already less than at Muheza Sisal Estate, due to the reduced surface area to volume ratio. At Muheza Sisal Estate the level of water fell continuously between June and September 1987, never actually disappearing but leaving areas of stranded plants and drying mud. Spores and larvae left stranded in this way would be lost. In addition the inter and intra-specific competition effects may have applied here as larvae were concentrated onto smaller numbers of plants.

Kisiwani was a site similar to Mamakingi, though the prevalence of infection was lower. At Mamakingi the water was regularly disturbed, by people collecting drinking and washing water, thus bringing spores from the

mud into the water column again, where transmission could occur. By comparison the water at Kisiwani was rarely disturbed.

At all sites a proportion of the plants with larvae had roots which hung in the silty bottom layers of the ponds. Here spores are likely to be found, and are thus brought into contact with new hosts.

4.6 DISCUSSION OF TRANSMISSION EXPERIMENTS

Transovarial transmission

Adult female Ma. africana from Muheza Sisal Estate infected with the smaller microsporodian (Species A) species produced progeny of which 13.8% carried a multisporous infection. Octosporous infections never developed, and no infections developed in controls except in one case when a female thought, retrospectively, to have had a low level infection must have been added inadvertantly. This demonstrated that small binucleate spores in the ovaries of adults are part of the same complicated life cycle as the infections terminating in multisporous sporophorous vesicles in larval progeny.

Spores in adult Ma. africana were binucleate and were presumed to inject their sporoplasms into developing oocytes. In the larvae developing from these ova, sporogonic development commenced with the separation of the 2 nuclei of the diplokaryon and continued with repeated nuclear and, ultimately cytoplasmic, divisions to form many uninucleate spores within a sporophorous vesicle. Occasional giant spores were seen by electron microscopy in one larva but they were rare in Giemsa stained smears, suggesting that they resulted from aberrant sporogonic divisions and did not represent a second spore type developing in larvae.

Only 13.8% of progeny of Ma. africana became infected with the multisporous species which is low in comparison with many Amblyospora and Parathelohania spp. infections where it is common for 100% of the progeny of an infected female to develop infections, as seen in the present study of P. africana from An. gambiae s.l. (Kumbamtoni). This provides one explanation of the 10.9% of Ma. africana adults infected with diplokarya while only 5.7% of larvae had the multisporous infections at Muheza Sisal Estate. These figures suggest that nearer 50% of progeny should become infected which is higher than observed. However, the artificial rearing conditions always became extremely polluted during the development of larvae from eggs, much more so than natural field conditions, and it is possible this

Figures 124 and 125 demonstrate the 2 putative microsporidium lifecycles thought to occur in Mansonia africana mosquitoes from Kisiwani, Muheza Sisal Estate and Mamakingi.

They have been inserted at this point to act as a reference for the reader during section 4.6, where the 2 lifecycles are discussed.

———— = known pathway

- - - - - - - - - - = suggested pathway

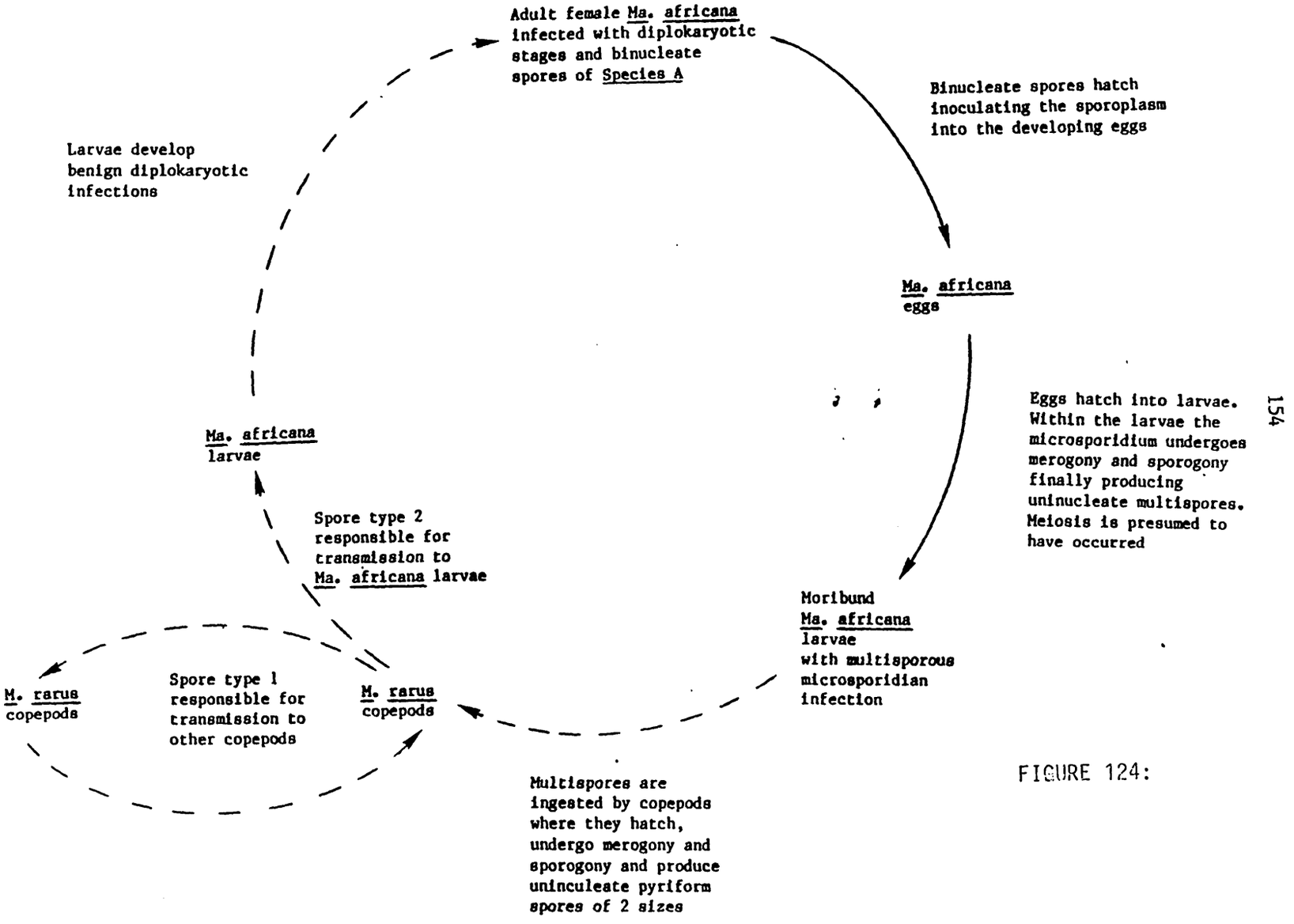

Adult female Ma. africana infected with diplokaryotic stages and binucleate spores of Species A
Binucleate spores hatch inoculating the sporoplasm into the developing eggs
Ma. africana eggs
Eggs hatch into larvae. Within the larvae the microsporidium undergoes merogony and sporogony finally producing uninucleate multispores. Meiosis is presumed to have occurred
Moribund Ma. africana larvae with multisporous microsporidian infection
Multispores are ingested by copepods where they hatch, undergo merogony and sporogony and produce uninculeate pyriform spores of 2 sizes
M. rarus copepods
Spore type 1 responsible for transmission to other copepods
M. rarus copepods
Spore type 2 responsible for transmission to Ma. africana larvae
Ma. africana larvae
Larvae develop benign diplokaryotic infections

FIGURE 124:

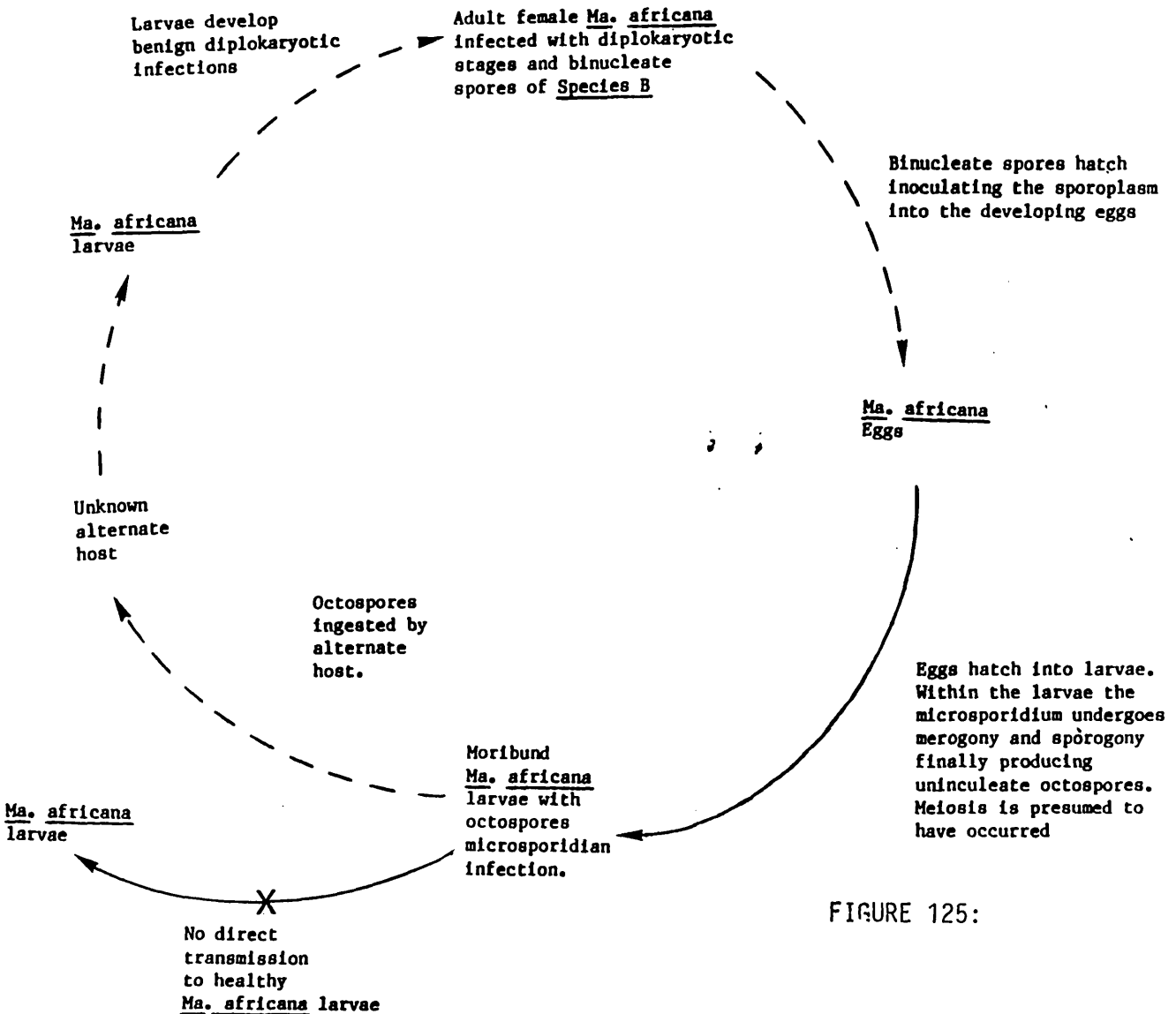

Larvae develop benign diplokaryotic infections
Adult female Ma. africana infected with diplokaryotic stages and binucleate spores of Species B
Binucleate spores hatch inoculating the sporoplasm into the developing eggs
Ma. africana larvae
Ma. africana Eggs
Unknown alternate host
Octospores ingested by alternate host.
Eggs hatch into larvae. Within the larvae the microsporidium undergoes merogony and sporogony finally producing uninculeate octospores. Meiosis is presumed to have occurred
Moribund Ma. africana larvae with octospores microsporidian infection.
Ma. africana larvae
No direct transmission to healthy Ma. africana larvae

FIGURE 125:

increased environmental stress acted particularly against infected larvae causing them higher mortality. This may also explain why so few larvae survived in Bowl 2 from the second infection trial (Table 9).

There remains the possiblity that the larger diplokaryotic infections in adults correspond to octosporous infections in larvae. The octosporous species, though differing morphologically from both Amblyospora and Parathelohania is clearly closely related to these genera. A mean of 5.7% of adults were infected with the larger diplokaryotic stages and 6.9% of the larvae had octosporous infections at Muheza Sisal Estate. If these 2 infections are linked then almost 100% of the progeny of female Ma. africana infected with large diplokaryotic stages should develop octospores. This is in accord with the infection levels achieved in the closely related genera Amblyospora and Parathelohania.

The transmission experiments have shown the multisporous infection to be dimorphic in the mosquito host and the octosporous infection is presumed to be the same. It is likely therefore, that they undergo a sexual cycle with meiosis at some point in their life cycles. Meiosis is recognised by the presence of synaptonemal complexes, as seen by electron microscopy in the two nuclei of the diplokarya as they separate in early sporonts. This occurs in the octosporous sequence of Amblyospora (Sweeney et al., 1988; Andreadis, 1988) and in the multisporous sequence of P. simulii (Canning and Hazard, 1982). Synaptonemal complexes were not seen in the Ma. africana material probably because the material had remained in buffer solution for several weeks or months after fixation, before final processing for electron microscopy. However, the separation of the two nuclei of the diplokaryon and subsequent development, producing uninucleate spores followed the pattern for octosporous and multisporous species in which meiosis has been demonstrated.

Horizontal Transmission

Attempts to transmit infections using octospores from mosquito larvae to healthy 1st instar larvae of the same species

Transmission was attempted using octospores from Ma. africana and An. gambiae s.l., but in neither case was transmission effected. This is in accord with previous observations on dimorphic genera such as Amblyospora and Parathelohania (Hazard and Anthony, 1975; Hazard and Oldacre, 1976).

Attempts to transmit octospores and multispores to Mesocyclops rarus copepods

Anopheles gambiae s.l. : Parathelohania africana

Attempts to transmit P. africana to M. rarus were not successful. The breeding grounds of the An. gambiae s.l., from which P. africana octospores were derived for transmission experiments, were not known and the larval habitats could not be sampled for copepods or other potential alternate hosts. Thus, the alternate host could be one of many other invertebrates rather than the M. rarus which was readily available for testing.

Mansonia africana : octosporous and multisporous infection

Many experimental modifications were made but there was no conclusive evidence that transmission had been effected.

Although not proven, transmission could have occurred, particularly as occasional copepods from several of the trials were found infected, long after the estimated four days after which field derived infections must have died out. Infections were found, not only in the tests with the octosporous and multisporous species, but also in control pots, where no spores had been added. Possible explanations for infections in control copepods are that spores were added inadvertently with the copepods from collection water, or that the species present in copepods is directly transmitted between copepods. Great care was taken to reduce the likelihood of the first by removing all the pond water from the copepods and rinsing them several times in fresh water before trials were initiated. Dickson (1988) found copepods infected wth two types of spores, one responsible for transmission to a mosquito host and the other responsible for transmission to other copepods. It is possible that the two types of spore in M. rarus also have different transmission cycles, one responsible for transmission to Ma. africana and the other for transmission to other copepods.

Cannibalism of moribund and dead copepods was seen to occur. If moribund copepods were infected, spores ingested by cannibalism would either pass directly through the copepod or infect the feeding copepod. If one of the spore types was directly transmissible the infection may spread through the copepods in the trial, perhaps resulting in the large numbers unaccounted for. However, this seems unlikely as more infections would have been expected during the trial. If this were the only transmission

cycle occurring, then theoretically the percentage unaccounted for would be the same between infected pots and control pots but this was not found to be the case. With only 4 exceptions (Tables 20, 21, 24, 28) and 1 case when it was marginal (Table 15) there was a much higher percentage unaccounted for in the infected pots. This was true for the experiments using octospores from Ma. africana and An. gambiae s.l. and for multispores. It is unlikely that all the copepods unaccounted for died of microsporidian infection. It is possible some may have become infected and died but the majority of deaths are likely to have been from other causes - natural, stress or other infections such as fungal or bacterial. Predation by other copepods is likely. Fungal strains were seen in some infected mosquito cadavers, even though anti-fungal and anti-bacterial solutions were used. Unfortunately, these contaminated larvae had to be used as sources of infection in trials as infected material was in short supply.

The most valuable trial was experiment 6 in which copepods were held singly with Ma. africana derived octospores. This prevented cannibalism. In addition, the spores were from fresh uncontaminated mosquito larvae reducing the effect of fungi and bacteria. None of the copepods became infected and although the numbers used were low, it seems likely that the octosporous species from Ma. africana does not infect M. rarus.

From experimental evidence it is difficult to say whether or not the multisporous species from Ma. africana infects M. rarus. However, evidence from field prevalences suggest that it does. Copepods at Kisiwani pond were never found infected. In addition the multisporous infection was not found there in 1985 or in 1987 until Ma. africana larvae from Muheza Sisal Estate had been added to the pond. Even then it was only found at low levels. Had collections been continued after September, infections may have arisen in the copepods.

Unfortunately, in the experiment attempting to infect Ma. africana larvae with spores from copepods none of the larvae survived. This was probably due to the variable conditions in culture bowls. When culturing Ma. africana in Britain and Tanzania, bowls treated similarly produced highly variable numbers of emerging adults.

Using morphological and experimental studies, it is possible to suggest two possible life cycles for the octosporous and multisporous infections of Ma. africana (Figs.124,125). The larger species infecting

adult mosquitoes (Species B) probably gives rise to octosporous infections in larval progeny, which die at 4th instar. Larvae were always heavily infected and moribund by 4th instar. These spores were not directly transmissible but the organism acting as alternate host was not identified.

The smaller species infecting adult mosquitoes (Species A) gave rise to multisporous infections in a percentage of progeny. These larvae were presumed to die at 4th instar as no multisporous infections were found in pupae or adults. The multisporous infection from larvae may be infective to M. rarus copepods which develop fatal dimorphic infections. One spore type may be responsible for transmission between copepods and the other for transmission to larvae where benign diplokaryotic infections could develop.

4.7 IMPLICATIONS IN BIOLOGICAL CONTROL

According to Anderson and May (1981) the qualities of a good biological control agent are moderate pathogenicity, long-term survival of infective stages and high transmission rate. Of all the protozoan pathogens of invertebrates the microsporidia offer the greatest potential as biocontrol agents due to their massive reproductive potential (Canning, 1982). In the case of directly transmissible species, once an insect becomes infected reproduction of the parasite is limited only by the resources of the host. As a result the hosts suffer larval mortality and reduced adult longevity and fecundity. Infected individuals are more susceptible to environmental stress. However, microsporidia do not produce toxins and the interval between infection and host destruction is usually long, therefore detracting from their potential as fast acting control agents (Canning, 1977b; 1981; 1982). In addition microsporidian spores are often not very resistant to heat, drying and ultra violet radiation (Maddox, 1973). When used in conjunction with parasitoids or when mixtures of microsporidian species or mixtures of microsporidia and viruses are used they can be antagonistic (Nordin and Maddox, 1972; Wilson, 1978; Hamm et al., 1983). There may be a vertical component to transmission as well as horizontal, the vertial component being highly beneficial in a biocontrol agent for recycling within a population.

It has been suggested that the usefulness of entomophilic protozoa will only be for the control of pests of grassland, forest, soil, stored

products and some crops such as tobacco, where high economic injury levels can be tolerated (Brooks, 1986; Fuxa and Brooks, 1979). These ecosystems offer good conditions for development of epizootics with maximum potential for dispersal and persistance of spores (Brooks, 1980). Microsporidia may recycle in these habitats by horizontal and vertical transmission causing epizootics at least for the first season. Even so, permanent control is unlikely, and repeated applications are required (Brooks, 1986). Nosema locustae has been used successfully against grasshoppers in America in conjunction with low levels of chemical insecticide (Henry, 1971; 1978; 1981; Henry and Oma, 1974; Henry et al., 1973).

Vector control however, requires far greater reductions of insect numbers as, for example, low levels of An. gambiae s.l. can still be responsible for high levels of malaria (Service, 1981, cited in Nugud and White, 1985). Studies on N. algerae, a microsporidian parasitising a wide range of anophelines, including An. gambiae s.l., initially proved favourable, though fairly high doses were required, and long term control was not achieved (Lacey and Undeen, 1986; Nugud and White, 1985). This was believed to be due to rapid settling of the inoculum, which was what had previously been found when it was used against An. albimanus (Anthony et al., 1978). Its use against An. gambiae s.l. would require application to the multitude of small puddles over large areas and would be as time consuming and costly as chemical insecticides with less efficiency. Its use in permanently irrigated rice fields against An. arabiensis and other species is also unlikely to succeed. Long term control of Cx. quinquefasciatus using Vavraia culicis was also not achieved (Reynolds, 1972) and the same problems would apply to the use of N. algerae against anopheline mosquitoes.

Dimorphic species have complex lifecycles involving transovarial transmission and an alternate host. No field trials have been attempted but in natural epizootics where conditions were presumably optimal, they have been seen to suppress mosquito populations significantly (Andreadis, 1983a,1983b; 1985b). The advantages of dimorphic (polymorphic) species as biocontrol agents are twofold. Firstly, horizontal and vertical transmission occur, the latter often producing 100% infection levels in the progeny, as seen in An. gambiae s.l. in this study. Secondly, they might recycle in the host populations more successfully than directly transmissible species as they can survive in more than one host. Within a

permanent site, levels of infection may increase, although in non-permanent sites repeated applications of spores or infected individuals would be needed to replace those lost.

Mass production of infective spores and safety testing on non-target biota provide further complications that must be overcome before microsporidia can be used as control agents. It is possible to raise some infections, for instance N. algerae, in other larger organisms (e.g. Heliothis zea) (Undeen and Maddox, 1973) making mass production of infective spores possible. Also in vitro culture techniques are now available for some species although this is more expensive (Undeen, 1975; Street, et al., 1980; Smith, et al., 1982). Henry (1981) suggested that due to the relatively low virulence of most microsporidia they would probably never be used alone against insect pests or vectors. This is still likely to be true, though dimorphic species used in permanent sites have yet to be investigated fully.

It is likely that microsporidia may be used in integrated control programmes providing short term control of key pests in certain ecosystems, or, as previously discussed, in situations where high levels of economic damage are acceptable. Their potential is summarised in a quotation from Brooks (1986) "They are not likely to generate high-volume sales potential and commercialisation will be most likely by cottage-type industries following adequate safety testing...". "As new species are described and their complex lifecycles revealed, the large number of available protozoa for use against many agricultural and medically important insects should enhance the potential for commercialisation in the next 10-20 years. In addition, as society continues to demand less ecologically disruptive alternatives for insect control than insecticides, we must strive to augment the already significant role the entomophilic protozoa play as natural mortality agents".

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APPENDIX I, TABLE A: Details of British sites visited where no infections were found

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
1) Cackle Street, Sussex	Woodland pools formed from old iron workings	17. 3.86	Pool 1	-	15-20	2nd	Aedes sp.	0	0	-
			Pool 2	-	40-50	4th	Aedes rusticus	0	Cyst like organism found in many larvae	-
			Pool 3	-	30-40	2nd	Aedes sp.	0	0	-
			Pool 4	-	40-50	2nd	Aedes sp.	0	0	
		11. 6.86	Pools 1-4	0	0	-	-	-	-	Dry
			Pool 5	0	0	-	-	-	-	Dry
			Pool 6	0	0	-	-	-	-	Dry
			Pool 7	1	1	4th	Aedes sp.	0	0	
				1	1	Pupa	-	0	0	
2) Hamm Court	Water filled ditches and a temporary pool	27. 3.86	Section 1 of ditch	0	30-40	1-2nd	-	0	0	Copepods found of species Acanthocyclops vernalis
			Section 2 of ditch	-	20-30	1-2nd	Aedes sp.	-	0	
			Temporary Pool in field	-	10-15	3-4th	Aedes sp.	0	0	
		17. 6.86	Section 1 of ditch	2	2	-	Aedes sp.	0	0	-
			Section 2	1	1	-	Aedes sp.	0	0	-
			Section 3	2	2	pupae	Aedes sp.	0	0	-
			Temporary pool	-	-	-	-	-	-	Overgrown

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APPENDIX I, TABLE A: Page 2

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
3)										
Isle of Grain	Salt marshes, freshwater ponds, ditches and slowly moving streams covering many miles	22. 4.86	British Petroleum Site 1 Large fleet of brackish water	50	20-30	3-4th	Aedes detritus	0	0	Many red copepods of species Acanthocyclops bicuspidatus bicuspidatus
			British Petroleum Site 2.Smaller fleet of brackish water.	50	20-30	3-4th	Aedes detritus	0	0	-
		22. 5.86	British Petroleum site.	-	-	-	-	-	-	Water present but highly polluted, no living aquatic invertebrates
		9. 7.86	Small freshwater pool remaining in the British Petroleum site.	28	28	3-4th	Culex pipiens	0	0	-
		22.4.86	'Clubs' Gravel pits - brackish water.	15	5-10	4th	Culiseta littorea	0	0	Few larvae found
		11. 9.86	'Clubs' Gravel Pits - brackish water	-	-	-	-	-	-	Dry

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APPENDIX I, TABLE A: Page 3

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
3) contd		22. 4.86	Ryfleet - freshwater ditch.	20	10-15	4th	Culiseta annulata	0	0	Larvae difficult to find as dispersed by rain Diaptomus castor, Cyclops strenuus and Acanthocyclops bicuspidatus lubbocki copepods found
		22. 4.86	Cliffe ditches filled with brackish water	35	20-30	3-4th	Anopheles claviger	0	0	A. bicuspidatus bicuspidatus, C. strenuus & A, vernalis copepods found
				20	10-20	4th	Culiseta littorea	0	0	
		22. 4.86	Pond	-	-	-	-	-	-	No larvae found
		22.. 4.86	Bus Park	6	6	4th	Culiseta sp.	0	0	Very few larvae.
		11. 9.86	Bus Park	4	4	3rd	Anopheles claviger	0	0	
				1	1	4th	A. maculipennis	0	0	
				5	5	3rd	A. maculipennis	0	0	
				1	1	2nd	A. maculipennis	0	0	
				5	5	pupae	-	0	0	

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APPENDIX I, TABLE A: Page 4

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
4) Sandwich	Area of salt marsh and brackish water	27. 5.86	Ditch 1	-	-	-	-	-	- larvae	Drying up - no larvae found
			Ditch 2 Site (a)	1	1	3rd	Aedes sp.	0	0	Very few larvae found
			Ditch 2 Site (b)	3	1	pupa	-	0	0	
			Ditch 2 Site (c)	1	1	3rd	Anopheles sp.	0	0	
				18	18	3-4th	Aedes sp.	0	0	
				5	5	2nd	Aedes sp.	0	0	
				48	40	pupae	Aedes sp.	0	0	
			Ditch 3	-	-	-	-	-	-	No larvae found
			Reeded area	-	-	-	-	-	-	
5) Pegwell Bay	Salt marsh area close to sea, with a nearby ditch	27. 5.86	Saltmarsh	-	-	-	-	-	-	No larvae found
			Ditch	5	5	3-4th	Culex pipiens	0	0	Very few larvae
				20	18	pupae	-	0	0	-
6) Epping Forest	Large forest with large bodies of temporary pools	28. 4.86	Site 10 (Fallis 1984)	2	2	pupae	-	0	0	No larvae found but many pupal cases seen
			Site 9 (Fallis 1984)	-	-	-	-	-	-	No larvae found

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APPENDIX I, TABLE A: Page 5

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
6) contd			Site 8 (Fallis 1984)	-	-	-	-	-	-	No larvae found
			Site 1 (Fallis 1984)	-	-	-	-	-	-	
			Temporary pools a)	30	15-30	3-4th	Aedes sp.	0	0	
			b)	20	15-20	3-4th	Aedes sp.	0	0	
			c)	10	5-10	3-4th	Aedes sp.	0	0	
			d)	20	15-20	3-4th	Aedes sp.	0	0	
7) Sites at Silwood Park	Estate with some wood-land (Onyeka 1980)	20. 4.86	Japanese pond	-	-	-	-	-	-	No larvae found
			House pond	-	-	-	-	-	-	No larvae found
			Lake	-	-	-	-	-	-	No larvae found
			Temporary Pond by lake	22	22	2-4th	Aedes sp.	0	0	
		18. 6.86	Paddling Pool	-	-	-	-	-	-	No larvae found
			Large tank	-	-	-	-	-	-	No larvae found
		13. 7.86	Water butt	22	18	2-4th	Culex pipiens	0	0	-
		14. 7.86		26	21	3-4th	Cx. pipiens	0	0	
		18. 7.86	Field pond	-	-	-	-	-	-	Very overgrown and silted up
		19. 6.86	Beech Tree holes	7	7	3rd	Aedes geniculatus	0	0	-

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APPENDIX I, TABLE A: Page 6

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
8)										
South Ascot	Woodland area with ditches and temporary pools	4. 5.86	Ditch	30	20-30	3-4th	Aedes sp.	0	0	-
			Pool 1	-	-	-	-	-	-	No larvae found
			Pool 2	20	10-20	3-4th	Aedes sp.	0	0	-
			Pool 3	20	10-20	3-4th	Aedes sp.	0	0	-
			Pool 4	20	10-20	3-4th	Aedes sp.	0	0	-
		18. 6.86	-	-	-	-	-	-	-	All pools dried up. No larvae found
9)										
Burnham Beeches	Large area of woodland with an old water-filled moat	14. 5.86	Sites around the moat							
			Site 1	40	20-30	3-4th	Aedes sp.	0	0	
			Site 2	40	20-30	3-4th	Aedes sp.	0	0	
			Site 3	40	20-30	3-4th	Aedes sp.	0	0	
			Site 4	40	20-30	3-4th	Aedes sp.	0	0	
			Site 5	40	20-30	3-4th	Aedes sp.	0	0	
			Site 6	40	20-30	3-4th	Aedes sp.	0	0	
			Site 7	40	20-30	3-4th	Aedes sp.	0	0	
			Nearby ditch	20	10-15	3-4th	Aedes sp.	0	0	
			Temporary pool	45	30-40	3-4th	Aedes sp.	0	0	
		25. 6.86	-	-	-	-	-	-	-	No larvae in sites 1-7 and ditch and pool, dried out
			Tree 4204	-	-	-	-	-	-	Holes dried out
			Tree 4208	-	-	-	-	-	-	" "

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APPENDIX I, TABLE A: Page 7

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
10) Wimbledon		6. 7.86	Garden pond	43 + numerous 1-2nds	30	3-4th	Culex pipiens	0	Possible fungal infection in one.	No copepods
				23	7	pupae	Cx. pipiens	0	0	
		15. 7.86		29 (9=2-3rds)	20	3-4th	Cx. pipiens	0	0	
				1	1	pupa	Cx. pipiens	0	0	
11) Winkfield		7. 7.86	Garden pond	41 + numerous lsts	32	3-4th	Cx. pipiens	0	0	No copepods
				12	3	pupae	Cx. pipiens	0	0	
		13. 7.86		18	12	3-4th	Cx. pipiens	0	0	
				10	4	pupae	Cx. pipiens	0	0	
		12. 8.86		12	12	4ths	Cx. pipiens	0	0	
				17 + numerous lsts	9	3rds	Cx. pipiens	0	0	
				11	3	pupae	Cx. pipiens	0	0	
		25. 7.86	Greenhouse water tank	30-40	6	4ths	Cx. pipiens	0	0	
12) Wimbledon Common	Area of scrubland woodland and several large ponds	15. 7.86	Kingsmere	-	-	-	-	-	-	No larvae, though many dragonfly larvae and adults

.../contd 8

APPENDIX I, TABLE A: Page 8

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
12) contd			Queensmere							
			Stretch 1	3	3	3-4th	Cx. pipiens	0	0	
				6	6	2nd	Cx. pipiens	0	0	
				8	8	1-2nd	Cx. pipiens	0	0	
				4	3	pupae	Cx. pipiens	0	0	
			Stretch 2	1	1	3-4th	Cx. pipiens	0	0	
13) Nower Wood (Dorking)	Woodland with temporary and permanent pools	17. 7.86	Top pond	-	-	-	-	-	-	No larvae found
			Marsh pond	-	-	-	-	-	-	" "
			Donkey pond	-	-	-	-	-	-	" "
			Stable pond	-	-	-	-	-	-	" "
			Young pond	13	13	3-4th	Cx. pipiens	0	0	
				14	14	2nd	Cx. pipiens	0	0	
				13	9	pupae	Cx. pipiens	0	0	
			9/159 Beech trees with water-filled holes	35	35	3-4th	Aedes geniculatus	0	0	
				14	3	2nd	Ae. geniculutas	0	0	
				23	17	pupae	Ae. geniculatus	0	0	
14) North Ascot	Children's Paddling pool	19. 7.86	Pool	28 (6=1-2nd)	30	3-4th	Cx. pipiens	0	0	
15) Cromer Norfolk,	-	29. 7.86	Garden Pool	4	4	1-2nd	Cx. pipiens	0	0	Very few in larvae. Copepods found

.../contd 9

APPENDIX I, TABLE A: Page 9

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
15) contd			Pond 1 Golf links	-	-	-	-	-	-	No larvae found
			Pond 2 Golf links	-	-	-	-	-	-	Dry
16) Southrepps Farm	Waterbutts in farm land, surrounding area had temporary water and ditches	4. 8.86	Ditch 1	-	-	-	-	-	-	No larvae found. 1 biting adult = Culiseta annulata
			Marshland	-	-	-	-	-	-	Dry
			Water butt 1	1	1	1st	Cx. pipiens	0	0	Very few larvae
			Water butt 2	4	4	2nd	Cx. pipiens	0	0	" "
			Water butt 3	12	12	2nd	Cx. pipiens	0	0	" "
17) Southrepps	Rural area	4. 8.86	Farm pond	3	3	pupae	-	0	0	No larvae found
			Village pond	52	36	3-4th	Cx. pipiens	0	0	Found copepods of species Eucyclops agilis
				17	2	2nd	Cx. pipiens	0	0	
				23	8	pupae	Cx. pipiens	0	0	

.../contd 10

APPENDIX I, TABLE A: Page 10

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
18)										
Northrepps	Rural area	5. 8.86	Garden	1	1	4th	Cx. pipiens	0	0	
			Water butt 1	6	6	3rd	Cx. pipiens	0	0	Copepods of
				5	5	2nd	Cx. pipiens	0	0	Acanthocyclops
				1	1	pupa	Cx. pipiens	0	0	vernalis found
			Water butt 2	3	3	4th	Cx. pipiens	0	0	
				1	1	2nd	Cx. pipiens	0	0	
				6	6	pupae	Cx. pipiens	0	0	
			Pond	-	-	-	-	-	-	No larvae found
			Village pond	-	-	-	-	-	-	" "
			Roadside pond	-	-	-	-	-	-	Inaccessible
19)										
Roughton area	Rural farmland	6. 8.86	Pond	-	-	-	-	-	-	No larvae found
			Water butt 1	1	1	pupa	-	0	0	
			Water butt 2	45	14	1-2nd	Cx. pipiens	0	0	
20)										
East Runton	Rural area	7. 8.86	Pond	100	50	4th	Cx. pipiens	0	0	
			Pond	-	-	-	-	-	-	No larvae found
21)										
Caterham		15. 8.86	Water butt 1	26	20	4th	Cx. pipiens	0	0	
				15	4	Pupae	Cx. pipiens	0	0	
			Water filled bowl	12	12	3-4th	Cx. pipiens	0	0	
				38 +9 lsts	11	2nds	Cx. pipiens	0	0	
				3	3	pupae	Cx. pipiens	0	0	

.../contd 11

APPENDIX I, TABLE A: Page 11

Main Area	Area Description	Date	Site	No. of Larvae Collected	No. of Larvae Examined	Instar	Species	Comments on Parasites: Micro-sporidian	Comments on Parasites: Other	Other Comments
22) Montpellier	Colony	10. 9.86	Colony	Many	6	3rd	Aedes detritus	0	0	

At a further 21 sites no mosquito larvae were collected, either because none were found or because the sites were dry, flooded or inaccessible. These sites were: Hamm Court, Sunningdale Recreation Ground, Ascot Station area, Virginia Water Station Area, Heatherdown site, Obelisk Pond (Windsor Great Park), Great Pond (Windsor), Brewers Pond (Windsor Great Park), Ascot Place, Folijohn Park, Pond at Camberley, Pond at Winkfield, Coulsdon Common (Surrey), Gainborough Road (Bracknell), Pond at Caterham (Surrey), Bodham Pit (Norfolk), Salthouse Marshes (Norfolk), Rural area at Edingthorpe (Norfolk), Pond at Gimingham (Norfolk), Pond at North Walsham (Norfolk), Beech trees at Newbury.

APPENDIX I, TABLE B: Details of sites visited in Tanzania during November and December 1985 and during June and September 1987 where no microsporidian infections were found.

Main Area	Site	Species Collected	Parasites	
			Microsporidia	Other
1) Between Tanga and Maramba 1985	Kibafuta Marshy pond	Anopheles sp. & Culex sp.	0	0
	Gombero	Anopheles sp.	0	0
	"Grasshopper" site	Anopheles sp. & Culex sp.	0	0
	"Purini" site	Culex sp.	0	0
	"Last" site	Anopheles sp. & Culex sp.	0	0
	Site 2 - Roadside pool beneath bridge	"	0	0
	Site 3 - Roadside pool.	"	0	0
2) Amani 1985	Pit latrine 1	Culex quinquefasciatus	0	0
	Pit latrine 2	" "	0	0
	Pit latrine 3	" "	0	0
	Pit latrine 4	" "	0	0
	Pit latrine 5	" "	0	0
	Pit latrine 6	" "	0	0
	Pit latrine 7	" "	0	0
3) Dar-es-Salam, 1985	Pit latrine	- -	-	-
	Flood area by rubbish tip	Anopheles sp. & Culex sp.	0 0	0 0
	Pool by rubbish tip	Culex quinquefasciatus	0	0
4) Between Muheza and Mombo, 1985	Flooded cow pasture	Anopheles sp. & Culex sp.	0 0	0 0
	Roadside pool	"	0	0
	Night storage pool - Mombo	"	0	0
	Private paddy field - Mombo	"	0	0
5) Muheza, 1985	"Reli" ditch	Anopheles sp. & Culex sp.	0 0	0 0
	Lily pond 1	"	0	0
	Lily pond 2	"	0	0
	Marsh	Anopheles sp.	0	0
	"Small Simba" site	Anopheles sp. & Culex sp.	0	0

.../contd 2

APPENDIX 1, TABLE B: (contd)

Main Area	Site	Species Collected	Parasites	
			Microsporidia	Other
5) Muheza, 1985, (contd)	Sewage works	Culex sp.	0	0
	Marshy Area	Anopheles sp. & Culex sp.	0	0
	Rice field by hospital	-	0	0
	"International" site	Anopheles gambiae	0	Numerous larvae were infected with a cyst forming organism, possibly Coelomomyces sp.
	Pond	Culex sp.	0	0
6) Muheza, 1987	Mzikitini pond	Mansonia africana	0	0
	Zimbiri Pond A	Anopheles sp.	0	0
	Pond B	"	0	0
	Pond C	"	0	0
	Pond D	"	0	0
	Kilimanguidu Pond A	"	0	0
	Pond B	"	0	0
	Mpangala School pond	"	0	0
	Kwamihayo Pond A	-	0	0
	Pond B	-	0	0
7) Mindu, 1987	Village huts	Adult Anopheles gambiae	0	0
8) Jitengi, 1987	Village huts	Adult Anopheles gambiae & An. funestis	0	0

APPENDIX I, TABLE C: *Mansonia uniformis* cultures

Mansonia uniformis (Sri Lanka)

Generation 1

Date	Cumulative Emergence		Cumulative Deaths		Comments
	Male	Female	Male	Female	
2. 4.86	6	1			
3. 4.86	9	3			
4. 4.86	12	6	4	1	
5. 4.86	14	6	4	1	BF 3
6. 4.86	-	-	-	-	
7. 4.86	25	6	4	1	
8. 4.86	34	9	7	3	
9. 4.86	58	15	11	3	
10. 4.86	74	26	12	4	BF 4
11. 4.86	88	40	14	5	BF 2
12. 4.86	93	48	14	6	BF 4
13. 4.86	93	54	16	6	
14. 4.86	93	59	19	6	
15. 4.86	93	64	19	7	(1 EBL)
16. 4.86	94	65	20	9	BF 0 (1 EBL)
17. 4.86	94	67	23	9	BF 2
18. 4.86	94	68	24	11	BF 0
19. 4.86	94	68	27	12	BF 4 (1 EBL)
20. 4.86	94	68	27	15	BF 5 (2 EBL)
21. 4.86	94	68	27	21	(2 EBL)
22. 4.86	94	68	27	21	BF 1

Total of (7 EBL) laid

BF = Blood fed

EBL = Egg batches laid

EBH = Egg batches hatched

Generation 2

Date	MALE Cumulative Emergence			Cumulative Total	FEMALE Cumulative Emergence			Cumulative Total	Cumulative Dead Male/Female		Comments
Bowl No:	1	2	3		1	2	3				
12. 5.86	46										
13. 5.86	73			73	22			22			
14. 5.86	84			84	39			39			
15. 5.86	95			95	60			60		2	
16. 5.86	99	5		104	74			74	2	3	BF 14
17. 5.86	102	17		119	81	3		84	4	7	
18. 5.86	104	19		123	88	5		93	9	14	
19. 5.86	107	21		128	95	5		100	16	22	BF 8
20. 5.86	107	23		130	99	8		107	25	22	(8 EBL)
21. 5.86	107	23	5	135	100	9		109	29	33	
22. 5.86	"	"	"	"	"	"		"	"	"	
23. 5.86	"	"	"	"	"	"		"	"	"	
24. 5.86	"	"	"	"	"	"		"	"	"	BF 0
25. 5.86	107	23	27	157	100	9	21	130	46	45	BF 5 (6 EBH)
26. 5.86	"	"	"	"	"	"	"	"	Total		
27. 5.86	107	23	31	161	100	9	42	151	26		
28. 5.86	107	23	33	163	100	9	54	163	38		
29. 5.86	"	"	"	"	100	9	59	168	50		
30. 5.86	"	"	"	"	100	9	62	171	"		(3 EBL)
31. 5.86	"	"	"	"	100	9	64	173	"		
1. 6.86	107	23	36	166	100	9	67	176	"		BF 13 2 EBH
2. 6.86	107	23	38	168	100	9	69	178	59		
3. 6.86	107	23	39	169	100	9	71	180	65		
4. 6.86	"	"	"	"	"	"	"	"	"		
5. 6.86	"	"	"	"	"	"	"	"	70		
6. 6.86	"	"	"	"	"	"	"	"	"		(6 EBL)
7. 8.86	"	"	"	"	"	"	"	"	"		
8. 6.86	"	"	"	"	"	"	"	"	"		(4 EBH)
9. 6.86	"	"	"	"	"	"	"	"	"		
10. 6.86	"	"	"	"	"	"	"	"	"		
11. 6.86	"	"	"	"	"	"	"	"	81		
12. 6.86	"	"	"	"	"	"	"	"	94		
13. 6.86	"	"	"	"	"	"	"	"	112		
14. 6.86	"	"	"	"	"	"	"	"	133		
15. 6.86	"	"	"	"	"	"	"	"	145		
16. 6.86	"	"	"	"	"	"	"	"	159		
17. 6.86	"	"	"	"	"	"	"	"	163		
18. 6.86	"	"	"	"	"	"	"	"	175		

Bowl 1 contained 2 egg batches
Bowl 2 contained 2 egg batches
Bowl 3 contained 3 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 3

Date	MALE Cumulative Emergence			MALE Cumulative Total	FEMALE Cumulative Emergence			FEMALE Cumulative Total	Cumulative Dead Male/Female	Comments
Bowl No:	1	3	4		1	3	4			
16. 6.86	43			43	1			1		
17. 6.86	73			73	12			12		
18. 6.86	100			100	26			26		
19. 6.86	126			126	45			45		
20. 6.86	137			137	57			57		BF 13
21. 6.86	142			142	75			75		
22. 6.86	142			142	75			75		
23. 6.86	145	23		169	88	4		92	20	BF 25
24. 6.86	147	42		189	96	9		105	50	
25. 6.86	148	65		213	108	26		134	78	
26. 6.86	148	67		214	111	29		140	107	(2 EBL)
27. 6.86	148	77		225	114	34		148	107	BF 26
28. 6.86	149	101		250	117	54		171	133	
29. 6.86	No collection									
30. 6.86	149	105	1	255	117	62		179	133	(1 EBL), (2EBH)
1. 7.86	149	105	2	257	117	71	1	189	184	
2. 7.86	149	105	4	259	117	77	2	196	213	2 EBL
3. 7.86	No collection									
4. 7.86	149	105	28	283	117	79	14	210	213	BF 7 (human)
5. 7.86	No collection									
6. 7.86	"									
7. 7.86	149	105	53	308	117	82	34	233	278	(1 EBH)
8. 7.86	149	105	61	316	117	82	42	241	287	BF 22
9. 7.86	No collection									
10. 7.86	"									
11. 7.86	149	105	62	317	117	82	50	249	287	BF 8 (2 EBL(h))
12. 7.86	"	"	"	"	"	"	"	"	312	(1 EBH)
13. 7.86	"	"	"	"	"	"	"	"	"	(2 EBH)
14. 7.86	"	"	"	"	117	82	51	251	323	(7 EBL)
15. 7.86	"	"	"	"	117	82	53	252	331	
16. 7.86	"	"	"	"	"	"	"	"	339	(5 EBL) (7 EBH)
17. 7.86	"	"	"	"	"	"	"	"	350	
18. 7.86	"	"	"	"	"	"	"	"	"	
19. 7.86	"	"	"	"	"	"	"	"	"	(2 EBH)

Bowl 1 contained 6 egg batches
Bowl 2 contained 2 egg batches (no emergence)
Bowl 3 contained 6 egg batches
Bowl 4 contained 4 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 4

Date	MALE Cumulative Emergence			MALE Cumulative Total	FEMALE Cumulative Emergence			FEMALE Cumulative Total	Comments
Bowl No:	1	2	3		1	2	3		
25. 7.86	6			6	2			2	
29. 7.86	"			"	"			"	
30. 7.86	8			8	6			6	
31. 7.86	8			8	6			6	
1. 8.86	10			10	7			7	
2. 8.86	No collection								
3. 8.86	"								
4. 8.86	"								
5. 8.86	11			11	11			11	
6. 8.86*		24	34	58		5	1	6	
7. 8.86	No collection								
8. 8.86	"								
9. 8.86	"								
10. 8.86		29	41	70		13	7	20	
11. 8.86		34	59	93		14	14	28	BF 12
12. 8.86		41	78	119		16	24	40	
13. 8.86		42	88	130		18	31	49	BF 9
14. 8.86		51	102	153		21	43	64	
15. 8.86		59	112	171		30	53	83	BF 17
16. 8.86		75	127	202		35	68	103	
17. 8.86		76	129	205		39	83	122	(10 EBL)
18. 8.86		76	130	206		53	95	148	BF 24 (3 EBL)
19. 8.86		79	131	210		61	107	168	
20. 8.86		80	131	211		62	110	172	(11 EBL)
21. 8.86		80	133	213		64	114	178	
22. 8.86		"	"	"		64	115	179	BF 18 (1 EBH)
23. 8.86		"	"	"		"	"	"	(9 EBH) (6 EBL)
24. 8.86		"	"	"		"	"	"	(1 EBH)
25. 8.86		"	"	"		"	"	"	(1 EBL)
26. 8.86		"	"	"		"	"	"	(5 EBH)
27. 8.86									3 EBL
28. 6.86	No collection								
29. 8.86	"								
30. 8.86		80	133	213		64	115	179	(1 EBL)
31. 8.86		"	"	"		"	"	"	(1 EBH)

Bowl 1 contained 3 egg batches
Bowl 2 contained 5 egg batches
Bowl 3 contained 8 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

* No more emerged from Bowl 1 after 5.8.85 and all those that had already emerged were lost through a hole which had formed in the cage.

Generation 5

Date	MALE Cumulative Emergence			Cumulative Total	FEMALE Cumulative Emergence			Cumulative Total	Comments
Bowl No:	1	2	3		1	2	3		
8. 9.86	10			10	2			2	
9. 9.86	24			24	6			6	
10. 9.86	37			37	13			13	
11. 9.86	No collection								
12. 9.86	"								
13. 9.86	"								
14. 9.86	43	3	2	48	17	3		20	
15. 9.86	46	18	8	72	18	12	2	33	
16. 9.86	50	32	9	91	23	18	4	45	BF 10
17. 9.86	53	37	22	112	24	26	10	60	BF 10
18. 9.86	56	38	23	117	27	27	12	66	BF 10
19. 9.86	56	41	26	123	27	28	14	69	
20. 9.86	57	43	30	130	27	30	15	72	BF 2
21. 9.86	57	44	31	132	28	30	18	76	(1 EBL) BF 3

Bowl 1 contained 9 egg batches
Bowl 2 contained 9 egg batches
Bowl 3 contained 10 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 6

Date	MALE			FEMALE			Comments
	Cumulative Emergence		Cumulative Total	Cumulative Emergence		Cumulative Total	
Bowl No:	1	2		1	2		
16.10.86	9		9				
17.10.86	24		24	1		1	
18.10.86	48		48	6		6	
19.10.86	63		63	12		12	
20.10.86	79		79	16		16	BF 3
21.10.86	94		94	18		18	
22.10.86	110		110	28		28	BF 2
23.10.86	121		121	42		42	
24.10.86	129	1	130	63		63	BF 13
25.10.86	No collection						
26.10.86		"					
27.10.86		"					
28.10.86	138	6	144	96	1	97	(3 EBL)
29.10.86	139	8	147	103	7	110	BF 19 (1 EBH)
30.10.86	141	14	155	114	11	125	(2 EBH)
31.10.86	141	24	165	115	16	131	BF 20
1.11.86	141	27	168	121	19	140	
2.11.86	141	31	172	121	23	144	(2 EBH)
3.11.86	"	"	"	121	27	148	BF 12 (10 EBL) (1 EBH)
4.11.86	"	"	"	121	32	153	
5.11.86	141	32	173	122	34	156	(7 EBL)
6.11.86	"	"	"	"	"	"	(5 EBH)
7.11.86	"	"	"	"	"	"	(2 EBL) (1 EBH)
8.11.86	No collection						
9.11.86	141	32	173	122	34	156	(8 EBH)
10.11.86	"	"	"	"	"	"	(3 EBL)
11.11.86	"	"	"	"	"	"	(3 EBH)
12.11.86	"	"	"	"	"	"	(1 EBH)

Bowl 1 contained 8 egg batches
Bowl 2 contained 8 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 7

Date	MALE Cumulative Emergence			Cumulative Total	FEMALE Cumulative Emergence			Cumulative Total	Comments
Bowl No:	1	2	3		1	2	3		
20.11.86	2			2	1			1	
21.11.86	5			5	7			7	
22.11.86	No collection								
23.11.86	"								
24.11.86	33			33	15			15	
25.11.86	44			44	22			22	
26.11.86	60	8		68	39			39	BF 9
27.11.86	No collection								
28.11.86	73	42		115	57	13		70	
29.11.86	No collection								
30.11.86	"								
1.12.86	76	71	6	153	66	34	2	102	BF 27 (6 EBL)
2.12.86	80	89	13	182	72	46	4	112	
3.12.86	80	103	22	205	76	65	6	147	(1 EBL)
4.12.86	81	107	28	216	79	72	9	160	(3 EBH)
5.12.86	81	114	36	231	79	79	12	170	(2 EBH)
6.12.86	No collection								
7.12.86	"								
8.12.86	81	121	54	256	82	85	21	188	(20 EBL) (11 EBH) (7 EBH)

Bowl 1 contained 7 egg batches
Bowl 2 contained 9 egg batches
Bowl 3 containing 9 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 8

Date	MALE Cumulative Emergence			Cumulative Total	FEMALE Cumulative Emergence			Cumulative Total	Comments
Bowl No:	1	2	3		1	2	3		
5. 1.87	33	15	14	62	26	20	11	57	BF 8
6. 1.87	38	25	19	82	38	36	17	91	
7. 1.87	42	27	21	90	44	46	24	114	
8. 1.87	No collection								
9. 1.87	"								
10. 1.87	"								
11. 1.87	"								
12. 1.87	44	31	28	103	48	48	26	122	6 EBL
13. 1.87	44	31	31	106	49	50	28	127	BF 12 (6 EBH)
14. 1.87	No collection								
15. 1.87	"								
16. 1.87	44	31	31	106	49	50	28	127	(1 EBL)
17. 1.87	No collection								
18. 1.87	"								
19. 1.87	44	31	31	106	49	50	28	127	(3 EBL)(1 EBH)
20. 1.87	No collection								
21. 1.87	44	31	31	106	49	50	28	127	(2 EBL)
22. 1.87	"	"	"	"	"	"	"	"	(2 EBH)
23. 1.87	No collection								
24. 1.87	"								
25. 1.87	44	31	31	106	49	50	28	127	(1 EBH)
26. 1.87	"	"	"	"	"	"	"	"	(1 EBH)

Bowl 1 contained 7 egg batches
Bowl 2 contained 8 egg batched
Bowl 3 contained 8 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 9

Date	MALE Cumulative Emergence		MALE Cumulative Total	FEMALE Cumulative Emergence		FEMALE Cumulative Total	Comments
Bowl No:	1	2		1	2		
6. 2.87	12		12				
7. 2.87	No collection						
8. 2.87	"						
9. 2.87	66		66	23		23	
10. 2.87	77		77	46		46	
11. 2.87	79		79	53		53	BF 18
12. 2.87	No collection						
13. 2.87	83		83	74		74	
14. 2.87	No collection						
15. 2.87	"						
16. 2.87	88	11	99	82		82	
17. 2.87	92	27	119	84	6	90	(15 EBL)
18. 2.87	92	35	127	87	11	98	
19. 2.87	92	38	130	90	19	109	(6 EBH)
20. 1.87	92	38	130	90	19	109	(8 EBH) (1 EBH)

Bowl 1 contained 7 egg batches
Bowl 2 contained 4 egg batches

Generation 10

Date	MALE Cumulative Emergence		MALE Cumulative Total	FEMALE Cumulative Emergence		FEMALE Cumulative Total	Comments
Bowl No:	1	2		1	2		
17. 3.87	88	46	134	26	9	35	
18. 3.87	109	54	163	38	27	65	
19. 3.87	126	68	194	51	38	89	
20. 3.87	139	82	221	74	55	129	BF 26
21. 3.87	No collection						
22. 3.87	"						
24. 3.87	148	94	242	105	68	173	16 EBL

Bowl 1 contained 7 egg batches
Bowl 2 contained 8 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 11

Date	MALE Cumulative Emergence		MALE Cumulative Total	FEMALE Cumulative Emergence		FEMALE Cumulative Total	Comments
Bowl No:	1	2		1	2		
27. 4.87	11	1	12	1		1	
28. 4.87	17	3	20	5		5	
29. 4.87	20	3	23	8		8	
30. 4.87	21	3	24	10	1	11	BF 7
1. 5.87	23	3	26	10	1	11	
2. 5.87	No collection						
3. 5.87	"						
4. 5.87	"						
5. 5.87	23	3	26	14	3	17	BF 5 (2 EBL)
6. 5.87	24	4	28	18	3	21	(2 EBL)
7. 5.87	26	4	30	21	3	24	(1 EBL) BF 1
8. 5.87	"	"	"	25	3	28	(1 EBH)
9. 5.87	26	5	31	"	"	"	(2 EBL) (1 EBH
10. 5.87	"	"	"	"	"	"	(1 EBH)
11. 5.87	"	"	"	26	3	29	1 EBL BF 6
12. 5.87	"	"	"	"	"	"	(1 EBL)
13. 5.87	No collection						
14. 5.87	26	5	31	"	"	"	(1 EBL)(1 EBH)
15. 5.87	"	"	"	"	"	"	(1 EBL)(1 EBH
16. 7.87	"	"	"	"	"	"	(1 EBH)
17. 5.87	"	"	"	"	"	"	(1 EBH)
18. 5.87	No collection						
19. 5.87	26	5	31	"	"	"	(1 EBH)

Bowl 1 contained 8 egg batches
Bowl 2 contained 8 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 12

Date	MALE Cumulative Emergence		MALE Cumulative Total	FEMALE Cumulative Emergence		FEMALE Cumulative Total	Comments
Bowl No:	1	2		1	2		
3. 6.87	3		3	3		3	
4. 6.87	9		9	6		6	
5. 6.87	No collection						
6. 6.87	"						
7. 6.87	"						
8. 6.87	16		16	15		15	BF 8
9. 6.87	23		23	20		20	
10. 6.87	29		29	21		21	
11. 6.87	33		33	24		24	
12. 6.87	"		"	"		"	BF 8 (3 EBL)
12. 6.87	No collection						
13. 6.87	"						
14. 6.87	"						
15. 6.87	36		36	29		29	
16. 6.87	37	1	38	30		30	(8 EBL)
17. 6.87	No collection						
18. 6.87	"						
19. 6.87	38	11	49	33	6	39	BF 17
20. 6.87	No collection						
21. 6.87	"						
22. 6.87	38	17	55	33	11	44	
23. 6.87	"	"	"	33	13	46	

Bowl 1 contained 7 egg batches
Bowl 2 contained 1 egg batch

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 13

Date	MALE Cumulative Emergence			MALE Cumulative Total	FEMALE Cumulative Emergence			FEMALE Cumulative Total	Comments
Bowl No:	1	2	3		1	2	3		
9. 7.87	2			2					
10. 7.87	3			3					
11. 7.87	No collection								
12. 7.87	"								
13. 7.87	12			12	7			7	
14. 7.87	"			"	9			9	
15. 7.87	12	1		13	"			"	
16. 7.87	12	4		16	9	3		12	
17. 7.87	No collection								
18.7.87	"								
19. 7.87	"								
20. 7.87	12	17		29	9	13		22	BF 11
21. 7.87	12	18		30	9	16		25	
22. 7.87	"	"		"	9	22		31	
23. 7.87	"	"		"	9	25		34	
24. 7.87	"	"		"	9	26		35	BF 11
25. 7.87	No collection								
26. 7.87	"								
27. 7.87	12	18	3	33	"	"		"	(6 EBL)
28. 7.87	12	19	4	34	"	"		"	
29. 7.87	"	"	"	"	"	"		"	BF 3 (1 EBL)(3 EBH)
30. 7.87	No collection								
31. 7.87	12	19	6	36	"	"		"	
1. 8.87	No collection								
2. 8.87	12	19	7	37	"	"		"	(2 EBL)
3. 8.87	No collection								
4. 8.87	12	19	7	37	"	"		"	BF 2
5. 8.87	No collection								
6. 8.87	12	19	7	37	"	"		"	(1 EBH)
7. 8.87	No collection								
8. 8.87	"								
9. 8.87	"								
10. 8.87	12	19	7	37	"	"		"	(2 EBL)

Bowl 1 contained 13 egg batches)
Bowl 2 contained 7 egg batches) not all had hatched
Bowl 3 contained 8 egg batches)

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 14

Date	MALE Cumulative Emergence		MALE Cumulative Total	FEMALE Cumulative Emergence		FEMALE Cumulative Total	Comments
Bowl No:	1	2		1	2		
1. 9.87	2		2				
2. 9.87	3		3				
3-7.9.87	No collection						
8. 9.87	3		3	1		1	
9. 9.87	No collection						
10. 9.87	3		3	1		1	BF 1
11-14.9.87	No collection						
15. 9.87	3		3	1		1	(1 EBL)

Colony lost as egg batch hatched but did not survive.

Bowl 1 contained 7 egg batches
Bowl 2 contained 4 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Mansonia uniformis (Tanzania)

Generation 1

Date	MALE Cumulative Emergence			Cumulative Total	FEMALE Cumulative Emergence			Cumulative Total	Cumulative Dead	Comments
Bowl No:	1	2	3		1	2	3			
29. 5.86			12	12						
30. 5.86			24	24						
31. 5.86			46	46			9	9		
1. 6.86	12		56	68			24	24		
2. 6.86	30	12	61	103	15		42	57	2	BF 1
3. 6.86	40	15	63	118	42	10	52	104	4	
4. 6.86	42	17	64	123	50	10	63	123	21	
5. 6.86	42	19	65	126	55	10	71	136	46	BF 10
6. 6.86	42	21	65	128	58	10	74	142	80	BF 9
7. 6.86	42	121	65	128	58	10	74	142		
8. 6.86	47	22	65	134	50	10	75	145	123	
9. 6.86	"	"	"	"	"	"	"	"	157	BF 3(4 EBL)
10. 6.86	No further collections									(4 EBL)
11. 6.86			"							(4 EBL)
12. 6.86			"							(2 EBH)
13. 6.86			"							(1 EBH)
14. 6.86			"							
15. 6.86			"							(1 EBH)
16. 6.86			"							(4 EBH)
17. 6.86			"							(1 EBH)

Bowl 1 contained 1 egg batch
Bowl 2 contained 1 egg batch
Bowl 3 contained 1 egg batch

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 2

Date	MALE Cumulative Emergence			MALE Cumulative Total	FEMALE Cumulative Emergence			FEMALE Cumulative Total	Cumulative Dead	Comments
Bowl No:	1	2	3		1	2	3			
7. 7.86	7			7	1			1		
8. 7.86	28	1		29						
9. 7.86	No collection									
10. 7.86	"									
11. 7.86	70	11		81	4	3		7		BF 3
12. 7.86	82	18		100	8	7		15	12	
13. 7.86	No collection									
14. 7.86	86	20		106	23			30	25	BF 2
15. 7.86	89	20		109	32	8		40	31	
16. 7.86	90	20		110	22	8		41	34	(2 EBL) BF 19
17. 7.86	"	"		"	34	8		42	42	
18. 7.86	"	"		"	35	9		44		3 BF (2 EBL)
19. 7.86	No collection									
19. 7.86	"									
21. 7.86	90	20		110	35	9		44		(1 EBH) (4 EBL)
22. 7.86	"	"		"	"	"		"		(1 EBH) (2 EBL)
23. 7.86	No collection									
24. 7.86	90	20		110	"	"		"		(2 EBH)
25. 7.86	"	"		"	"	"		"		(2 EBH)
26. 7.86	"	"		"	"	"		"		(2 EBH)

Bowl 1 contained 5 egg batches
Bowl 2 contained 5 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 3

Date	MALE Cumulative Emergence		MALE Cumulative Total	FEMALE Cumulative Emergence		FEMALE Cumulative Total	Comments
Bowl No:	1	2		1	2		
10. 8.86	1		1				
11. 8.86	1		1	2		2	
12. 8.86	2		2	3		3	
13. 8.86	8		8	6		6	BF
14. 8.86	8	1	9	"		"	BF 4
15. 8.86	20	9	29	12		12	
16. 8.86	29	26	55	21	4	25	
17. 8.86	39	30	69	24	7	31	
18. 8.86	46	40	86	31	16	47	BF 15 (3 EBL)
19. 8.86	55	47	102	36	26	72	
20. 8.86	62	52	114	37	35	72	
21. 8.86	64	56	120	37	40	77	BF 17
22. 8.86	64	59	123	39	40	79	
23. 8.86	64	61	125	39	45	84	(9 EBL)(2 EBH)
24. 8.86	"	"	"	40	47	87	
25. 8.86	"	"	"	42	47	89	
26. 8.86	"	"	"	"	"	"	(3 EBH)
27. 8.86	"	"	"	"	"	"	(3 EBH)(4 EBL)
28. 8.86	"	"	"	"	"	"	.(1 EBL)
29. 8.86	No collection						
30. 8.86	64	61	125	"	"	"	(2 EBH)
31. 8.86	"	"	"	"	"	"	(3 EBH)

Bowl 1 contained 4 egg batches
Bowl 2 contained 4 egg batches

BF = Blood fed
EBL = Egg batches laid
EBH = Egg batches hatched

Generation 4

Date	MALE Cumulative Emergence		MALE Cumulative Total	FEMALE Cumulative Emergence		FEMALE Cumulative Total	Comments
Bowl No:	1	2		1	2		
28.10.86	5		5	2		2	
29.10.86							
30.10.86							
31.10.86				1			Colony had become mixed with Sri-Lankan, ans so was sacrificed

Bowl 1 contained 5 egg batches
Bowl 2 contained 8 egg batches

APPENDIX II

Chlorella culture:

An inoculum of algae was introduced to a vigorously aerated solution of salts and allowed to grow. The salt solution was changed every 6 weeks.

Paramecium culture:

Concentrated lettuce juice (100g lettuce leaves scalded then blended with 200ml distilled water and filtered through 8 layers of muslin) diluted in Dryl's solution and autoclaved, provided the basic nutrient medium. To approximately 40ml of lettuce juice 0.4ml of 1.5mM $CaCl_2$ was added, and the resulting solution seeded with Klebsiella sp. bacteria (grown on MacConkey's Agar). This was incubated at 27°C for 24 h to encourage the growth of bacteria, on which the paramecium feed. Existing colonies of Paramecium tetraurelia were then divided, and made up to their original volumes with fresh lettuce and bacteria solution. Paramecium fed weekly in this way thrived on the Klebsiella provided.

Modified Karnovsky's fluid

Mix:	Paraformaldehyde	2 g
	$CaCl_2$	25 mg
	Distilled water	20 ml

Heat to 65°C; add 2 drops of 1 M NaOH;

Stir until clear; cool to room temperature;

Add gluteraldehyde (25%), 10 ml; and

Make up to 50 ml 0.2 M sodium cacodylate buffer, pH 7.4.