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NOTE

Observations on a new intranuclear virus-like particle infecting larvae of a black fly *Simulium vittatum* (Diptera: Simuliidae)

G. Charpentier[1]*, C. Back[2], S. Garzon[3] & H. Strykowski[3]

[1] Groupe de Recherche sur les Insectes Piqueurs, Université du Québec à Trois-Rivières, C. P. 500, Trois-Rivières, Québec G9A 5H7, Canada

[2] Department of Entomology, Macdonald College of McGill University, 21 111 Lakeshore Rd., Ste-Anne-de-Bellevue, Québec H9X 1C0, Canada

[3] Départment de Microbiologie et d'Immunologie, Université de Montréal, C. P. 6128, Succursale A, Montréal, Québec H3C 3J7, Canada

ABSTRACT: Only 3 types of viruses have yet been isolated from black flies: cytoplasmic polyhedrosis viruses, iridescent viruses and a densonucleosis virus. In 1983, a viral infection was detected in a winter developing population of *Simulium vittatum* near Trois-Rivières, Canada. About 1 % of the larvae showed a translucent band in their mid-portion. Electron microscopy showed in these larvae a virus-like particle in the nucleus of columnar cells of the gut epithelium. The particles are 38 nm in diameter, a characteristic which together with morphological and biological characteristics sets them apart from other known black fly viruses. Further studies on this new type of virus are in progress.

Black flies are among the insects of foremost medical importance around the world, as they are vectors of a filarial nematode causing onchocerciasis in inhabitants of tropical regions (Molloy 1982). They also have a veterinary importance, because they transmit Protozoa and Nematoda to animals, and are therefore a nuisance, reducing the productivity of livestock and other farm animals (Nat. Res. Council of Canada 1982). In North America, black flies are mostly known for their impact on human outdoor activities and consequent effects on the tourist industry (Molloy 1982), but there is one report on transmission of an arbovirus to humans (Ritter & Feltz 1974). In East Africa (Uganda), black flies are also considered possible vectors of the Kaposi sarcoma (Williams & Williams 1966).

In recent years there have been increased demands for the biological control of black flies (WHO 1983), but only 3 kinds of viruses have yet been isolated from black fly larvae which can be considered as potential microbiological control agents: cytoplasmic polyhedrosis viruses (the most frequent), iridescent viruses and a densonucleosis virus (Weiser & Undeen 1981). Attempts to infect larvae with viruses in the laboratory gave erratic results (Bailey 1977). Certain other pathogens have been used for biological control (Gaugler & Finney 1982) or have been found in black fly (Weiser & Undeen 1981), mainly microsporidians (Microspora: Microsporida) (Maurand 1975). In Canada, viruses (Bailey et al. 1975) and microsporidians (Vávra & Undeen 1981) have been found.

In November 1983, black fly larvae were sampled in the Yamachiche River (46° 30′ 25″ N, 72° 57′ 20″ W), about 35 km northwest of Trois-Rivières (Québec, Canada). The biological material consisted of an almost pure sample of about 1,000 larvae of *Simulium (Psilozia) vittatum* Zetterstedt. *S. vittatum* is a species complex developing all year round, and in the Trois-Rivières area 2 siblings of this species complex may occur (Rothfels & Featherston 1981). The larvae were medium-sized (ca 5 mm) and probably midway through their larval development. This sample of black flies was kept at 5 °C in the laboratory in a closed rearing apparatus (Lacoursière et al. unpubl.). Upon examination, 1 larva showed a whitish, translucent band in its mid portion (Fig. 1) compared to normal specimens showing a darker and colored mid portion. Infected larvae showed no iridescence. Behavior, though, was normal and feeding was sustained, as shown by the regular excretion of fecal pellets and by the full digestive tract. We do not know whether or not the infection had any influence on fecundity or longevity. After 4 mo of continuous rearing, a total of 10 larvae showed the same symptoms. The cumulative rate of infection was thus around 1 % or less. For electron microscopy, larval tissues were excised, cut

* Addressee for reprint requests

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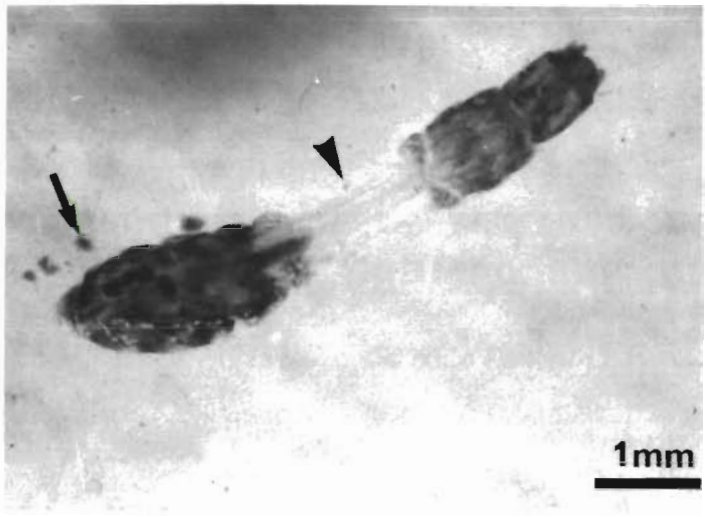

Fig. 1. *Simulium vittatum.* Live larva with symptoms of advanced infection. Note whitish translucent band in mid portion (►) and normal excretion of fecal pellets (—►)

into small pieces, fixed with 2 % glutaraldehyde (Lab. Meca, Canada) in phosphate buffer (0.067M, pH 7.4) at 4 °C for 2 h, postfixed with 1 % OsO_4 (Lab. Meca, Canada) in the same buffer at 4 °C for 1 h, dehydrated, and embedded in Araldite 502 (Ladd Research Industries, USA) (Luft 1961). Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963), and examined in a Philips 300 electron microscope. The magnification was calibrated with a line grating replica having 2160 lines mm^{-1}. Some larvae showing this band were kept at −80 °C for further studies and reinfection assays.

The virus-like particles were only observed inside the nucleus of the columnar cells of the gut epithelium (Fig. 2) and never observed in the cytoplasm. Surrounding tissues such as other epithelium cells, fat cells, muscular cells, and tracheal tissue were free of viruslike particles. Attempts at detection by electron microscopy of these viruslike particles in other parts of the larvae were unsuccessful.

The particles were scattered in the columnar-cell nuclei, isolated or in clusters, and never found in paracrystal arrays. Different stages of infection could be observed. In the early stage, the nucleolus is prominent and clusters of virus-like particles are observed around it. Later, the nucleolus disappears and the virus-like particles are much more abundant. The viruslike particles are about 38 nm (± 6 nm) in diameter. They frequently show a densely stained outer layer (9 nm thick) and a less densely stained central nucleoid (20 nm in diameter) (Fig. 2).

Capsid fragments are occasionally seen. The viral morphology is apparently icosahedral since pentagonal and hexagonal particles are seen in ultrathin section (Fig. 2, inset).

The intranuclear virus-like particle described here may be compared with a virus-like particle found in another dipteran, *Drosophila melanogaster* (Drosophilidae). Felluga et al. (1971) described this particle as 'doughnut-shaped' with a dense outer layer. The virus that we observed, by its size (38 nm compared to 20 nm) and morphological characteristics, is different from the densonucleosis virus described in black fly by Federici (Federici 1976, Federici & Lacey 1976). In our observations, the viral infection is restricted to the gut epithelium columnar cells, differing from the densonucleosis of black fly that infects midgut epithelial cells and to a lesser extent fat body cells (Federici 1976). Further studies are needed to characterize this virus-like particle and its effects on black fly larvae, particularly its effects on fecundity and longevity of black fly. This could be of interest for the microbiological control of black flies. More over, the size of the particles is similar to the size of polyoma virus (Maltern et al. 1966). This suggests that the presence of virus-like particles in the adult black fly should be investigated because of the eventual transmission of the particles to vertebrates.

Further studies on this new kind of virus-like particle are in progress, especially laboratory trials to infect larvae.

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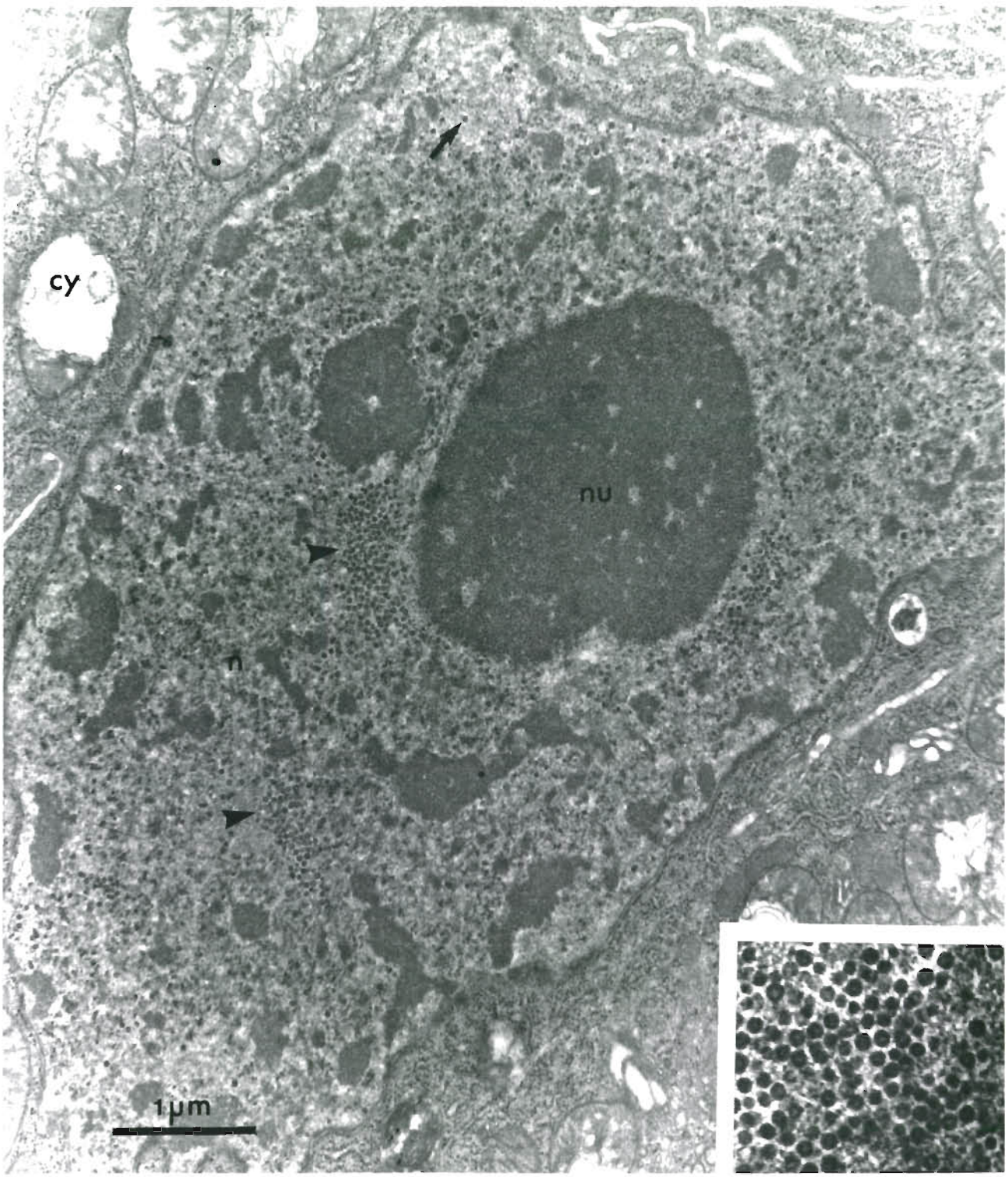

Fig. 2. *Simulium vittatum*. Electron micrograph of an infected columnar cell of gut epithelium. Note presence of virus-like particles in clusters (►) associated or not with the nucleolus (nu) or isolated (⟶) in the nucleus (n), and their absence in the cytoplasm (cy). Inset: magnification (79,300×) of the virus-like particles showing the 'doughnut-shaped' appearance

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