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Fatty acid profiles of invertebrate iridescent viruses

Brief Report

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Summary. Eight invertebrate iridescent viruses (IVs) from diverse host taxa were grown in a common lepidopteran host, *Galleria mellonella*. The lipid composition of purified virus was assessed by fatty acid methyl esterase (FAME) analysis using a gas-liquid chromatograph. IV fatty acid profiles were markedly different from those of the host tissues. The interrelationships among the IVs did not follow previous serological and genetic findings. We conclude that FAME analysis is not a useful technique for revealing phylogenetic relationships among these viruses.

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The need for comparative studies to clarify relationships among an increasing number of iridescent virus (IVs) isolates mostly from insects, has been repeatedly recognized [1–8]. IVs are icosahedral particles, containing a dsDNA genome of some 150–200 kbp. The particles assemble in host cytoplasm and may proliferate to form crystalline arrays in heavily infected cells producing a characteristic opalescent hue [9, 10]. Recently, comparative genomic studies have led to a proposal for a reclassification of invertebrate IVs [7, 8]. The new classification names isolates according to their place of origin and assigns them to one of three complexes of inter-related isolates with the *Iridovirus* genus, based on DNA hybridization and other characteristics. The new proposed names are used here with the historical type numbers in brackets for clarity. The iridoviruses from vertebrates, frog virus 3 and flounder lymphocystis disease virus, were not affected by the new proposals and remain separate and mutually distinct species within the family *Iridoviridae*.

Iridescent viruses are typically isolated from hosts associated with moist or aquatic habitats. They appear highly stable in water [2] and this probably arises from their structure. The icosahedral outer shell is composed of a major

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structural (capsid) protein, of some 50 K which forms numerous triangular and pentagonal subunits [11–14]. Complexes of protein pass through an intermediate lipid layer to link capsid and core polypeptides. The core of the virion is highly hydrated and contains a number of DNA binding proteins [15, 16]. The diversity of polypeptides in iridoviruses is remarkable [17, 18], and a number of virion-associated enzymes have also been reported [19–21]. IVs also contain an internal lipid membrane. The lipid membrane is approximately 4 nm thick and comprises 3.9% to 9.0% of the virion by weight [22, 23].

The composition of the IV lipid layer differs from that of the host cells and about three quarters of the lipid appears to be phospholipid [23–25]. It is not known exactly how iridescent viruses sequester their lipid content but IV lipid envelopes are not acquired as a result of budding through particular cell organelle membranes, as occurs in many virus families. Consequently, differences between host cell and virus lipid composition make comparative studies of this type particularly relevant.

Previous comparative studies have used serological and physico-chemical factors to describe the relationships among invertebrate IV isolates. Recently, genetic investigations [7, 8] and protein sequence comparisons have been performed [6]. Gas-liquid chromatographic analysis of cellular fatty acid methyl esters (FAME) has become a standard technique for identification of bacteria. FAME profiles can also be used for comparative studies provided the organisms have been grown under standardized, reproducible conditions [26–30]. In this paper, we compare the FAME profiles of IV isolates from each of the hybridization complexes within the *Iridovirus* genus to present new information on the chemotaxonomic relationships among these viruses.

Details of the origin, history, propagation and characterization of IV isolates follow those given in Williams and Cory [7]. Briefly, eight IV isolates were propagated by injection into *Galleria mellonella* (Lepidoptera: Pyrallidae) which is highly permissive in many IVs. All material was checked against original stock by restriction endonuclease analysis using *Hin*dIII. Virus was extracted and purified following the method of Kelly and Tinsley [31], involving repeated centrifugation steps followed by sucrose gradient separation and further centrifugation. Host material for comparison was obtained either as a crud homogenate, or in a semi-purified state following the centrifugation steps above but without sucrose gradient treatment, there being no clear equivalent to the virus bands following sucrose gradient centrifugation of the host material alone.

The fatty acid content of 25 mg triplicate samples of pelleted, purified virus or host cellular material was analyzed following the methods described in Thompson et al. [28]. Briefly, the extraction involved saponification using methanol and hydroxide followed by methylation using acidified methanol and separation into an organic phase by hexane treatment. This organic phase was base washed and analyzed in a Hewlett Packard II gas chromatograph 5890 equipped with a fused silica gel capillary column.

FAME peaks were measured and identified by a Microbial Identification Software (MIS) program (Newark, DE). The MIS program was used to calculate

Table 1. Fatty acid analysis of invertebrate iridescent viruses (mean % composition ± SD)

	Polyiridovirus complex						Oligo-iridovirus complex	Crustaceo-iridovirus complex	Host (*Galleria mellonella*)	
Fatty acid	Plowden (IV1)	Tia (IV2)	Nelson (IV9)	Aberystwyth (IV22)	San Miguel (*Anticarsia* IV)	Stoneville (IV30)	Ntondwe (IV21)	Riverside (IV31)	low purification	high purification
14:0									0.32 ± 0.05	0.33 ± 0.03
16:1 (8)			0.21 ± 0.01			0.71 ± 0.03			0.64 ± 0.06	0.54 ± 0.03
16:1 (cis 9)	5.10 ± 0.09	6.54 ± 0.15	5.64 ± 0.28	4.13 ± 0.23	2.07 ± 0.05	4.46 ± 0.36	2.20 ± 0.10	2.00 ± 0.04	2.85 ± 0.32	2.17 ± 0.08
16:0	16.33 ± 0.14	19.38 ± 0.15	25.08 ± 1.15	17.03 ± 0.36	17.63 ± 0.51	14.96 ± 0.21	21.16 ± 0.33	22.04 ± 0.74	33.15 ± 0.84	30.35 ± 0.44
18:1 (cis 9)	33.28 ± 0.18	36.01 ± 0.25	30.93 ± 0.17	34.81 ± 0.08	43.23 ± 0.75	39.91 ± 0.66	39.53 ± 0.16	34.10 ± 0.66	47.68 ± 1.35	40.46 ± 0.18
18:0	13.21 ± 0.18	9.17 ± 0.18	7.32 ± 0.76	7.77 ± 0.66	7.29 ± 0.49	5.75 ± 0.52	8.54 ± 0.27	10.63 ± 0.58	2.10 ± 0.48	4.82 ± 0.19
19:1 (iso I)										0.35 ± 0.03
20:0										0.22 ± 0.02
Unknown	31.58 ± 0.36	27.75 ± 0.19	29.28 ± 0.84	34.83 ± 0.84	29.34 ± 0.28	33.89 ± 1.46	27.81 ± 0.21	30.29 ± 1.05	11.53 ± 1.26	19.78 ± 0.22

a generalized similarity coefficient [32] and a Euclidean distance between pairs of isolates [33], based on percentage peak area values. Clustering of isolates was performed using an unweighted pair group method for arithmetic means [34].

The fatty acid content of the iridescent viruses was similar among the isolates tested and was clearly distinguishable from the host material (Table 1). The major fatty acid for all isolates, and for the host material was *cis*-Octadec-9-enoate (C18:1cis9) which ranged between 30.9% in Nelson IV (IV9) and 43.2% in San Miguel IV from *Anticarsia gemmatalis* and between 40.4–47.7% in host tissues. The second major fatty acid was Hexadecanoate (C16:0) which was markedly lower in the virus samples (14.9–25.1%) compared to the host samples (30.4–33.2%). Also present in the virus samples were C18:0 (5.7–13.2%), C16:1 *cis*9 (2.0–6.5%) and for Nelson IV (IV9) and Stoneville IV (IV30), very low levels of C16:1 8(0.2–0.7%). In addition, host tissues contained low levels of C14:0, C19:1, and 20:0. The incidence of unresolvable features was similar among the IV isolates and ranged between 27.8–34.8%.

A dendrogram of Euclidean distances clearly demonstrated the differences between the lipid contents of the IVs and the host larvae (Fig. 1). The viruses fell into two main clusters, one containing Aberystwyth IV (IV22), Stoneville IV (IV30), Ntondwe IV (IV21) and San Miguel IV (*Anticarsia* IV), the other containing Nelson IV (IV9), Plowden IV (IV1), Tia IV (IV2) and Riverside IV (IV31). This was contrary to previous genetic and serological findings which indicated that most isolates within the *Iridovirus* genus were interrelated, apart from Ntondwe IV (IV21) and Riverside IV (IV31), which were assigned to distinct and separate complexes within the genus.

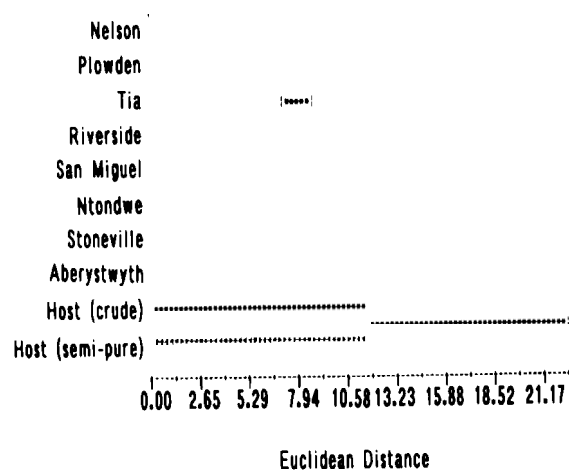

Fig. 1. Dendrogram drawn from similarity coefficients generated by comparison of FAME profiles for all iridescent virus isolates tested and host material. Isolates named following the proposed nomenclature of Williams and Cory [7] for which the historical type numbers are as follows: Nelson IV = IV9, Plowden IV = IV1, Tia IV = IV2, Riverside IV = IV31, San Miguel IV = *Anticarsia* IV, Ntondwe IV = IV21, Stoneville IV = IV30, Aberystwyth IV = IV22

Willis and Granoff [35] reported that the lipid composition of a vertebrate iridovirus, frog virus 3, was virtually identical to that of the host fathead minnow cells. However, in the invertebrate isolate, Dazaifu IV (IV6) the major fatty acids in order of abundance were reported as: C18:1 (39.0%), C16:0 (20.0%), C18:3 (15.5%), C18:2 (12.5%) and C18:0 (11.0%). This was a markedly different ratio from that of the host cells, which derived from *Choristoneura fumiferana* [25]. The differences in the fatty acids reported by these authors and those of the present study probably arise from differences in the virus isolates under study, the host species, and the system in which the viruses replicated, i.e. cell culture viruses whole body infections. Clearly the composition of viruses reflects to some degree the environment in which they replicate. Invertebrate iridoviruses are believed to acquire their lipid component during de novo assembly in the cytoplasm. A similar mechanism was also believed to exist in poxviruses [36]. However, recently Schmelz et al. [37] have presented evidence that vaccinia virus inner lipid envelopes are derived from an intracellular compartment between the Golgi apparatus and the rough ER, whereas outer membranes are derived from a cellular cisterna originating from a late Golgi or post Golgi compartment. The second of these wrapping events is facilitated by virally encoded proteins which appear in the trans Golgi network shortly after infection. Other lipid-containing viruses acquire their membranes by budding. Thus, the composition of the viral lipid layer often reflects the composition of the organelle membranes through which the viruses have passed: herpesviruses bud from the nuclear membrane, bunyaviruses bud through the Golgi apparatus, etc.

The ability of IVs to sequester host lipids in proportions which differ from those present in the cytoplasmic environment means that comparative studies using genetically distinct isolates from diverse invertebrate taxa may reveal intriguing differences in the fine structure of IV particles. Such differences may influence the physical stability of particles or affect ecological factors such as the efficacy of transmission of invertebrate iridoviruses. However, for the purposes of taxonomy, the use of fatty acids as biochemical indicators in comparative studies seems inappropriate.

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