Introduction

The microsporidian parasite Cougourdella sp. (Fig. 1A) is known to infect populations of the caddisfly Glossosoma nigrior (Trichoptera: Glossosomatidae; Fig. 1B) in coldwater trout streams in Michigan and Maine. Infections invariably result in host death before hosts reach the adult stage. Glossosoma is a strong interactor in trout stream ecosystems, and has pronounced effects on food web structure and function (McAuliffe 1984, Kohler 1992). Where present, Cougourdella has keystone effects on food web structure through its impacts on Glossosoma population dynamics (Kohler and Wiley 1997) (Fig. 2). Whether such effects occur more generally is unknown in part because Cougourdella’s distribution has not been described. Our objectives were to survey coldwater streams in the eastern United States from Georgia to Vermont to determine: 1) if Cougourdella occurs throughout the range of Glossosoma nigrior, 2) if microsporidiosis in Glossosoma is produced by a single species of Cougourdella, and 3) whether disease epizootics occur in streams outside of Michigan and Maine.

We attempted to collect G. nigrior larvae and pupae from five streams in Georgia, 14 in Virginia, 17 in Pennsylvania, and 10 in Vermont. At each site we tried to collect 100 individuals. All individuals collected were preserved immediately in 70% ethanol.

To screen for Cougourdella infection, individuals were homogenized in ~ 0.5 mL distilled water in 1.5 mL centrifuge tubes. Homogenates for each individual were examined for infection using phase contrast microscopy at 400x. If no infections were detected, the remaining larvae were screened in groups of two (i.e., 2 larvae per centrifuge tube). Larvae and pupae were tested separately because disease prevalence is often lower in pupae. Cougourdella prevalence in each G. nigrior population was estimated using procedures described in Hoenig et al. (1987).

We used DNA sequencing of the SSU rDNA gene to determine if the same species of Cougourdella was present in G. nigrior populations in the eastern United States as in Michigan. DNA was extracted from Cougourdella sporonts from isolates obtained in Georgia, North Carolina, and Pennsylvania, amplified by PCR using microsporidian-specific, primers, and gel-extracted PCR products were sequenced at the University of Michigan DNA Sequencing Core.

Results

SSU rDNA gene sequences from Georgia, North Carolina, Pennsylvania and Michigan Cougourdella isolates were nearly identical (> 98% similarity), which suggests that a single Cougourdella species infects G. nigrior populations in the eastern United States.

Cougourdella was present in streams supporting Glossosoma nigrior populations in all regions surveyed (Table 1). Overall, 75.6% of G. nigrior populations were infected by Cougourdella. The proportion of populations infected was very similar for the subset of streams where ≥ 10 G. nigrior were collected (74.2%; Table 1).

Conclusions

The microsporidian parasite Cougourdella sp. is associated with Glossosoma nigrior populations throughout G. nigrior’s range in the eastern United States.

Available evidence suggests that a single species of Cougourdella is responsible for disease epizootics in G. nigrior populations.

Nearly 50% of infected G. nigrior populations exhibited relatively high disease prevalence. This suggests that disease epizootics are common, and that Cougourdella likely has keystone effects on stream food web structure throughout the eastern United States.

Future Research

We will apply pyrosequencing techniques to the Cougourdella genome to search for microsatellite markers.

Candidate markers will be screened and a set of markers will be used to determine the genetic structure of Cougourdella populations throughout G. nigrior’s range in the eastern United States.

Analysis of Cougourdella population genetic structure over this broad spatial scale should help to answer questions about the history of the host-parasite relationship and Cougourdella’s life cycle.

Acknowledgements

Funding for this research was provided by SFSA and FRACAA awards from WMU and from grants from the National Science Foundation. Julie Ryan and Phil Sawatzki performed the DNA analyses. Jason Glatz prepared the map.

References


Table 1. The number of streams sampled in each state in which Glossosoma was found, the number of streams in which at least 10 Glossosoma individuals were collected, and the number of those streams in which Cougourdella was detected.

<table>
<thead>
<tr>
<th>State</th>
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<th>≥ 10 Glossosoma</th>
<th>All Streams with Cougourdella</th>
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<tr>
<td>Georgia</td>
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<td>Virginia</td>
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<td>Vermont</td>
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Figure 1. A) Electron micrograph of a Cougourdella spore in cross-section (approximate dimensions: 2.5 x 7 µm). B) Glossosoma larva (length ~ 5 mm).

Figure 2. Glossosoma density, expressed relative to the mean pre-collapse density, as a function of disease prevalence for two streams in southwest Michigan, based on > 15 year time series for each stream. Each data point represents 1 Glossosoma generation. Piecewise linear regression (using SiZer in R) suggests prevalence ≥ 10% (95% CI = 2.36) is sufficient to maintain populations at low density.

Figure 3. Incidence and prevalence of Cougourdella in Glossosoma nigrior populations.

Distribution and Prevalence of a Keystone Parasite in the Eastern United States

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Cougourdella prevalence in G. nigrior populations ranged from 0 to 100% (Fig. 3). Disease prevalence was ≥ 20% in 15 of 31 streams (48.4%) in which Cougourdella was present. G. nigrior populations supporting high disease prevalence were present in all regions surveyed (Fig. 3). For comparative purposes, mean disease prevalence in Michigan and Maine streams that have been surveyed for > 10 years is shown in Figure 3. The incidence of disease in Michigan and Maine G. nigrior populations (62 of 70; 88.6%) and prevalence levels are quite similar to those observed in the streams surveyed in 2009 (Fig. 3).

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