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STUDIES ON THE LIFE CYCLE AND TRANSMISSION OF
COUGOURDELLA SP., A MICROSPORIDIAN PARASITE OF
GLOSSOSOMA NIGRIOR (TRICHOPTERA: GLOSSOSOMATIDAE)

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ABSTRACT

The trichopteran *Glossosoma nigrior*, the dominant benthic invertebrate grazer in Michigan trout streams, hosts a microsporidian (Protozoa) pathogen, *Cougourdella* sp., which strongly regulates the population density of larvae in the stream. In order to better understand the interactions between these two species, two possible modes of pathogen transmission, oral and transovum, were investigated. While both sexes of adult *G. nigrior* were found to be infected with mature environmental spores, spores were not found associated with reproductive tissue. This suggests that transovum transmission does not occur in this system. *Glossosoma nigrior*, when exposed to viable spores taken from infected larvae, did not produce *Cougourdella* sp. infections, which suggests that oral transmission also does not occur. It is possible that an intermediate host is required.

The trichopteran *Glossosoma nigrior* (Banks) is the dominant benthic invertebrate grazer in cold-water Michigan trout streams (Kohler and Wiley 1992). The larvae build small, saddle-shaped cases out of rock and silk (Fig. 1, Wiggins 1996) and feed as scrapers in the shallow erosional habitats or riffles of the stream (Merritt and Cummins 1996). There are at least 283 streams in Michigan's lower peninsula which have suitable conditions to support populations of *G. nigrior* (M. Wiley, pers. comm.). Larvae can attain mean population densities of over 1,000 individuals/m^2 (Kohler and Wiley 1992).

Glossosoma nigrior populations host a microsporidian (Protozoa) pathogen. Microsporidia, important primary pathogens of insects, are obligate intracellular parasites (Federici and Maddox 1996). The undescribed microsporidium which infects *G. nigrior* populations is probably in the genus *Cougourdella* (Hesse) (Fig. 2, Kohler and Wiley 1992). This placement is based on the gross and ultrastructural morphology of the environmentally resistant spore which is roughly tear-shaped and is nearly identical to the description by Larsson (1989) of a *Cougourdella* sp. isolated from *Polycentropus* (J. Maddox, pers. comm.). When *G. nigrior* populations become infected with *Cougourdella* sp., the host populations decline precipitously (Kohler and Wiley 1992). In the lower peninsula of Michigan, *Cougourdella* sp. infections

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Figure 1. *Glossosoma nigrior* larvae, with and without case; bar = 6 mm.

have been found in 49 of 62 examined streams supporting *G. nigrior* populations (M. Wiley, pers. comm.). The pathogen has also been found in the upper peninsula of Michigan in 4 of 21 examined streams, and in Maine in 10 of 13 examined streams (M. Wiley, pers. comm.). Subsequent to epizootics, which were first observed in 1988, several previously undetected species of caddisflies were observed in streams that had been dominated by *G. nigrior,* such as *Agapetus hessi* and *Neophylax oligius.* The timing of their appearance suggests that these species were competitively excluded by *G. nigrior* (Kohler and Wiley 1997). Recurrent *Cougourdella* sp. epizootics appear to be responsible for maintaining *G. nigrior* populations at low but detectable levels (Kohler and Wiley 1992) and increasing the faunal diversity among filter-feeder and grazer feeding guilds (Kohler and Wiley 1997). In the laboratory, we observed that infected *G. nigrior* usually lyse after death, releasing large numbers of environmentally resistant spores (environmental spores). These environmen-
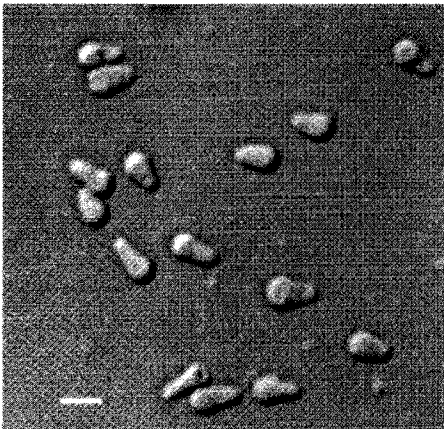

Figure 2. *Cougourdella sp.* environmental spores from *G. nigrior* larvae; bar = approximately 3 µm. Photo courtesy of J. V. Maddox, Illinois Natural History Survey.

tal spores, when introduced into an artificial stream system appear to become trapped in the polysaccharide matrix produced by the substrate-attached diatomaceous algae upon which the insects feed. We have found pupal cases in natural streams that contained *Cougourdella* sp. environmental spores.

Little else is known about the life cycle of this important pathogen, including how *Cougourdella* sp. is transmitted between *G. nigrior* individuals. Four major modes of transmission are employed by microsporidia: vertical transmission from parent, usually maternal, to offspring, horizontal transmission between conspecific individuals, mechanical transmission via parasitoids, and transmission via an obligate intermediate host ("indirect horizontal transmission" [Becnel and Andreadis 1999]). In this paper, we report the results of studies in which we examined two possible modes of transmission of *Cougourdella* sp. in *G. nigrior*, vertical and horizontal.

MATERIALS AND METHODS

Vertical transmission. To evaluate the possibility that *Cougourdella* sp. is vertically transmitted, 52 *G. nigrior* pupae were collected from Seven
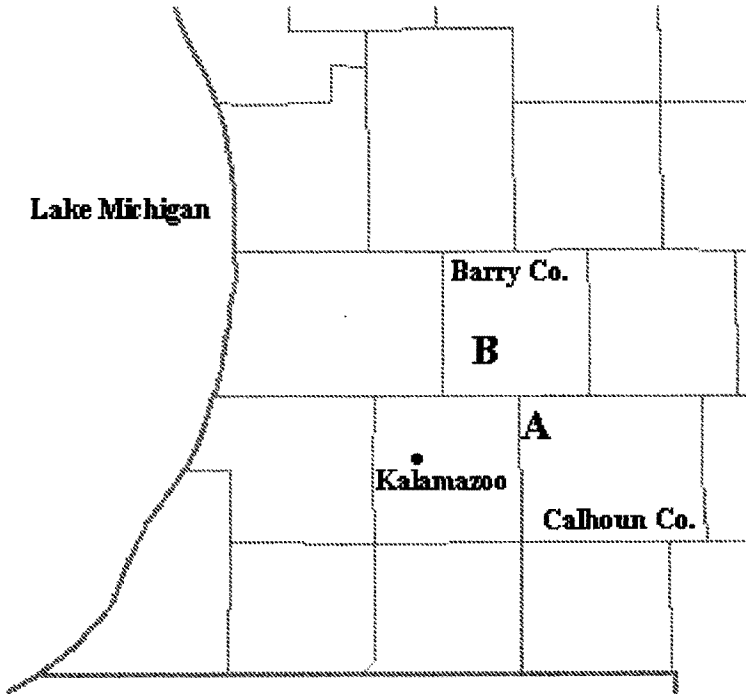

Figure 3. Map of southwestern Michigan. A = Seven Mile Creek, B = Little Pine Lake Creek.

Mile Creek (Calhoun Co., MI; Fig. 3), a site known to support *Cougourdella* sp. infections. At the same time, pathogen prevalence was estimated as per Kohler and Wiley (1992). The pupae were reared to eclosion in the laboratory. Fat body and reproductive tissues from emergent adults, as well as the contents of pupal cases from non-emergent pupae, were examined under 400X phase-contrast microscopy for the presence of microsporidia.

Horizontal transmission. To determine whether horizontal transmission occurs in this system, *Cougourdella* sp. environmental spores were collected by centrifugation from infected homogenized *G. nigrior* larvae, mixed with algae, and allowed to settle in cups for 48 h. The microsporidian spores became trapped in the polysaccharide matrix secreted by the diatoms. Forty-six *G. nigrior* larvae from Little Pine Lake Creek (Barry Co., MI) (Fig. 3), a site where no infections have been found, were allowed to feed upon the algal-spore mixture for 3 h at 8°C. Three larvae were examined at 3 h post inoculation (pi) to obtain an estimate of the number of spores ingested. A squash preparation was made of the larval midgut and contents were examined under 400X phase contrast microscopy. Spores were scored as "germinated" or "ungerminated" as per Undeen (1997).

The remaining larvae were held in a flume with constant water flow at 8° C. Three to five larvae were sampled every 12 h pi for the first 72 h and then every 24 h until 240 h pi (Andreadis 1994, Johnson et al. 1997, Solter and Maddox 1998). Only midgut tissues were sampled between 12–48 h pi, because most *per os* microsporidian infections in insects are initiated in these tissues (Maddox et al. 2000). Both midgut and fat body tissues were sampled between 60–240 h pi, because our dissections indicated that fat body is the target organ for the production of *Cougourdella* sp. environmental spores. Fresh tissues were examined under 400X phase-contrast microscopy, and Giemsa stains were made (Undeen and Vavra 1997) to detect the presence of meronts and environmental spores under 400X bright field microscopy.

Latency of infection. In order to determine whether *Cougourdella* sp. infections are latent in female *G. nigrior* larvae as they are for many *Amblyospora* species in female mosquitoes, 50 *G. nigrior* pupae were sexed and squash preparations were made of pupal tissues and examined under 400X phase-contrast microscopy.

RESULTS

Vertical transmission. The prevalence of *Cougourdella* sp. in *G. nigrior* larvae at the time pupae were collected from the stream was determined to be 70%. Of the 52 pupae reared in the laboratory, 28 emerged as adults. Of those 28 adults, 39% (11 adults) were infected. Eighteen of the 24 non-emergent pupae (75%) were infected (Table 1). Both sexes of adults were found to be infected and the environmental spores appeared morphologically identical to those found in infected larvae. The spores that were found in the adults were associated with the fat body tissues. Reproductive tissues were not infected.

Horizontal transmission. At 3 h pi, the number of spores in the midgut lumen of *G. nigrior* larvae ranged from 650 spores in one small larva to 5,000 and 8,000 in the two larger larvae. The percentage of germinated spores ranged from 80–95%. At 12 h pi, *Cougourdella* sp. spores were absent in the midgut lumen of *G. nigrior* larvae. Neither vegetative stages nor environmental spores were detected in the midgut and fat body tissues at any other time period pi.

Table 1. Prevalence of *Cougourdella* sp. infection in *Glossosoma nigrior* pupae and survivorship to eclosion in the laboratory.

	Infected	Uninfected	Total
Emerged	11	17	28
Non-emergent	18	6	24
Survivorship	0.3793103	0.739130435	

Latency of infection. Heavily infected fat body tissues were found in both male and female pupae.

DISCUSSION

In order for vertical transmission to be the main transmission mechanism in epizootics of *Cougourdella* sp. in the *G. nigrior* host, infection should be found in a relatively high percentage of adults and the pathogen should be able to infect the eggs and/or the accessory glands of infected females. The prevalence of *Cougourdella* sp. infections in adult *G. nigrior* was 39%, which is considerably lower than the 70% prevalence in larvae from Seven Mile Creek at the time of collection. Additionally, 62% of the infected pupae were unable to eclose which, along with the observed precipitous host population declines, suggests a sizable reduction in survival due to infection. While some infected adults eclosed, neither vegetative forms nor environmental spores were found associated with the reproductive organs of either sex. If transovarial transmission occurs in this system, the mechanism is not clear.

It is possible that the microsporidian spores have a different form in female larval caddisflies as occurs in the genus *Amblyospora* (Hazard and Oldacre), a mosquito pathogen (Johnson et al. 1997). However, approximately 60,000 *G. nigrior* larvae were examined for parasitism by *Cougourdella* sp. and no alternate spore forms were observed at the level of light microscopy. We do not know if transovum transmission occurs, or is even important in this system. Only one report of an observation of *G. nigrior* eggs exists. This anecdotal report by Cummins (Anderson and Bourne 1974) did not identify the stream where the eggs were observed, and *G. nigrior* has not been successfully reared in the laboratory. The possibility of transmission of the parasite on the surface of the eggs cannot be dismissed. While some pathogens may also be transmitted venereally from male to female host, this is rare for microsporidia (Tanada and Kaya 1993) and was not evaluated for this system. The 39% of infected adults is unlikely to produce the high levels of mortality observed in the field (Kohler and Wiley 1992) if *Cougourdella* sp. is transmitted by vertical transmission alone. Anderson and May (1981) showed in theoretical models that vertical transmission cannot maintain microparasitic infections in systems where the parasite kills its host before the host reproduces. While some of the infected *G. nigrior* do survive to maturity, most in our experiment did not, which suggests that a different mechanism is at least partially responsible for transmission of the pathogen.

The absence of *Cougourdella* sp. vegetative forms or spores in the midgut or fat body tissues of inoculated host larvae despite high germination rates suggests that the spores produced in *G. nigrior* larvae are unable to initiate reproduction in the tissues of conspecific larvae. There are no known cases of a single population of the natural host being immune to microsporidia and the mechanical nature of spore germination makes this unlikely to occur.

Other aquatic microsporidia have been shown to infect 100% of exposed, susceptible hosts at 20% of the dosage used in this experiment (Andreadis 1994). These results suggest that, despite the germination of the environmental spores in the midgut lumen, horizontal transmission between *G. nigrior* larvae does not occur.

Vertical transmission alone is unlikely to initiate an epizootic of the magnitude observed in the streams we studied, and no horizontal transmission was observed in laboratory experiments. It is possible that *Cougourdella* sp., like several *Amblyospora* spp. pathogens of mosquitoes (Andreadis 1985a, Becnel 1992), requires an intermediate host in which to complete its life cycle. The life cycle of *Cougourdella* sp., however cannot be assumed to be identical to that of *Amblyospora*, because the respective hosts have different life cycles. Mosquito hosts of *Amblyospora* spp. have discrete seasonal life cycles, and sporulation in female mosquitoes is induced by a blood meal (Andreadis 1985b). In contrast, the *G. nigrior* larval hosts of *Cougourdella* sp. are present in streams throughout the year and the adults of *G. nigrior* do not appear to possess a functional gut, suggesting that they do not feed. Nevertheless, some level of comparison between these two systems is useful, as both hosts are aquatic and neither host appears to be susceptible to horizontal transmission.

Many of the *Amblyospora* spp. which infect mosquitoes produce latent infections in female hosts. Environmental spores of *Cougourdella* sp. were found in the fat body of female *G. nigrior* pupae, suggesting that in this system, latent infections do not occur.

Microsporidia in both *Cougourdella* sp. and *Amblyospora* sp. have been found to infect members of the Copepoda (Sprague 1977, Andreadis 1985a). *Cougourdella* species are only known from trichopterans and copepods (Sprague 1977), and it is possible that the intermediate host will be found in one of these groups. Currently, we are examining microsporidian-infected copepods for the presence of this pathogen.

ACKNOWLEDGMENTS

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