OPERATIONAL AND SCIENTIFIC NOTES

THE DISTRIBUTION OF *AMBLYOSPORA* (MICROSPORA) SP.—INFECTED OENOCYTES IN ADULT FEMALE *CULEX SALINARIUS:* SIGNIFICANCE FOR MECHANISM OF TRANSOVARIAL TRANSMISSION[1]

DONALD W. HALL

Department of Entomology & Nematology, University of Florida, Gainesville, FL 32611

The microsporidian parasite *Amblyospora* sp. of *Culex salinarius* Coquillett exhibits dimorphic development (Andreadis and Hall 1979a). In male hosts the parasite invades fat body, undergoes meiosis (Hazard et al. 1979) and forms large numbers of haploid spores killing most male larvae as fourth instars. In females, infection is restricted to larval oenocytes, and the parasites do not multiply significantly until late pupal or early adult stages at which time they gradually fill the oenocytes. When the host takes a blood meal, the parasites form spores (diploid) and subsequently infect the developing oocytes thereby ensuring transovarial transmission to the next generation (Andreadis and Hall 1979a).

Amblyospora sp. infects only the large larval oenocytes and not the smaller adult oenocytes. The larval oenocytes form groups of 5 or 6 closely packed cells on each side of abdominal segments 1 to 8 lying in a pocket of the lateral fat body (Christophers 1960, Clements 1963).

A similar life cycle exists in *Parathelohania anophelis* (Kudo) (= *Thelohania legeri* Hesse), a microsporidian parasite of *Anopheles quadrimaculatus* Say. Hazard and Anthony (1974) reported that *P. anophelis*-infected oenocytes adhere to the ventral diverticulum and anterior midgut cells of the adult female host. When the mosquito takes a blood meal, the oenocytes dislodge and migrate to the ovaries where they become involved in the transovarial transmission of the parasite to the progeny of the infected female. The present study was initiated to determine whether the same phenomenon occurs in *Amblyospora* sp.-infected *Cx. salinarius.*

Healthy and *Amblyospora*-infected *Cx. salinarius* were colonized by James Haeger, Florida Medical Entomology Laboratory, Vero Beach, FL. Larvae were reared in 33 × 21 × 15 cm enameled pans, each with 75 individuals. One hundred ml of tap water and approximately 30 mg of koi-goldfish food (Aquatrol Inc., Anaheim, CA) were added at hatch and at 2 and 5 days post-hatch. On alternate days thereafter until pupation or death of larvae, 15 ml of 10 mg/ml brewer's yeast suspension were added. Both larvae and adults were maintained at 25±2°C under natural photoperiod. Caged adults were given 5% sucrose and raisins as carbohydrate sources, and females were blood-fed on guinea pigs. Since almost all males from the infected colony died during the 4th larval stadium, males from the healthy colony were used to inseminate infected females.

Mosquitoes for histological studies were fixed in Carnoy's solution and embedded in paraffin. Thick serial sections were cut at 12 μm (to facilitate oenocyte counts) and stained with Delafield's hematoxylin and eosin Y. Sections were scanned at 100x magnification and clumps of oenocytes were differentiated at 400x. Syncytia were counted as single oenocytes. Adult females of 3 age groups were examined. These were 4–7, 10–13, and 18–21 days post-emergence. The 18–21 day post-emergence group was fed blood 77 hr prior to fixation.

In healthy mosquitoes, the larval oenocytes break down during the pupal and early adult stages, but in infected individuals they persist, undergoing fusion during the pupal stage to form syncytia containing two to seven nuclei (Fig. 1) per syncytial oenocyte. Also during this period, the infected oenocytes break loose from the fat body and begin to circulate throughout the hemocoel. Examination of serial sections revealed the presence of infected oenocytes in all regions of the hemocoel (Table 1) including the head and particularly the thorax in the vicinity of the foregut. The distribution data in Table 1 are not amenable to statistical analysis

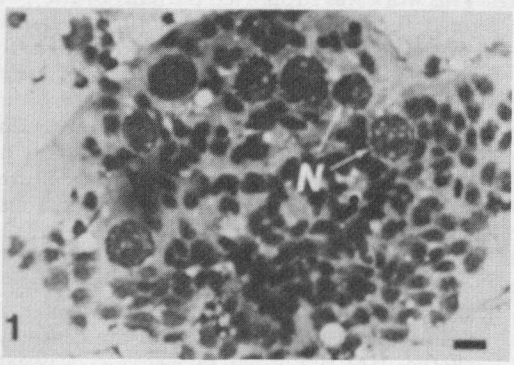

Fig. 1. *Amblyospora*-infected syncytial oenocyte with seven nuclei (N). Giemsa stain (line = 20 μm).

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Table 1. Distribution of *Amblyospora*-infected oenocytes in adult female *Culex salinarius*.

Age in days (Post-emergence)	Mean number of oenocytes/body regions								
			Abdominal segments						
	Head	Thorax	I	II	III	IV	V	VI	VII–VIII
4–7 (n = 8)	0.9	15.9	3.0	1.8	2.9	2.8	3.1	3.1	2.0
10–13 (n = 1)	0.3	14.4	1.1	2.7	2.1	2.5	3.2	2.7	0.5
18–21* (n = 9)	0.7	12.3	2.7	2.7	2.3	1.3	0.8	2.4	0.9

* Fed on blood 77 hr prior to fixation.

because of the inability to determine the frequency of fusion of the oenocytes into syncytia in the different body segments and the fact that the hemocoel compartments of the various body regions are not comparable in volume. The data are presented merely to demonstrate the wide distribution of the oenocytes in adult females of different ages, and that there is no marked difference in distribution in blood-fed mosquitoes.

The microsporidia have a specialized tubular structure, the polar filament, which is coiled within the spore. Under appropriate conditions the polar filament is forcibly extruded, often penetrating the host tissue, and the sporoplasm then exits the spore through the polar filament and enters the host tissue. Andreadis and Hall (1979b) reported that a very high percentage (ca. 90% over 5 gonotrophic cycles) of the progeny of an infected *Cx. salinarius* female are infected. The exact mechanism of infection of the developing oocytes is not known. For the oocytes to be penetrated directly by the extruded polar filaments, the oenocytes would have to be clustered around the ovaries. This has been suggested as one possible mechanism for infection of the oocytes (Andreadis and Hall 1979a). Although oenocytes were sometimes observed adjacent to the ovaries in the present study, this occurrence did not seem to be sufficiently common to account for infection of most of the developing oocytes. There is certainly no preferential migration of the infected oenocytes to the ovaries in blood-fed *Cx. salinarius* as reported for *P. anophelis*-infected oenocytes in *An. quadrimaculatus*.

An alternative hypothesis for oocyte infections is that the spores extrude their polar filaments and release the sporoplasms into the hemolymph. The sporoplasms could then be carried to the ovaries. Extensive electron microscopic studies of mosquitoes during the period 72–96 hr after a blood meal will be required to elucidate the exact mechanism of infection of the oocytes.

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EVALUATION OF A PORTABLE CO_2 GENERATOR FOR SAMPLING BLACK FLIES

J. L. SHIPP

Research Station, Agriculture Canada, Lethbridge, Alberta T1J 4B1

Black fly attacks on cattle are a serious problem in Alberta and Saskatchewan, Canada, affecting beef and milk production, decreasing the calving rate and causing death (Fredeen 1977, Haufe and Croome 1980). Permethrin, registered in Canada for on-farm control of adult black flies, protects cattle for only 10 days (Shemanchuk 1981). Because black fly attacks can occur any time from June through September, accurate monitoring of the adult black fly population is useful for predicting when to apply chemical treatments to cattle.

The dry ice-baited silhouette trap has been shown to be an effective method for sampling populations of adult black flies (Shipp 1985). This trap, however, must be examined daily to