

## 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: Pyralidae) which is highly permissive in many IVs. All material was checked against original stock by restriction endonuclease analysis using *HindIII*. 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 crude 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  $\pm$  SD)

| Fatty acid   | Polyiridovirus complex |                  |                  |                    |                                    | Oligo-iridovirus complex | Crustaceo-iridovirus complex | Host ( <i>Galleria mellonella</i> ) |                  |                   |
|--------------|------------------------|------------------|------------------|--------------------|------------------------------------|--------------------------|------------------------------|-------------------------------------|------------------|-------------------|
|              | Plowden (IV1)          | Tia (IV2)        | Nelson (IV9)     | Aberystwyth (IV22) | San Miguel ( <i>Anticarsia</i> IV) |                          |                              |                                     | low purification | high purification |
| 14:0         |                        |                  |                  |                    |                                    |                          |                              |                                     | 0.32 $\pm$ 0.05  | 0.33 $\pm$ 0.03   |
| 16:1 (8)     |                        |                  | 0.21 $\pm$ 0.01  |                    |                                    | 0.71 $\pm$ 0.03          |                              |                                     | 0.64 $\pm$ 0.06  | 0.54 $\pm$ 0.03   |
| 16:1 (cis 9) | 5.10 $\pm$ 0.09        | 6.54 $\pm$ 0.15  | 5.64 $\pm$ 0.28  | 4.13 $\pm$ 0.23    | 2.07 $\pm$ 0.05                    | 4.46 $\pm$ 0.36          | 2.20 $\pm$ 0.10              | 2.00 $\pm$ 0.04                     | 2.85 $\pm$ 0.32  | 2.17 $\pm$ 0.08   |
| 16:0         | 16.33 $\pm$ 0.14       | 19.38 $\pm$ 0.15 | 25.08 $\pm$ 1.15 | 17.03 $\pm$ 0.36   | 17.63 $\pm$ 0.51                   | 14.96 $\pm$ 0.21         | 21.16 $\pm$ 0.33             | 22.04 $\pm$ 0.74                    | 33.15 $\pm$ 0.84 | 30.35 $\pm$ 0.44  |
| 18:1 (cis 9) | 33.28 $\pm$ 0.18       | 36.01 $\pm$ 0.25 | 30.93 $\pm$ 0.17 | 34.81 $\pm$ 0.08   | 43.23 $\pm$ 0.75                   | 39.91 $\pm$ 0.66         | 39.53 $\pm$ 0.16             | 34.10 $\pm$ 0.66                    | 47.68 $\pm$ 1.35 | 40.46 $\pm$ 0.18  |
| 18:0         | 13.21 $\pm$ 0.18       | 9.17 $\pm$ 0.18  | 7.32 $\pm$ 0.76  | 7.77 $\pm$ 0.66    | 7.29 $\pm$ 0.49                    | 5.75 $\pm$ 0.52          | 8.54 $\pm$ 0.27              | 10.63 $\pm$ 0.58                    | 2.10 $\pm$ 0.48  | 4.82 $\pm$ 0.19   |
| 19:1 (iso I) |                        |                  |                  |                    |                                    |                          |                              |                                     |                  | 0.35 $\pm$ 0.03   |
| 20:0         |                        |                  |                  |                    |                                    |                          |                              |                                     |                  | 0.22 $\pm$ 0.02   |
| Unknown      | 31.58 $\pm$ 0.36       | 27.75 $\pm$ 0.19 | 29.28 $\pm$ 0.84 | 34.83 $\pm$ 0.84   | 29.34 $\pm$ 0.28                   | 33.89 $\pm$ 1.46         | 27.81 $\pm$ 0.21             | 30.29 $\pm$ 1.05                    | 11.53 $\pm$ 1.26 | 19.78 $\pm$ 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 (C18:1*cis*9) 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.

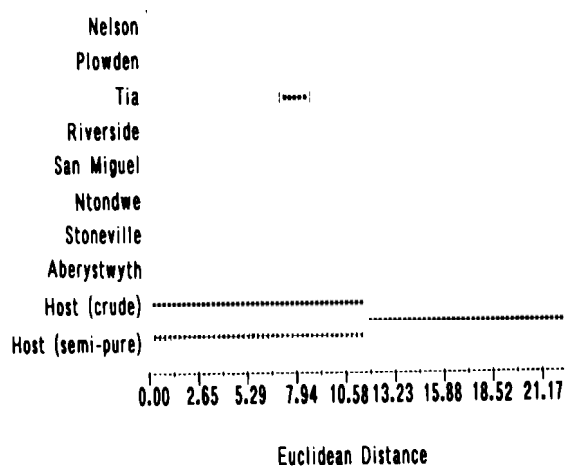


Fig. 1. Dendrogram drawn from similarity coefficients generated by comparison of FAME profiles for all iridescent virus isolates tested and host material. Isolates named following the proposed nomenclature of Williams and Cory [7] for which the historical type numbers are as follows: Nelson IV = IV9, 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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### References

1. Kelly DC, Edwards ML, Robertson JS (1978) The use of enzyme-linked immunosorbent assay to detect and discriminate between small iridescent viruses. *Ann Appl Biol* 90: 369–374
2. Kelly DC (1985) Insect iridescent viruses. *Curr Topics Microbiol Immunol* 116: 23–35
3. Willis DB (1990) Taxonomy of iridoviruses. In: Darai G (ed) *Molecular biology of iridoviruses*. Kluwer, Boston, pp 1–12
4. Ward VK, Kalmakoff J (1991) Invertebrate iridoviridae. In: Kurstak E (ed) *Viruses of invertebrates*. M. Dekker, New York, pp 197–226

5. Stohwasser R, Raab K, Schnitzler P, Janssen W, Darai G (1993) Identification of the gene encoding the major capsid protein of insect iridescent virus type 6 by polymerase chain reaction. *J Gen Virol* 74: 873–879
6. Schnitzler P, Darai G (1993) Identification of the gene encoding the major capsid protein of fish lymphocystis disease virus. *J Gen Virol* 74: 2143–2150
7. Williams T, Cory JS (1994) Proposals for a new classification of iridescent viruses. *J Gen Virol* 75: 1291–1301
8. Williams T (1994) Comparative studies of iridoviruses: further support for a new classification. *Virus Res* 33: 99–121
9. Klug A, Franklin RE, Humphreys-Owen SPF (1959) The crystal structure of *Tipula* iridescent virus as determined by Bragg reflection of visible light. *Biochim Biophys Acta* 32: 203–219
10. Hemsley AR, Collinson ME, Kovach WL, Vincent B, Williams T (1994) The role of self-assembly in biological systems: evidence from iridescent colloidal sporopollenin in *Selaginella* megaspore walls. *Phil Trans R Soc London Ser B* 345: 163–173
11. Wrigley NG (1969) An electron microscope study of the structure of *Sericesthis* iridescent virus. *J Gen Virol* 5: 123–134
12. Wrigley NG (1970) An electron microscope study of the structure of *Tipula* iridescent virus. *J Gen Virol* 6: 169–173
13. Manyakov VF (1977) Fine structure of the iridescent virus type 1 capsid. *J Gen Virol* 36: 73–79
14. Moore NF, Kelly DC (1980) A comparative study of the polypeptides of three iridescent viruses by N terminal analysis and surface labelling. *J Invert Pathol* 36: 415–422
15. Cuillel M, Tripper F, Braunwald J, Jacrot B (1979) A low resolution structure of frog virus 3. *Virology* 99: 277–285
16. Cerutti M, Devauchelle G (1985) Characterisation and localisation of CIV polypeptides. *Virology* 145: 123–131
17. Carey GP, Lescott T, Robertson JS, Spencer LK, Kelly DC (1978) Three African isolates of small iridescent viruses. *Virology* 85: 307–309
18. Barry S, Devauchelle G (1979) Study of structural polypeptides of *Chilo* iridescent virus (Iridovirus type 6). *Can J Microbiol* 25: 841–849
19. Kelly DC, Tinsley TW (1973) Ribonucleic acid polymerase activity associated with particles of iridescent virus types 2 and 6. *J Invertebr Pathol* 22: 199–202
20. Monnier C, Devauchelle G (1980) Enzyme activities associated with an invertebrate iridovirus: protein kinase activity associated with iridescent virus type 6 (*Chilo* iridescent virus). *J Virol* 35: 444–450
21. Farara T, Attias J (1986) Further characterisation of an alkaline protease activity associated with iridescent virus type 6. *Arch Virol* 87: 307–314
22. Wagner GW, Paschke JD, Campbell WR, Webb SR (1973) Biochemical and biophysical properties of two strains of mosquito iridescent virus. *Virology* 52: 72–80
23. Kelly DC, Vance DE (1973) The lipid content of two iridescent viruses. *J Gen Virol* 21: 417–423
24. Black PN, Blair CD, Butcher A, Capinera JL, Happ GM (1981) Biochemistry and ultrastructure of iridescent virus type 29. *J Invert Pathol* 38: 533–534
25. Balange-Orange N, Devauchelle G (1982) Lipid composition of an iridescent virus type 6 (CIV). *Arch Virol* 73: 363–367
26. Stoakes L, Kelly T, Schieven B, Harley D, Ramos M, Lannigan R, Groves D, Hussain Z (1991) Gas liquid chromatographic analysis of cellular fatty acids for identification of Gram-negative anaerobic bacilli. *J Clin Microbiol* 29: 2636–2638

27. Cacciopuoti B, Ciceroni L, Attard Barbini D (1991) Fatty acid profiles – a chemotaxonomic key for classification of strains of Leptospiraceae. *Int J Syst Bacteriol* 41: 295–300
28. Thompson IP, Bailey MJ, Ellis RJ, Purdy KJ (1993) Subgrouping of bacterial populations by cellular fatty acid composition. *FEMS Microbiol Ecol* 102: 75–84
29. Livesley MA, Thompson IP, Bailey MJ, Nuttall PA (1993) Comparison of the fatty acid profiles of *Borrelia*, *Serpulina* and *Leptospira* species. *J Gen Microbiol* 139: 889–895
30. Livesley MA, Thompson IP, Gern L, Nuttall PA (1993) Analysis of intra-specific variation in the fatty acid profiles of *Borrelia burgdorferi*. *J Gen Microbiol* 139: 2197–2201
31. Kelly DC, Tinsley TW (1972) Proteins of iridescent virus types 2 and 6. *J Invertebr Pathol* 19: 273–275
32. Gower JC (1966) Some distance properties of latent root vector methods used in multivariate analysis. *Biometrika* 53: 325–338
33. Boe B, Gjerde J (1980) Fatty acid patterns in the classification of some representatives of the families Enterobacteriaceae and Vibrionaceae. *J Gen Microbiol* 116: 41–49
34. Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. Freeman, San Francisco, pp 230–234
35. Willis DB, Granoff A (1974) Lipid composition of frog virus 3. *Virology* 61: 256–269
36. Palade GE (1983) Membrane biogenesis: an overview. *Methods Enzymol* 96: 24–55
37. Schmelz M, Sodeik B, Ericsson M, Wolffe EJ, Shida H, Hiller G, Griffiths G (1994) Assembly of vaccinia virus: the second wrapping cisterna is derived from the trans Golgi network. *J Virol* 68: 130–147

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