

The Effect of Parasite Gregarine on the Histology of the Stonefly *Paragnetina media*

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ABSTRACT

The sporozoan gregarine is usually found in the haemocoel or gut lumen of a certain species of crustacea or insects in an extracellular parasitic status. Those gregarines found in the stonefly *Paragnetina media* Walker, measured 200-250 μ long. Large amount of glycogen was shown in the endoplasm of adult gregarines by means of PAS staining and, meanwhile, glycogen was hardly seen in the midgut cells of the host. Furthermore, seriously infection of the gregarine leads to midgut cell necrosis, delay molting and eclosion; and consequently misses the exact time of mating.

Introduction

The order Plecoptera (stonefly) is of interest because its members are among the most primitive of winged insect; their nymphs are aquatic and are important in the food chain. In general they populate only well aerated waters and are sensitive to the presence of pesticides.

Paragnetina media is a common stonefly found in a wide variety of streams and even in lakes and ponds (Claassen 1931). The early instars live in deep water on aquatic vegetation, while the older instars are voracious carnivores, living under stones in shallow ripples or bank (Tarter and Krumholz 1971). Adults do not feed, living for about 10 days after emergence.

A species of gregarines was found in the gut lumen of the nymphs of *Paragnetina media* collected from a tributary of the Grand River in Connestogo, Ontario, Canada. This study examines the histopathological evidence of the parasite effect on the midgut

cells of the stonefly.

Material and Methods

Nymphs of *Paragnetina media* were collected in shallow streams by moving rocks and letting the nymphs drift on to a screen held a few feet downstream.

Excised gut tissue was rapidly fixed in Rossman's fluid at 0-4°C for 24 h. Fixed tissue was dehydrated in 50%, 100% cellosolve with 0.5% iodine, and then in 100% cellosolve and then embedded in glycol methacrylate in gelatin capsules (Bennett 1976). They were sectioned at 2 μ with glass knives, and stained with PAS reagent, and if desired use toluidine blue as counter stain.

Results

The gregarines found in the midgut lumen of the stonefly nymphs belong to sporozoans of Phylum Protozoa, and of extracellular parasites.

The young adults, cephalonts, push their

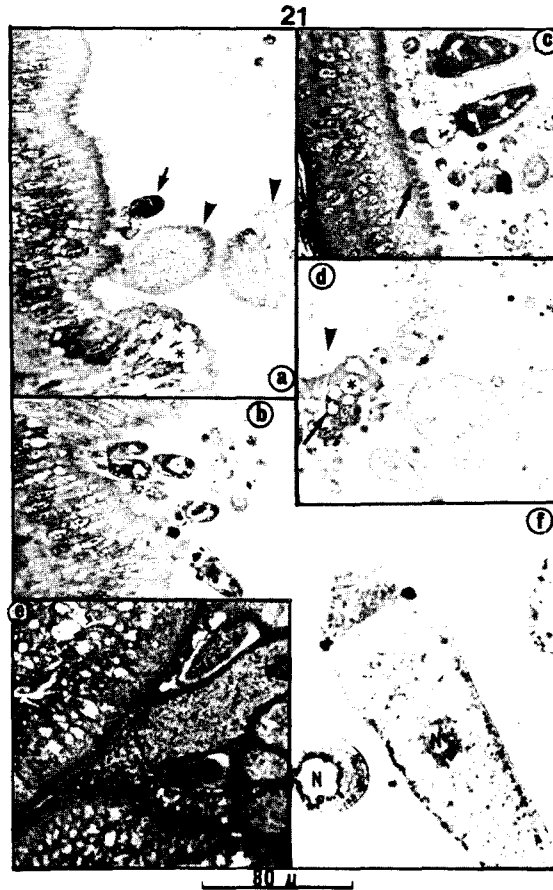


Figure 2. Gregarines (2)

- a. Cephalont (arrow) is more darkly stained than sporonts (arrow heads).
- b.c. Cephalonts, and epimerites (arrow in c).
- d. Degenerated midgut epithelial cells (arrow head) containing nuclei and spores (arrow).
(*)-vacule where epimerite resided.
- e. A sporont with its protomerite inserted into depression of the epithelial wall.
- f. Glycogen concentration is highest in vicinity of nucleus (N) in sporont.

epimerites into host's epithelial cells, damaging the brush border, and remaining attached so as to absorb cytoplasmic fluid continuously. They absorbed most of the nutrient, especially carbohydrates of midgut cells leaving the apical region empty (Fig. 1, 2)

and do not store glycogen (Compare Fig. 1a and 1b).

The mature adults, sporonts, are about 200μ to 250μ in length, and are free in the lumen, with large amount of storing glycogen granules (Fig. 1b, 1e, 2f) for the

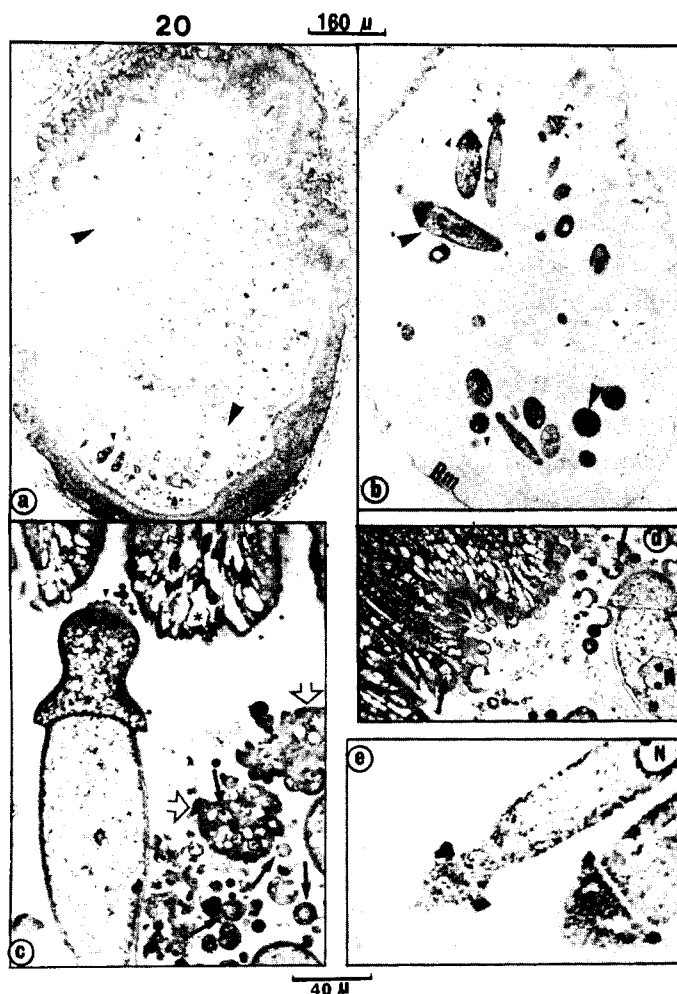


Figure 1. Gregarines (1)

- a. T.s. of ventriculus, containing parasitic gregarines. All epithelial cells are damaged seriously; large lesions occur in apical cytoplasm due to the activity of the cephalonts, causing a decrease in cell volume.
- b. PAS staining to show depletion of glycogen in midgut epithelium but increased in sporonts (large arrow heads). The cephalonts (small arrow heads) are continuously sucking cytoplasmic fluid by inserting their epimerites into host cells; they do not store glycogen.
- c.d. Higher magnification of part of a.
- c. After detaching from an epithelial cell, scars (small arrow head) remain on the protomerite. The host cells have been damaged and appear empty at apex(*).
- Hollow arrow-rupture of cyst and release spores.
- Arrows-spores, with gelatinous coats. (Mano-Sabaratnam 1971)
- d. Epimerites (small arrow heads) remain in apical cytoplasm after formation of sporonts.
- Arrows-spores(or hyaline inclusions). (Canning 1956)
- e. Higher magnification of part of b, showing the stored glycogen in the cytoplasm of sporonts but not in nucleus (N).

development into multiple spores (Fig. 1c) later.

The multiple spores enclosed in a thick gelatinous coat will be ruptured and let single spores release out (Fig. 1c). Those spores will be brought outside the host and continue infect other aquatic insects of the same population or community.

Discussion

The gregarines absorbed most of the nutrient in the midgut. Concomitantly, the cell volume, the glycogen depositions and the fat bodies were greatly decreased in infected nymphs. Gregarine infection also retarded the growth rate and molting rate of the host (resulted from ecological data of infected and normal colonies); this resulted in delayed emergence of imagoes and probably a consequent loss of the chances of mating in the very short synchronous pattern of the emergence period (about 10 days). This in turn, likely led to a decimation of the population. The extracellular (parasite, a gregarine, described here is expected) to have a lower degree of host specificity than tissue parasites, allowing the species to be transferred among different orders of

aquatic insects in the same community. The physiological symptoms in *P. media* infected with the gregarin are not known, but since a large population of the parasite exists in the midgut of the nymphs examined, the effects on impairment of nutritional and reproductional functions must be serious.

References

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簇蟲 (Gregarine) 對石蠅 *Paragnetina media* 中腸的影響

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摘 要

簇蟲是屬於原生動物門，孢子蟲綱。寄生於昆蟲的血體腔或消化道內，屬於胞外寄生，在石蠅 *Paragnetina media* 的中腸內所發現的簇蟲成長時體長約 200 至 250 μ ，若以 PAS 染色法則可見其細胞質內貯存了大量的醣類，同時在寄主中腸細胞中則少有醣類存在，嚴重感染的寄主中腸細胞體積減小，因部分細胞漿已被簇蟲吸收，尤有甚者，因過度感染而導致細胞群壞死脫落。

感染簇蟲的石蠅較正常石蠅生長得慢，延後羽化時間。如此極易錯過交尾時間，減少其繁殖率。