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SHORT COMMUNICATION

An epizootic of patent iridescent virus disease in multiple species of blackflies in Chiapas, Mexico

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Abstract. *Simulium* blackfly larvae (Diptera: Simuliidae) were collected from rivers and streams at 500–1500 m a.s.l. in Chiapas State of southern Mexico. Among 45 sites surveyed over an area of 2300 km^2 (around 15°15′ N 92°20′ W), some *Simulium* larvae from three sites were opalescent violet-blue, interpreted as patent infection with invertebrate iridescent virus (IIV). Dissection confirmed the presence of putative *Iridovirus* particles, 130 nm diameter, but no IIV isolates were obtained from homogenates injected into *Galleria mellonella* (L) larvae (Lepidoptera: Pyralidae). All *Simulium* with patent IIV infection died before metamorphosis, whereas ~60% of asymptomatic *Simulium* survived to adulthood in the laboratory.

During 1997, standard monthly samples from two parallel rivers 42–50 km north-west of Tapachula comprised the following species proportions (and rates of patent IIV infection): 41.8% (47%) *Simulium mexicanum* Bellardi complex, 31.3% (31.4%) *S. rubicundum* Knab, 10.1% (13.1%) *S. paynei*, 6.5% (2.9%) *S. callidum* (Dyar & Shannon), 6.3% (5.1%) *S. ochraceum* Walker complex, 3.1% (0.7%) *S. downsi* Vargas *et al*., 0.7% *S. samboni* Jennings and 0.2% *S. metallicum* Bellardi complex, showing a strong correlation between blackfly abundance and the prevalence of patent infection. An epizootic of IIV in January and February (infection rates 41–100%) was followed by absence of larvae (March–August) until the end of the rainy season, when numbers collected on nylon strings rose to ≈ 1/cm with patent IIV infection rates of 0–12.5% during September–December. Further investigations are underway to isolate this IIV and assess its potential usefulness for biological control of *Simulium* pests and vectors of onchocerciasis.

Key words. *Iridovirus*, *Simulium* spp., bioassay, biological control, blackfly larvae, epizootic, iridescent virus, onchocerciasis vectors, population density, Chiapas, Mexico.

In the southern Mexican state of Chiapas there are two foci of human onchocerciasis: Chamula and Golondrinas = El Soconusco (Rodríguez-Perez & Rodríguez, 1994), with some 286 000 persons at risk of infection in 947 communities (Martin-Tellaeche *et al*., 1998). The local vectors of *Onchocerca volvulus* (Leuckart) causing human onchocerciasis are blackflies of the *Simulium ochraceum* complex, *S. metallicum* complex and *S. callidum* (Ortega & Oliver, 1984, 1985; Millest *et al*., 1999). Immature stages of Simuliidae develop in fast-flowing rivers and streams that abound in this upland region. Elsewhere, *Simulium* larvae have been found infected with diverse microorganisms, including cypoviruses, densoviruses and iridescent viruses (Weiser & Undeen, 1981).

Invertebrate iridescent viruses (IIVs – Family Iridoviridae, genus *Iridovirus*) are icosahedral particles with an internal lipid layer and a dsDNA genome of 150–200 kbp. Typically IIVs infect invertebrates in humid or aquatic habitats (Williams, 1996). Patent infections are lethal, causing a dramatic opalescent violet-blue colour change in the host, due to formation of paracrystalline arrays of IIV particles in the cytoplasm of infected cells. Although striking in appearance, the prevalence of patent IIV infection in blackfly populations is usually low (usually < 10%) or extremely low (<< 1%) (Table 1).

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Table 1. Prevalence of patent IIV infections reported from blackfly larvae world-wide (in chronological order).

Species of Simuliidae	Maximum percent patent infection observed	Country	Reference
S. ornatum	<< 1.0	Czech Republic	Weiser (1968)
Simulium sp.	<< 1.0	Wales, U.K.	Batson *et al*. (1976)
S. rubicundulum	0.8	Guatemala	Takoaka (1980)
S. callidum	10.1	Guatemala	Takoaka (1980)
S. earlei	2.9	Guatemala	Takoaka (1980)
Prosimulium sp.	8.8	U.S.A.	Avery & Bauer (1984)
S. neornatipes	<< 1.0	New Caledonia	Batson (1986)
S. vittatum	17.0	Canada	Erlandson & Mason (1990)
S. luggeri	<< 1.0	Canada	Erlandson & Mason (1990)
S. variegatum	<< 1.0	Wales, U.K.	Williams & Cory (1993)

Using a sensitive insect bioassay, PCR amplification or DNA hybridization, covert non-lethal IIV infections have been detected in a natural population of *S. variegatum* Meigen (Williams, 1993, 1995) and in laboratory studies of a mosquito (Diptera: Culicidae) and of Lepidoptera (Ward & Kalmakoff, 1991; Marina *et al*., 1999).

The present study was performed during 1996–97 in order to determine the natural distribution and prevalence of IIV infections in *Simulium* species around the onchocerciasis focus of Golondrinas, Chiapas, Mexico.

A total of 45 river sites were visited over an area of $\approx 2300\,km^2$ (centre of study area at 15°15′N 92°20′W) between the towns of Motozintla in the north, Tapachula to the south, Mapastepec to the west and the Mexico-Guatemala border to the east. The rivers and streams from which samples were taken were all between 500 and 1500 m a.s.l. and varied considerably in terms of size, flow rate, vegetation, etc. Large rivers were usually sampled at several points. The rainy season in this region starts in April through to November (typically 300 mm rainfall per month). In the dry season rainfall is virtually zero.

At all sample sites, between three and six traps were placed to estimate the abundance of *Simulium* juvenile stages. Traps consisted of a nylon string 62 cm in length, tied to tree branches trailing in the water, in areas suitable for *Simulium* larvae: as determined by the pre-existing presence of *Simulium* larvae on submerged rocks and vegetation (Colbo, 1987). Traps were inspected after 48 h in the water, and those bearing *Simulium* larvae were placed directly in plastic bags with a little formalin (5%). Larvae were also collected from natural substrates, placed in plastic 1.5 mL Eppendorf tubes, labelled and transported to the laboratory in an insulated box, and stored at −20°C until required. At all sites water temperature, pH, turbidity and dissolved oxygen were measured *in situ* using a water quality meter (Horiba Inc., Irvine, CA, U.S.A.). When abundant patent IIV infections were observed, larvae ($n \sim 300$) were also placed in plastic bags with river water, transported to the laboratory and held at 22°C in 2 L beakers (with air bubbling from an aquarium pump) to monitor mortality. Food was supplied as ground aquarium fish food and water was changed daily. Control larvae came from sites where patently infected larvae were not observed.

In the laboratory, larvae from traps were counted and identified to species using the key of Onishi *et al*. (1977). Frozen larvae were thawed and individually homogenized in 130 µL of antibiotic solution (0.2% aureomycin). Each homogenate was subjected to centrifugation at 490 *g* to pellet debris, transferred to a disposable 1 mL syringe and 10 µL volumes were injected into individual third-instar moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). This insect is highly susceptible to the majority of IIVs and employed as the standard laboratory host for producing these viruses (Williams, 1998). Injected *G. mellonella* larvae were maintained on semi-synthetic diet at 25 ± 1°C and checked for patent infection at 8–14 days post-injection.

Patently infected *Simulium* larvae were observed at only three of the 45 sites. At a small stream 20 km north-east of Tapachula, among plentiful *S. mexicanum* immatures, a single larva with patent IIV symptoms was observed in July 1996. Patent IIV infections were abundant among *Simulium* larvae at the other two sites, in the Rio Nueva America and the Rio Guadalupe, respectively, some 50 and 42 km north-west of Tapachula. These rivers run almost parallel down adjacent valleys, 5 km apart at their closest point. Both sites were therefore sampled monthly during 1997.

Prevalence of IIV infection was extremely high in *Simulium* larvae at both sites in January (larvae 41–100% patently infected) and February (100% patent infection) in each sample of several hundred larvae (Fig. 1). The numbers of larvae collected on the nylon string traps were also high. By March, however, the population of *Simulium* larvae had fallen to an undetectable level, presumably due to IIV-induced mortality. Rains commenced in March–April, so the intermittent spate of river flow may have contributed to the disappearance of *Simulium* larvae by scouring, although it was not possible to quantify river volume and such data are not collected by the regional water authority. Immature *Simulium* densities remained extremely low until September–October, when an increase in larval populations was detected by trapping. This coincided with a reduction in precipitation, as the end of

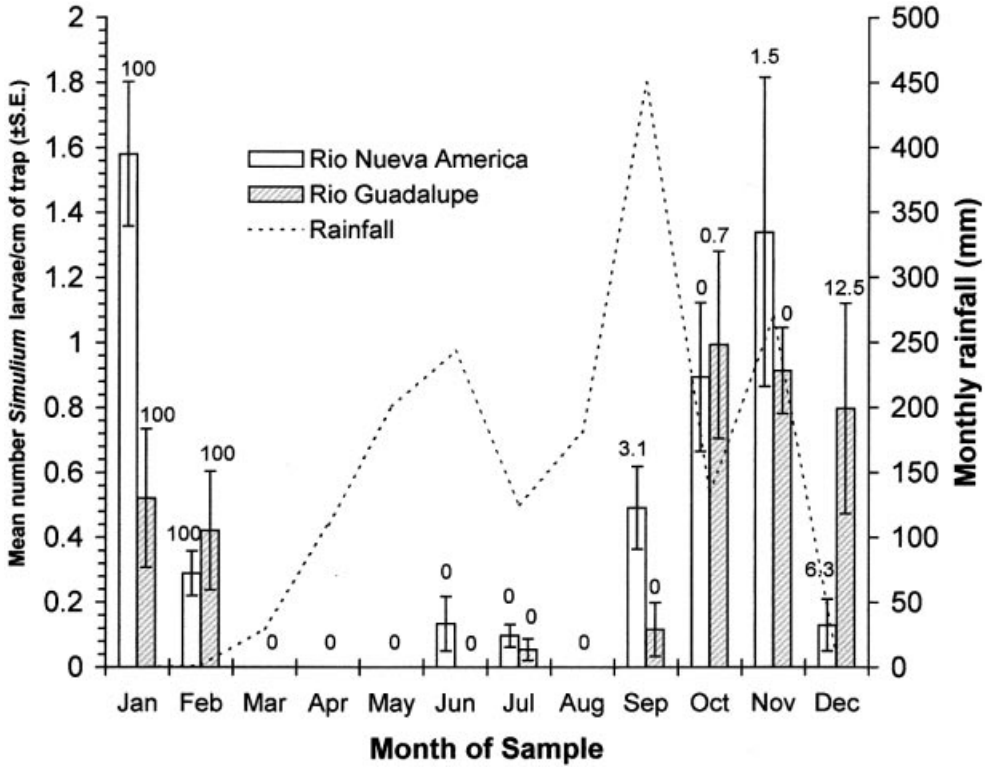

Fig. 1. Abundance (mean + SE) of *Simulium* larvae (all species) in 1997 monthly samples from sites in two rivers (Rio Nueva America and Rio Guadalupe) with patent IIV epizootic observed in January–February. Figures above histogram columns indicate maximum percent patent infection in larvae collected each month. Dotted line indicates total monthly rainfall measured in Tapachula.

season rains are sporadic (Fig. 1). In the period September–December 1997, patently infected *Simulium* larvae reappeared in the population at moderate levels (0–12.5%).

Identification of apparently healthy *Simulium* larvae among samples from both localities revealed that the most abundant species was *S. mexicanum* Bellardi *sensu lato* (41.8%) followed by *S. rubicundulum* Knab (31.3%), *S. paynei* Vargas (10.1%), *S. callidum* (Dyar & Shannon)(6.5%), *S. ochraceum* Walker *sensu lato* (6.3%), *S. downsi* Vargas *et al.* (3.1%), *S. samboni* Jennings (0.7%) and *S. metallicum* Bellardi *sensu lato* (0.2%). Prevalence of patent IIV infection was roughly proportional to host species abundance, the most commonly infected species being *S. mexicanum* (47.0%) followed by *S. rubicundulum* (31.4%), *S. paynei* (13.1%), *S. ochraceum* (5.1%), *S. callidum* (2.9%) and *S. downsi* (0.7%). Of these, *S. mexicanum*, *S. paynei*, *S. ochraceum* and *S. downsi* are new host records for IIV infection.

Changes in the prevalence of infection were apparently not related to physico-chemical aquatic factors measured in this study, found to be very similar between the two rivers. During the course of the year, water temperature varied by only 6°C (17–23°C); dissolved oxygen 6.8–11.8 mL/L O_2 and pH 6.9–8.0 also varied little, depending on site and month of sample (data not shown).

Figure 2 compares the mean density of *Simulium* larvae/trap (all species) between sites with presence of patent IIV

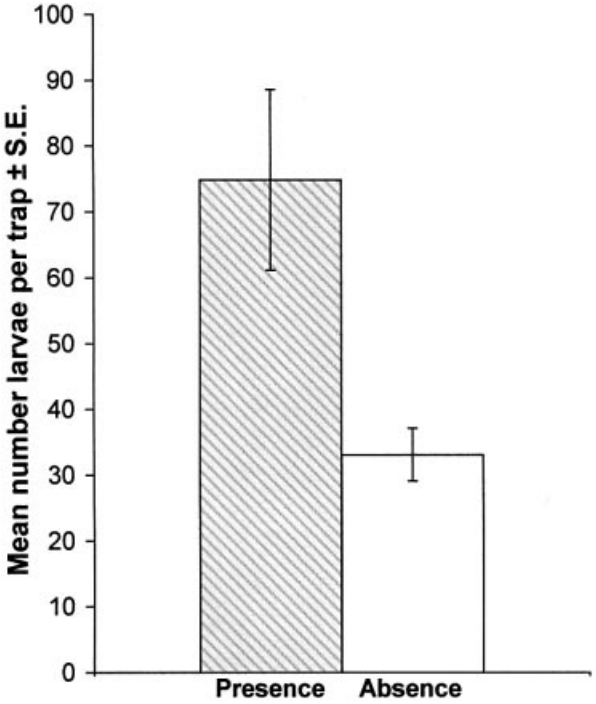

Fig. 2. Overall mean + SE numbers of *Simulium* larvae (all species) per trap in the presence (two study sites) or absence (43 other sites) of patently infected larvae during the sampling period of 12 months at two sites.

infections (Rio Guadalupe and Rio Nueva America) and sites without patent infections (43 other sites). The presence of patent IIV infection was found to be correlated with significantly higher densities of larvae ($t = 3.30$, d.f. = 387, $P = 0.001$).

These *Simulium* species cannot be readily reared in the laboratory and control mortality (of larvae from sites without patent IIV infection) was relatively high (~40%). Successful eclosion of blackfly adults was achieved only in beakers containing control larvae. None of the patently infected larvae reared in the laboratory attained adulthood.

Virus presence in infected *Simulium* larvae was confirmed by observation of particles of ≈ 130 nm diameter, using latex spheres of 100 nm and 460 nm diameter for reference (Sigma Chemical Co., St Louis, MO, U.S.A.), at × 20 000 magnification with a scanning electron microscope (Topcon SM-510, Tokyo, Japan). This particle size is typical for genus *Iridovirus*. To bioassay for covert IIV infections, a total of 5713 apparently healthy *Simulium* larvae were homogenized and injected into *G. mellonella*, but none showed positive. Likewise, injection of semipurified homogenates of 185 patently infected *Simulium* larvae failed to produce patent infection of this host. Evidently the type(s) of IIV causing patent disease of *Simulium* larvae in Chiapas does not replicate in *G. mellonella* larvae.

The only previous report of invertebrate IIV disease in Mesoamerica was by Takaoka (1980), who observed a low prevalence of infection in larvae of three *Simulium* species in a stream at an altitude of 700 m in Guatemala. As in our study, these infections were observed in January, although no data on larval density were given. In Wales (U.K.), the prevalence of IIV infection fluctuated markedly over the course of a 7-month study; patently infected *S. variegatum* larvae were observed only in May and September. Patent infections were not concurrent with high larval densities, but they followed, or coincided with, moderate levels of non-lethal covert infection in the larval population, as detected by *G. mellonella* bioassay and/or PCR (Williams, 1993, 1995).

How IIVs persist in host populations during periods of low host density remains an elusive issue, and our inability to detect covert infection in the present study did nothing to elucidate that. Studies in progress aim to find a suitable host for production of the IIV isolate(s) reported here as a means of starting a characterization study, including the possibility of designing primer oligonucleotides to the major capsid protein gene of the Chiapas IIV isolate in order to detect covert infections by PCR amplification. If this is a single virus species, it is clear that it has a high virulence to multiple species of *Simulium* but, like IIV3 from the mosquito *Aedes taeniorhynchus* (Diptera), IIV16 from the beetle *Costelytra zealandica* (Coleoptera) and IIV24 from the bee *Apis cerana* (Hymenoptera), the host range does not extend to *G. mellonella* (Williams, 1998).

This is the first report of an epizootic of patent IIV disease in wild *Simulium* populations and, as far as we are aware, the highest observed prevalence of IIV infection in any invertebrate population. In general, due to the routinely low incidence of patent IIV infections in insect populations, these viruses have not been considered useful as agents of biological control. Recognizing the impact of covert IIV infection in insects of medical importance (Marina *et al*., 1999), evidenced by the high prevalence in *Simulium* and mortality rates reported here, warrants further evaluation of these effulgent pathogens for biological control purposes.

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References

Avery, S.W. & Bauer, L. (1984) Iridescent virus from *Prosimulium* collected in Maine. *Journal of Invertebrate Pathology*, **43**, 430–431.

Batson, B.S. (1986) A small iridescent virus (Iridovirus) from *Simulium neornatipes* Dumbelton (Diptera: Simuliidae) in the South Pacific island of New Caledonia. *Journal of Invertebrate Pathology*, **48**, 384–387.

Batson, B.S., Johnston, M.R.L., Arnold, M.K. & Kelly, D.C. (1976) An iridescent virus from *Simulium* sp. (Diptera; Simuliidae) in Wales. *Journal of Invertebrate Pathology*, **27**, 133–135.

Colbo, M.H. (1987) Problems in estimating blackfly populations in their aquatic stages. *Blackflies: Ecology, Population Management and Annotated World List* (ed. by K. C. Kim and R. W. Merritt), pp. 77–89. Pennsylvannia State University Press, U.S.A.

Erlandson, M.A. & Mason, P.G. (1990) An iridescent virus from *Simulium vittatum* (Diptera: Simuliidae) in Saskatchewan. *Journal of Invertebrate Pathology*, **56**, 8–14.

Marina, C., Arredondo-Jiménez, J., Castillo, A. & Williams, T. (1999) Sublethal effects of iridovirus disease in a mosquito. *Oecologia*, **119**, 383–388.

Martin-Tellaeche, A., Ramirez-Hernandez, J., Santos-Preciado, J.I. & Mendez-Galvan, J. (1998) Onchocerciasis: changes in transmission in Mexico. *Annals of Tropical Medicine and Parasitology*, **92** (Suppl.), S117–119.

Millest, A.L., Cheke, R.A. & Greenwood, R. (1999) Distribution of the *Simulium metallicum* complex in Mexico in relation to selected environmental variables. *Medical and Veterinary Entomology*, **13**, 139–149.

Onishi, O., Okazawa, T. & Ochoa, J.O. (1977) Clave gráfica para la identificación de los simúlidos del área de San Vicente Pacaya, por los caracteres externos de larvas y pupas. *Gjcrcpo-Mensap*, **2**, 1–11.

Ortega, G.M. & Oliver, C.M. (1984) Entomologia de la oncocercosis en El Soconusco, Chiapas. I. Estudio sobre habitos de picadura de las tres especies de simulidos considerados transmisores de oncocercosis en el foco sur de Chiapas. *Folia Entomologica Mexicana*, **62**, 125–140.

Ortega, G.M. & Oliver, C.M. (1985) Entomologia de la oncocercosis en El Soconusco, Chiapas. II. Estudio sobre dinamica de poblacion de las tres especies de simulidos considerados transmisores de oncocercosis en el foco sur de Chiapas. *Folia Entomologica Mexicana*, **66**, 119–136.

Rodríguez-Perez, M.A. & Rodríguez, M.H. (1994) Oncocercosis.

Enfermedades Tropicales de México (ed. by J. L. Valdespino-Gómez, O. Velasco-Castrejón, A. Escobar-Gutiérrez, A. del Río-Zolezzi, S. Ibañez-Bernal and C. Magos-López), pp. 325–333. Instituto Nacional de Diagnostico y Referencia Epidemiologico Secretaría de Salud, Mexico D.F., Mexico.

Takaoka (1980) Pathogens of blackfly larvae in Guatemala and their influence on natural populations of three species of onchocerciasis vectors. *American Journal of Tropical Medicine and Hygiene*, **29**, 467–472.

Ward, V.K. & Kalmakoff, J. (1991) Invertebrate Iridoviridae. *Viruses of Invertebrates* (ed. by E. Kurstak), pp. 197–226. Marcel Dekker, New York.

Weiser, J. (1968) Iridescent virus from the blackfly *Simulium ornatum* Meigen in Czechoslovakia. *Journal of Invertebrate Pathology*, **12**, 36.

Weiser, J. & Undeen, A.H. (1981) Diseases of blackflies. *Blackflies: the Future for Biological Methods in Integrated Control* (ed. by M. Laird), pp. 181–198. Academic Press, London.

Williams, T. (1993) Covert iridovirus infection of blackfly larvae. *Proceedings of the Royal Society B*, **251**, 225–230.

Williams, T. (1995) Patterns of covert infection by invertebrate pathogens: iridescent viruses of blackflies. *Molecular Ecology*, **4**, 447–457.

Williams, T. (1996) The Iridoviruses. *Advances in Virus Research*, **46**, 347–412.

Williams, T. (1998) Invertebrate iridescent viruses. *The Insect Viruses* (ed. by L. K. Miller and L. A. Ball), pp. 31–68. Plenum Press, New York.

Williams, T. & Cory, J. (1993) Evidence for DNA restriction fragment polymorphism in iridovirus isolates from individual blackfly (Diptera: Simuliidae) hosts. *Medical and Veterinary Entomology*, **7**, 199–201.

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