ELSEVIER

Virus Research 33 (1994) 99–121

Virus Research

Comparative studies of iridoviruses: further support for a new classification

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(Received 10 January 1994; revised 15 March 1994; accepted 16 March 1994)

Abstract

Changes in the classification of invertebrate iridoviruses (IVs) (Iridoviridae) have recently been proposed (Williams and Cory, 1994). The previous system of naming isolates according to the host and sequence of discovery (IV type 1, IV2, IV3, etc.) is not adequate for the purposes of taxonomy, since iridovirus isolates may infect many species, including hosts from diverse invertebrate orders. The new system of invertebrate iridovirus nomenclature, as with several other virus families, is based on geographical origin. Proposals have been made, based on DNA hybridization and other characteristics, by which invertebrate iridovirus isolates can be assigned to one of four recognized complexes, or considered as candidates for alternative assignations. This study reports comparative data on the DNA of 14 invertebrate iridovirus isolates used in the Williams and Cory study plus the two type vertebrate iridoviruses, frog virus 3 and flounder lymphocystis disease virus. DNA studies support the validity of assigning several isolates a common name and of grouping the known isolates into four complexes. The detection of such complexes is in broad agreement with previous serological studies. A previously undescribed isolate (San Miguel IV) obtained from the lepidopteran pest *Anticarsia gemmatalis* (Lep.: Noctuidae) has been initially characterized following the procedures recommended by Williams and Cory. DNA hybridization and Southern blot analysis identified this isolate as a new member of the Polyiridovirus complex. The San Miguel IV MSP gene was identified and a central fragment of ca. 719 bp was recovered by PCR amplification. The restriction endonuclease profiles (5 enzymes) of this isolate were distinct from others previously described.

Key words: Invertebrate iridescent virus; Iridoviridae; Classification; Characterization; *Anticarsia gemmatalis*; Lepidoptera

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SSDI 0168-1702(94)00029-C

1. Introduction

Until recently, the inter-relationships among invertebrate iridescent viruses (IVs) were obscure. The paucity of comparative data amongst an increasing number of IV isolates was partly due to a decline of interest in IVs as candidate insect biocontrol agents in the 1960s/1970s. This was followed by a dramatic increase in the molecular techniques for characterization that are now routine. Most reports of invertebrate IVs have comprised a record of the host species, a description of icosahedral particles of some 130 nm diameter, a mention of the spectacular opalescent hues which result from the superabundance of virus particles in infected tissues, and often an estimate of the incidence of infection in the local host population; which is usually extremely low. A growing number of these records prompted Tinsley and Kelly (1971) to propose a numerical system of nomenclature in which isolates were sequentially allocated numbers and host species descriptions, e.g., *Tipula paludosa* iridescent virus type 1. However, this system is not useful for the purposes of taxonomy since serologically identical strains of virus have been isolated from different hosts and allocated different numbers.

Recently, the classification and nomenclature of iridescent viruses has been revised to account for genetic and serological relationships among invertebrate IVs (Williams and Cory, 1994). The invertebrate isolates were allocated to four distinct hybridization groups (Fig. 1). Three of these complexes lie within the *Iridovirus* genus. The largest group, the Polyiridovirus complex, contains Plowden IV (originally isolated from *Tipula paludosa*, previously IV1), and 8 related isolates from diverse host species. The other complexes; Oligoiridovirus and Crustaceoiridovirus, have fewer members. The genus *Chloriridovirus* contains one member, Vero Beach IV, from the mosquito *Aedes taeniorhynchus*. This system of classification is in broad agreement with the serological studies (summarized by Kelly et al., 1979), in which a large group of 11 inter-related isolates was described, and an additional 3 isolates were described as having intermediate (Fort Collins IV [IV29]) or low (Dazaifu IV [IV6], and Srinagar IV [IV24]) serological relatedness to the main group.

A comparatively large number of isolates, especially from mosquitoes, remain to be characterized (Table 1). Prior to the study of Williams and Cory (1994), only 11 out of the 68 IV isolates reported had genomic data available (restriction fragment profiles, etc.), while a further 7 isolates had some degree of serological characterization. In general, the iridescent viruses have had only curiosity value, and with the passage of time, many isolates have been lost. However, recent studies have indicated that IV-host relationships may be far more interesting than previously supposed (Williams, 1993; Williams et al., 1994).

All iridovirus genomes investigated to date display circular permutation and terminal redundancy, which implies that viral DNA is cleaved after packaging into the virion; in much the same way as the 'headful' mechanism seen in bacteriophages (Goorha, 1982). Reflecting this, the Dazaifu IV genome has at least six origins of replication identified to date (Handermann et al., 1992). The vertebrate

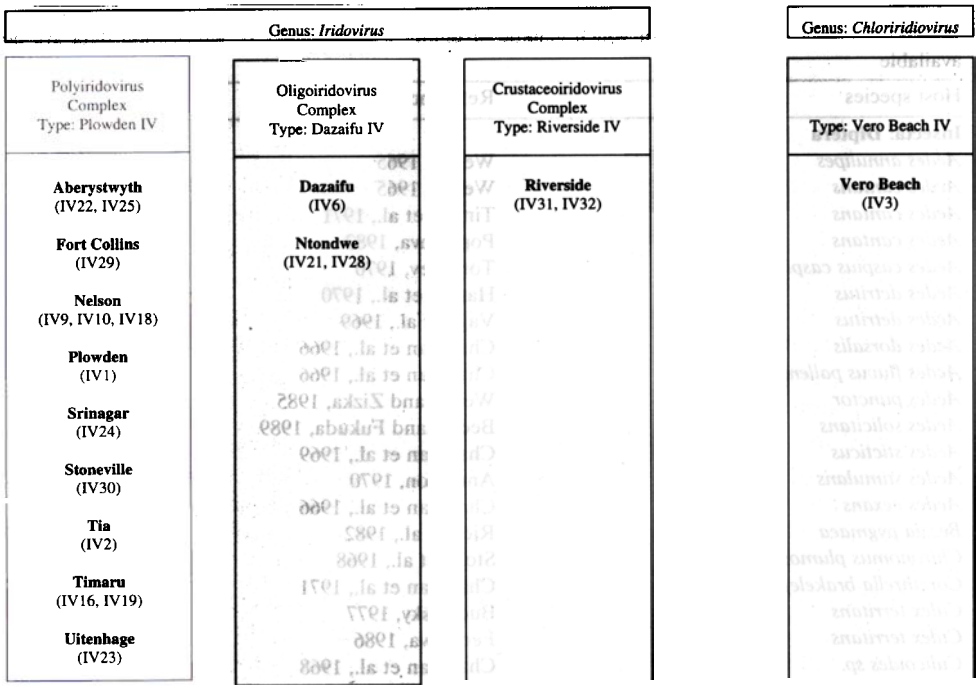

Fig. 1. New classification of invertebrate iridoviruses as proposed by Williams and Cory (1994). Isolates are named according to geographical origin, and assigned to one of four hybridization complexes.

iridoviruses, Frog Virus 3 (FV3) and Flounder Lymphocystis Disease Virus (FLCDV), have genomes that are highly methylated at cytosine residues and which, at least in FV3, appear to depend on the presence of a viral encoded *trans*-acting protein to assist in transcription by host RNA polymerase (Willis et al., 1990). Fortunately, the common occurrence of a major structural protein among all the members of the Iridoviridae has provided a useful genetic marker and conserved feature for comparative studies (Schnitzler and Darai, 1993).

This study is complementary to, and in support of, the study of Williams and Cory (1994), in which the new system of invertebrate iridovirus classification was proposed. Comparative data are presented on 14 invertebrate iridescent viruses from major insect and crustacean orders. The two type vertebrate viruses: FLCDV and FV3 were also included. Genomic characterization and Southern blot analysis using an iridovirus-specific gene probe was used to demonstrate the validity of the new classification system. In addition, a previously undescribed isolate from *Anticarsia gemmatalis* (herein named San Miguel IV) has been initially characterized following the recommendations given in the new classification. All nomenclature uses the new classification, but virus isolate numbers (IV1, IV2 etc.) are included where this improves clarity.

Table 1
Invertebrate iridovirus isolates for which genetic or detailed serological characterization data are not available

Host species	Reference
Insecta: Diptera	
Aedes annulipes	Weiser, 1965
Aedes cantans	Weiser, 1965
Aedes cantans	Tinsley et al., 1971
Aedes cantans	Popelkova, 1982
Aedes caspius caspius	Torybaev, 1970
Aedes detritus	Hassan et al., 1970
Aedes detritus	Vago et al., 1969
Aedes dorsalis	Chapman et al., 1966
Aedes fluvus pallens	Chapman et al., 1966
Aedes punctor	Weiser and Zizka, 1985
Aedes solicitans	Becnel and Fukuda, 1989
Aedes sticticus	Chapman et al., 1969
Aedes stimulans	Anderson, 1970
Aedes vexans	Chapman et al., 1966
Bezzia pygmaea	Rieb et al., 1982
Chironomus plumosus	Stoltz et al., 1968
Corethrella brakeleyi	Chapman et al., 1971
Culex territans	Buchatsky, 1977
Culex territans	Fedorova, 1986
Culicoides sp.	Chapman et al., 1968
Culicoides clastrieri	Rieb et al., 1982
Culicoides cubitalis	Rieb et al., 1982
Culicoides odibilis	Rieb et al., 1982
Culiseta annulata	Buchatsky, 1977
Dixid sp.	Fedorova, 1986
Prosimulium sp.	Avery and Bauer, 1983
Psorophora columbiae (confinnis)	Chapman, 1974
Psorophora ferox	Chapman et al., 1966
Psorophora horrida	Chapman et al., 1969
Psorophora mathesoni (varipes)	Chapman et al., 1969
Simulium callidum	Takoaka, 1980
Simulium earlei	Takoaka, 1980
Simulium neornatipes	Batson, 1986
Simulium ornatum	Weiser, 1968
Simulium rubicundulum	Takoaka. 1980
Other insect orders	
Acrolophus sp. (Lepidoptera)	Federici, 1984
Phyllophaga anxia (Coloeptera)	Poprawski and Yule, 1990
Pterostichus madidus (Coleoptera)	Robertson (unpublished)
Ephemoptera sp. (Trichoptera)	Federici (unpublished)
Formica lugubris (Hymenoptera)	Steiger et al., 1969
Scapteriscus borellii (Orthoptera)	Fowler, 1989
Crustacean orders	
Simocephalus expinosus (Cladocerca)	Federici and Hazard, 1975
Armadillidium vulgare (Isopoda)	Poinar et al., 1985
Porcellio scaber (Isopoda)	Poinar et al., 1985
Porcellio laevis (Isopoda)	Grosholz, 1992

Table 1 (continued)

	Reference
	Montanie et al., 1993
	Lightner and Redman, 1993
	Poinar et al., 1980
	Runnger et al., 1971

2. Materials and methods

All the procedures follow those given in Williams and Cory (1994). Iridovirus isolates were obtained from sources worldwide (Table 2) and were selected to be representative of a broad range of insect hosts and to include each of the recognized IV genera: *Iridovirus*, *Chloriridovirus*, *Ranavirus* and *Lymphocystivirus*. The majority of invertebrate iridoviruses were amplified in *Galleria mellonella* wherever possible, and purified by centrifugation to semi-pure suspensions prior to DNA extraction. Standard phenol-chloroform and dialysis protocols were used to extract DNA from virus particles following treatment with Proteinase K and SDS. DNAs were stored at −20°C until use.

2.1. REN characterization

The fidelity of all material amplified in *G. mellonella* was checked by comparison of original and amplified material restriction profiles following digestion with *Hin*dIII and electrophoresis in 0.6% agarose in TBE buffer containing ethidium bromide. Likewise, isolates were characterized by treatment with *Eco*RI or *Sal*I followed by electrophoresis as above. An iridovirus isolate from *Anticarsia gemmatalis* (Sieburth and Carner, 1987; now named San Miguel IV) which had not been included in the study of Williams and Cory (1994) was characterized by treatment with *Hin*dIII, *Eco*RI, *Bam*HI, *Asp*718 and *Pst*I. Sizes of restriction fragments were estimated using the program MolMatch (Glasgow University), which also generated coefficients of similarity for pairwise comparisons of restriction profiles.

2.2. Southern blot analysis

After photography, gels were denatured, neutralized and blotted overnight onto a Hybond membrane (Amersham Ltd). Blots were baked at 80°C for 2 h followed by prehybridization at 37°C in 50% formamide, 50% hybridization buffer (50 mM HEPES, 0.02% Ficoll 400, 0.02% BSA, 0.02% PVP, 0.1% SDS) for 4 h. A *Sal*I fragment containing 91% of the Aberystwyth IV (IV22) major structural protein (MSP) gene (Cameron 1990) was radiolabelled by nick translation to high specific activity and used to probe the blot overnight at the conditions described above. Blots were washed in 2 × SSC at room temperature and twice at 60°C (each for 1 h) before autoradiography. Blots were then stripped using 0.4 M NaOH at 45°C for 30 min, washed twice in 200 mM Tris-HCl (pH 7.0), 0.1 × SSC, 0.1% SDS, and

Table 2
Sources of material used in this study with details of the original host, passage history and revised nomenclature of Williams and Cory (1994)

Previous IV type number and new name	Original host species (order)	Virus grown in:	Source of material	Characterization reference
1 Plowden IV	*Tipula paludosa* (Diptera)	*G. mellonella*	IVEM (C.F. Rivers)	Tajbakhsh et al., 1986
2 Tia IV	*Sericesthis pruinosa* (Coleoptera)	*G. mellonella*	IVEM	Day & Mercer, 1964
3 Vero Beach IV	*Aedes taeniorhychus* (Diptera)	*A. taeniorhynchus*	S. Avery, USDA, USA	Wagner & Paschke, 1977
6 Dazaifu IV	*Chilo suppresalis* (Lepidoptera)	*Choristoneura fumiferana* cells (CF-124)	G. Darai, Heidelberg, Germany	Delius et al., 1984
9 Nelson IV	*Wiseana cervinata* (Lepidoptera)	*G. mellonella*	IVEM	Ward & Kalmakoff, 1987
10 Nelson IV	*Witlesia sabulosella* (Lepidoptera)	*G. mellonella*	IVEM	Fowler & Robertson, 1972
18 Nelson IV	*Opogonia* sp. (Coleoptera)	Original material?	IVEM	Kelly & Avery, 1974
21 Ntondwe IV	*Helicoverpa armigera* (Lepidoptera)	*G. mellonella*	IVEM	Carey et al., 1978
22 Aberystwyth IV	*Simulium* sp. (Diptera)	*G. mellonella*	IVEM	Hibbin & Kelly, 1980
24 Srinagar IV	*Apis cerana* (Hymenoptera)	*A. mellifera*	M. Allen, Rothamstead, UK.	Bailey et al., 1976
28 Ntondwe IV	*Lethocerus columbiae* (Hemiptera)	*G. mellonella*	IVEM	Carey et al., 1978
29 Fort Collins IV	*Tenebrio molitor* (Coleoptera)	*G.mellonella*	IVEM	Black et al., 1981
30 Stoneville IV	*Helicoverpa zea* (Lepidoptera)	*G. mellonella*	P. Christian, CSIRO, Australia.	Stadelbacher et al., 1978
31 Riverside IV	*Armadillidium vulgare* (Isopoda)	*G. mellonella*	B. Federici, Riverside, USA.	Cole & Morris, 1980
San Miguel IV	*Anticarsia gemmatalis* (Lepidoptera)	*G. mellonella*	G. Kinard, Clemson, USA	This study
FV3	*Rana pipiens* (Anura)	Chick embryo	A.M. Aubertin, ULP-INSERM, France.	Lee & Willis, 1983
FLCDV	*Pleuronectes flesus* (Acanthopterygii)	Flounder lesions: field material	B. Hill, MAFF, UK.	Darai et al., 1983

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prehybridized and hybridized as before but at a lower stringency: 20% formamide, 37°C, followed by washes at 40°C.

2.3. Additional characterization of the San Miguel IV

Dot blot hybridization against DNA from each of the three iridovirus hybridization groups: Plowden and Aberystwyth IV (Polyiridovirus complex) Dazaifu IV (Oligoiridovirus complex) and Riverside IV (Crustaceoiridovirus complex) was

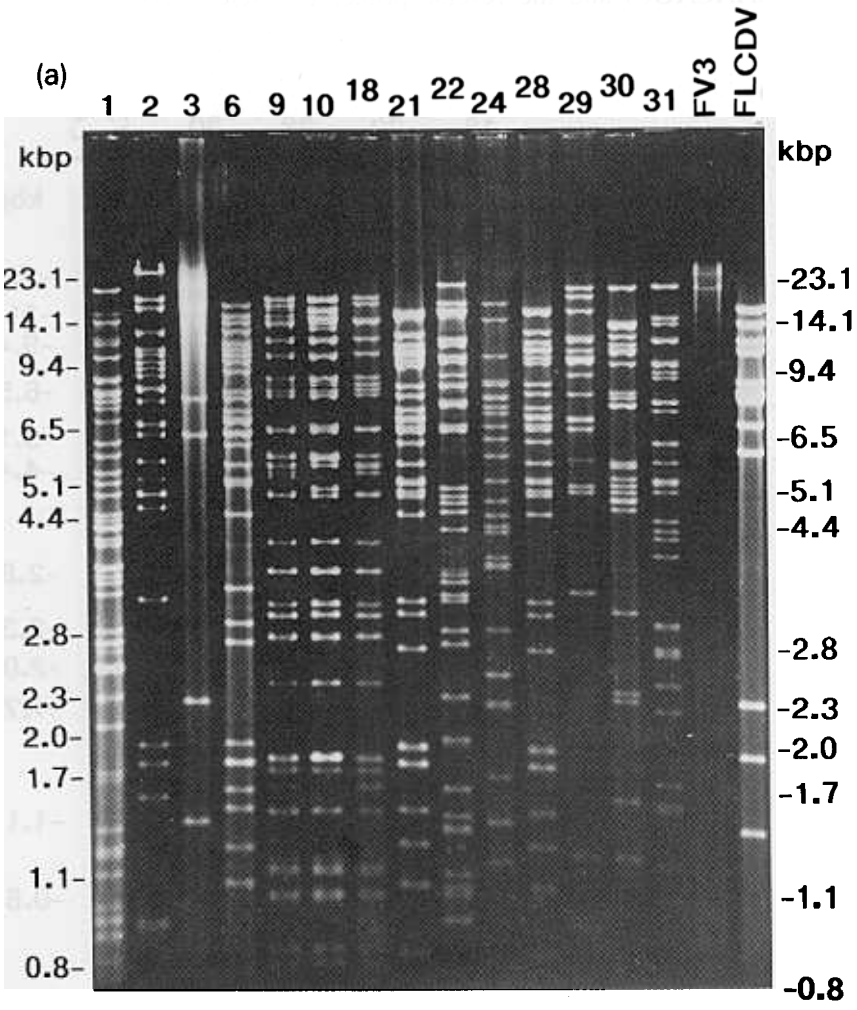

Fig. 2. (a) REN profiles in 0.6% agarose for iridescent virus DNA following digestion by *Eco*RI (sizes in kbp). Lanes are number according to the original type numbers given in Table 2.

performed. Dots of 100 ng DNA, replicated 5 times, were probed with San Miguel IV DNA radiolabelled with ^{32}P by nick translation. The hybridization conditions were: 37°C in 40% formamide, 60% hybridization buffer (above) followed by critical washes in 2 × SSC at 55°C. Salmon sperm DNA was used as a control for non-specific binding. Dots were counted for 3 min in a scintillation counter and the results used to calculate hybridization relative to homologous DNA after correction for background.

The ability of PCR primer sequences targeted at the MSP gene, to recognize and successfully amplify the MSP gene region of the isolate from San Miguel IV was also tested under cycling conditions of high stringency. The forward primer was designed to hybridize at base numbers 733–753 (5′ GGCGGCC-CAACAGCAACAGC) and the reverse primer (5′ GGCACAACCCATTCTAC-

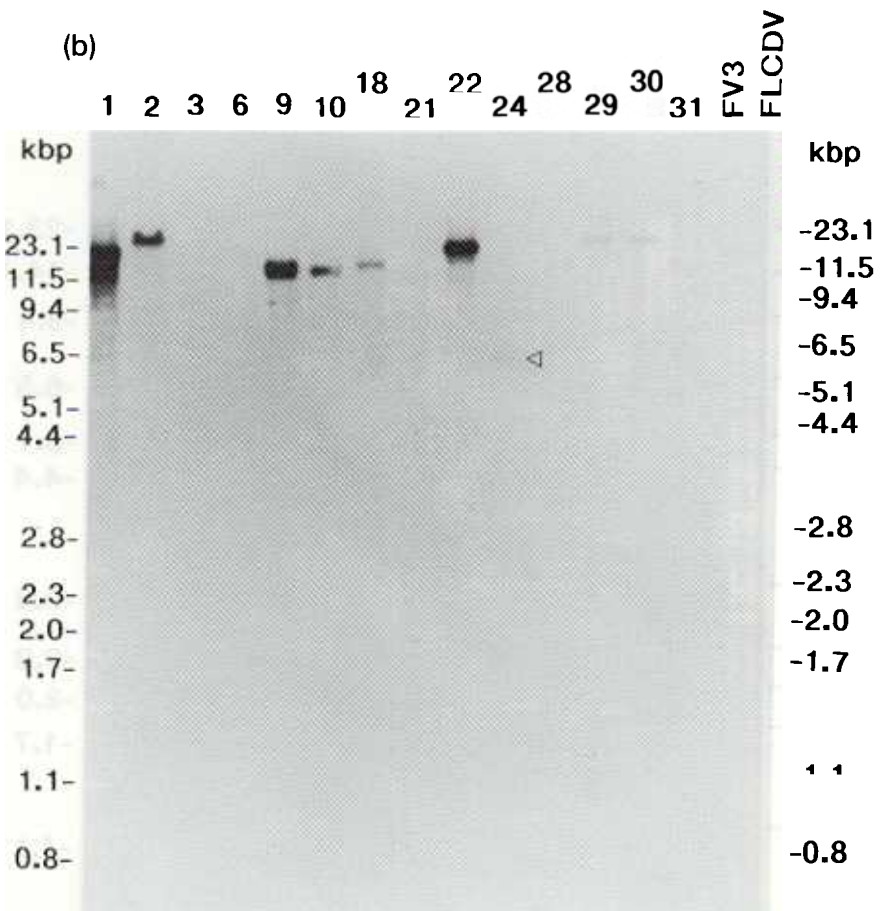

Fig. 2 (b) Southern blot of *Eco*RI gel probed at high stringency (50% formamide, 37°C) with Aberystwyth IV (previously IV22) MSP gene fragment. Weak bands are highlighted by arrowheads for clarity.

GACG) to the complement of bases 1452–1431 of the Aberystwyth IV MSP gene sequence (Cameron 1990). The amplification program involved 40 cycles all consisting of: 95°C for 1 min, 60°C for 1 min and 72°C for 30 s. The PCR product was subject to digestion by *Xho*I for which a cleavage site exists two-thirds along the length of the gene sequence of the Plowden and Aberystwyth isolates.

3. Results

3.1. REN characterization

The REN analysis (Table 2) was consistent with the results of Williams and Cory (1994). It was not possible to size accurately in some cases from the *Sal*I gel,

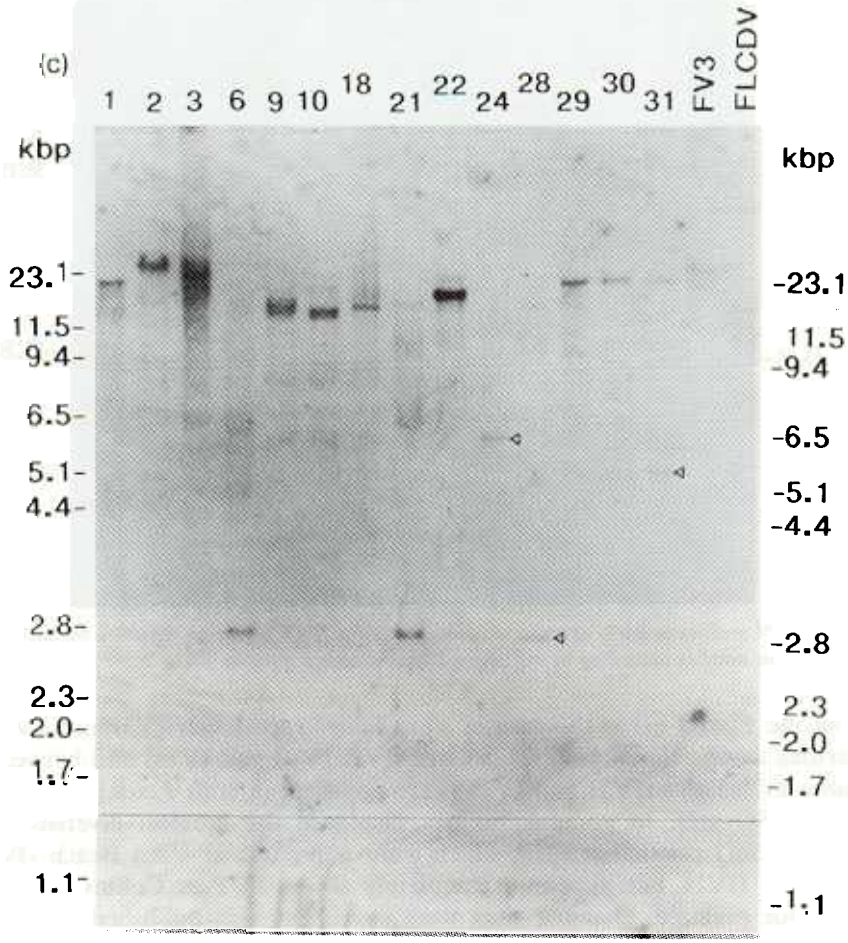

Fig. 2 (c) As above, but reprobed at low stringency (20% formamide, 37°C). The affinity of the Dazaifu IV (IV6) and Ntondwe IV (IV21 and IV28) isolates for the probe are evident only at low stringency.

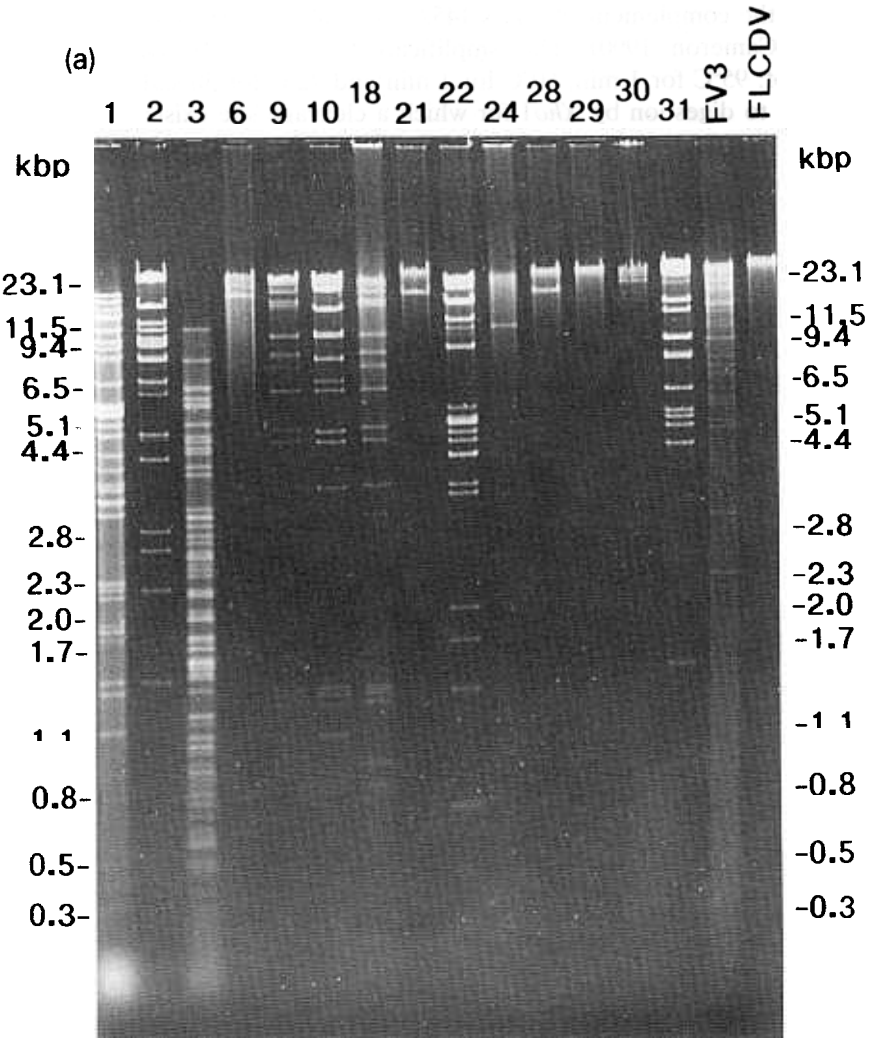

Fig. 3. (a) REN profiles in 0.6% agarose for iridescent virus DNA following digestion by *Sal*I (sizes in kbp). Lanes are number according to the original type numbers given in Table 2.

or from the *Eco*RI gel in the case of Vero Beach (previously IV3) and FV3. The similarities among the Nelson IV isolates (IV9, IV10 and IV18) and between the Ntondwe IV isolates (IV21 and IV28) were apparent in both *Eco*RI (Fig. 2a) and *Sal*I (Fig. 3a) gels. In general, isolates displayed the greatest diversity in the number of *Sal*I restriction sites; which were numerous in Vero Beach (IV3) or Plowden IV (IV1), but appeared completely absent in Fort Collins (IV29) and FLCDV for example. Genome sizes were consistent with published values, with the exception of Vero Beach IV (IV3) estimated as 160 kbp using *Sal*I, but which was previously reported to have an exceptionally large genome of 383kbp (Wagner

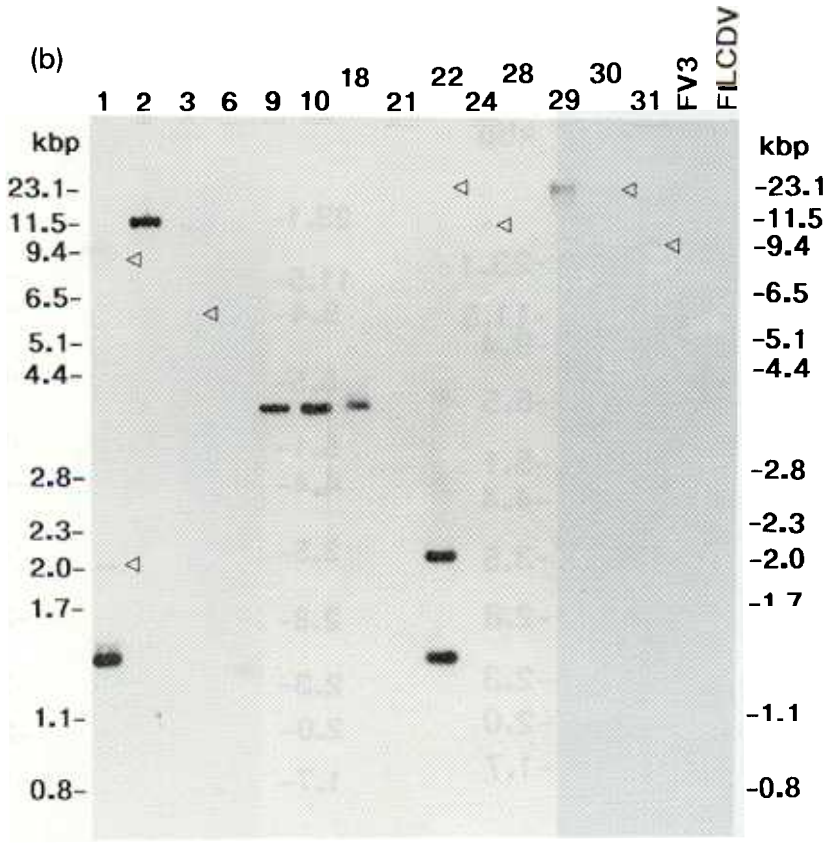

Fig. 3 (b) Southern blot of *Sal*I gel probed at high stringency (50% formamide, 37°C) with Aberystwyth IV (previously IV22) MSP gene fragment. Weak bands are highlighted by arrowheads for clarity.

and Paschke, 1977). The coefficients of similarity between isolates from the *Eco*RI digest were: IV9:IV10 88.5%, IV9:IV18 82.1%, IV10:IV18 91.5%, IV10:IV28 69.1%, IV10:IV31 67.8%, IV21:IV28 92%, IV22:IV28 68.9%, and from the *Sal*I digest were: IV9:IV10 76.5%, IV9:IV18 77.8%. These coefficients of similarity (Dice coefficients) reinforced the apparent similarities among the Nelson IV and Ntondwe IV isolates, but also suggested relationships between more distant isolates such as Nelson and Riverside IVs (IV10 and IV31), for example, in the *Eco*RI digests. This highlights the importance of using Dice coefficients derived from a range of restriction enzymes (Grothues and Tümmler, 1991). The mean genome size of the San Miguel IV isolate, calculated from restriction fragment sizes, was 176.3 kbp (Fig. 4a, Table 2). Dice coefficients derived from REN profiles from this study and those published by Williams and Cory (1994) indicated no apparent similarity between the San Miguel IV isolate and any other. San Miguel IV:Tia IV (IV2) values were 67.4% for *Hin*dIII and $< 66\%$ for all other profile comparisons.

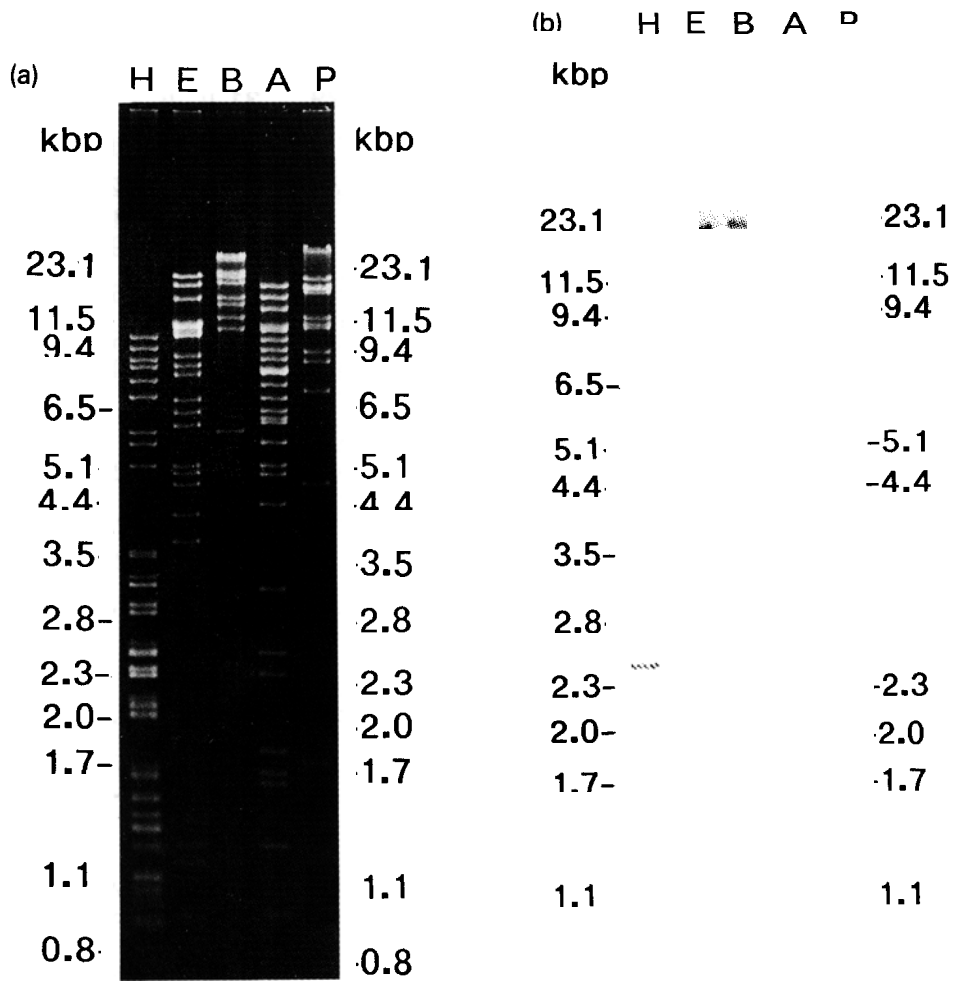

Fig. 4. (a) REN profiles in 0.6% agarose for San Miguel IV DNA following digestion by *Hin*dIII(H), *Eco*RI(E), *Bam*HI(B), *Asp*718(A) or *Pst*I(P) (sizes in kbp). (b) Southern blot of this gel probed at high stringency (50% formamide, 37°C) with Aberystwyth IV (previously IV22) MSP gene fragment. Weak bands are highlighted by arrowheads for clarity.

3.2. Southern blot analysis

The Aberystwyth IV MSP gene probe showed consistent hybridization among blots (Figs. 2b and 3b). The Nelson IV isolates (IV9, IV10, IV18) had virtually identical fragments hybridizing to this probe at high stringency; as did the Ntondwe IV isolates (IV21 and IV28) at low stringency. Dazaifu IV (IV6) showed similar behaviour to the Ntondwe IV isolates in the location and strength of the hybridization response to this gene probe. Similarities between Fort Collins IV (IV29) and Stoneville IV (IV30) were also apparent in the size of the REN fragments with affinity to the MSP gene. Vero Beach IV (IV3) showed very weak hybridization to

a 5.6kb *Sal*I fragment (highlighted in Fig. 3b), but did not produce an observable response in the *Eco*RI blot at either stringency. The San Miguel IV isolate showed a clear affinity for the probe at high stringency, across all REN digests (Fig. 4b). Neither of the vertebrate isolates, FV3 and FLCDV, showed any affinity for the probe. The *Sal*I blot did not produce consistent results when probed at low stringency and so is not included here.

3.3. Additional characterization of the San Miguel IV isolate

The San Miguel IV isolate hybridized solely to members of the Polyiridovirus complex. Relative dot-blot hybridization values recorded were: 16% for Plowden IV (IV1), 12% for Aberystwyth IV, and $< 0.2\%$ for Dazaifu IV (IV6), and Riverside IV (IV31) at the intermediate stringency used, after correction for background hybridization.

This isolate was recognized by the oligonucleotide primers derived from the Aberystwyth IV MSP gene sequence (Cameron 1990), and a product virtually identical in size to the Aberystwyth IV product (i.e., 719bp) was detected. However, unlike the Aberystwyth and Plowden isolates, the amplicon was not cleaved by *Xho*I.

4. Discussion

The data presented here have illustrated the relationships among a broad range of IV isolates spanning all the genera of the Iridoviridae currently recognized. The observed affinities for the MSP gene probe in the Southern blots analysis supported the findings of Williams and Cory (1994), who detected distinct hybridization groups within the *Iridovirus* genus. The grouping of the Nelson IV isolates (IV9, IV10 and IV18) under a common name was supported by the REN analysis, Dice coefficients and Southern blot results of this study; likewise for the Ntondwe IV isolates (IV21 and IV28). The remaining isolates were distinct from one another. Southern blot analysis was consistent with the observation of discrete hybridization complexes, inasmuch as isolates showed a high (Polyiridovirus complex), low (Oligoiridovirus complex), or no apparent affinity (Crustaceoiridovirus complex) to the Aberystwyth IV MSP gene probe.

The results of this study were generally consistent with previous serological studies (e.g., Glitz et al., 1968; Fowler and Robertson 1972; Kalmakoff et al., 1972; Elliott et al., 1977; Carey et al., 1978; Kelly et al., 1979; Cole and Morris, 1980) The only differences to the serological studies relate to Dazaifu IV (IV6) and Srinagar IV (IV24), both of which were previously described as unrelated to other isolates (Bailey et al., 1976; Kelly et al., 1979). However, the Ntondwe IV isolates (IV21 and IV28) appeared related to Dazaifu IV (IV6), whereas Srinagar IV (IV24) showed clear affinities to the large Polyiridovirus complex (see also Williams and Cory, 1994).

The vertebrate iridoviruses, FV3 and FLCDV showed no DNA similarities to any of the invertebrate IVs. These vertebrate isolates were consistently negative in

Table 3
Restriction fragment and genome sizes calculated by MolMatch program with identification of fragments which hybridized to the Aberystwyth IV (IV22) MSP gene probe ** at high stringency and * at low stringency

					Vero Beach IV IV3		Nelson IV IV9			
		*Sal*I		*Sal*I	*Sal*I		*Eco*RI	*Sal*I		*Sal*I
A	**19.8** **	17.74	**26.4** **	22.23	12.01	16.47	17.08	22.05	17.01	24.3
B	13.57	16.02	17.3	22.23	9.12	14.67	16.23	22.05	15.12	24.3
C	13.57	14.58	15.9	14.91	7.11	13.76	15.22	22.05	**14.1** **	22.06
D	11.82	13.47	12.74	14.91	7.11	12.58	**14.48** **	19.79	13.36	19.1
E	11.44	13.47	**10.3**	**12.48** **	6.4	11.3	**13.98** **	17.75	12.88	19.1
F	10.32	11.53	9.7	11.2	6.22	10.86	**13.98** **	15.94	11.69	14.46
G	8.9	10.2	9.26	10.45	6.14	10.48	11.83	14.91	10.42	10.64
H	8.82	9.65	8.99	10.01	**5.62** **	9.93	10.51	14.58	9.08	10.64
I	8.16	8.61	8.52	9.73	5.19	9.23	9.08	10.67	8.72	8.66
J	8.16	6.94	7.88	8.51	5.03	8.23	8.28	8.8	8.43	7.11
K	7.96	5.84	7.88	8.39	4.86	7.86	8.11	7.98	8.13	6.71
L	7.61	5.84	7.34	7.13	4.7	7.38	6.74	6.59	6.84	5.1
M	7.08	5.54	6.58	6.39	4.37	7.11	6	5.12	6.76	4.85
N	6.01	5.54	6.25	4.98	4.19	6.99	5.79	4.83	5.95	**3.79** **
O	5.58	5.2	5.6	4.88	3.48	6.56	5.59	**3.76** **	5.79	3.27
P	5.32	4.89	4.8	4.35	3.36	6.48	5.45	3.27	5.2	1.6
Q	5.11	4.42	4.8	3.04	3.36	6.26	5.06	1.59	5.09	1.56
R	4.64	4.12	4.55	2.71	3.25	5.65	4.13	1.56	4.11	1.48
S	4.57	3.77	3.21	2.36	3.08	5.33	3.68	1.49	3.67	1.32
T	4.31	3.68	1.97	1.62	3	5.24	3.24	1.17	3.24	1.06
U	4.31	3.46	1.86		2.88	4.6	3.11	1.05	3.12	0.82
V	4.12	3.46	1.65		2.79	3.43	2.84	0.81	2.84	
W	4.08	3.26	1.09		2.79	3.04	2.38		2.37	
X	3.73	2.42	1.09		2.72	**2.83** *	1.75		1.74	

Aa

Table 3 (continued)

	Nelson IV IV 18		Ntondwe IV IV 21	Aberystwyth IV IV 22		Srinagar IV IV24	Ntondwe IV IV28	Fort Collins IV IV29	Stoneville IV IV30
	*Eco*RI	*Sal*I	*Eco*RI	*Eco*RI	*Sal*I	*Eco*RI	*Eco*RI	*Eco*RI	*Eco*RI
A		20.1			22.07				
B		19.3			22.08				
C		16.5			18.65				
D		16			18.65				
E		14.9			15.41				
F		14.6			15.41				
G		10.6			15.41				
H		8.81			12.63				
I		8.01			9.51				
J		5.09			9.33				
K		4.82			5.88				
L		**3.77** **			5.41				
M		3.27			5.41				
N		1.6			5.41				
O		1.56			5.06				
P		1.51			4.84				
Q		0.95			4.48				
R		0.83			4.48				
S					3.82				
T					3.66				
U					**2.19** **				
V					1.91				
W					**1.58** **				
X					0.77				
Y					0.48				

Z	1.33		1.91	2.09			1.33		
a	1.22		1.65	1.79			1.13		
b	1.01		1.46	1.63			0.91		
c	0.95		1.27	1.56					
d	0.87		1.01	1.55					
e	0.7			1.35					
f	0.62			1.28					
g				1.23					
h				1.15					
i				0.91					
j				0.71					
k									
l									
m									
n									
o									
p									
q									
r									
s									
t									
u									
v									
w									
x									
y									
z									
Aa									
Total:	179.81	152.2	181.3	205.9	214.6	150	192.2	171.4	187.9

Table 3 (continued)

	Riverside IV IV31	FLCDV	San Miguel IV				
	*Sal*I	*Eco*RI		*Eco*RI	*Bam*HI	*Asp*718	*Pst*I
A	26.31	14.94	10.6	**20.02** **	34.8	17.49	36.2
B	26.31	12.9	9.65	17.6	32	**15.46** **	19.45
C	21.32	11.38	8.93	15.23	**22.67** **	13.9	**17.03** **
D	15.46	10.52	8.6	11.46	20.83	11.65	**17.03** **
E	15.46	8.54	7.89	11.46	18.26	11.29	16.07
F	13.71	8.08	7.22	11.17	15.56	10.68	12.39
G	13.71	7.94	6.02	10.78	14.45	9.87	11.66
H	**9.47** **	7.71	5.76	10.78	12.52	9.27	**11.53** **
I	**9.47** **	6.84	5.17	9.25	11.47	8.51	9.71
J	8.14	6.08	3.71	8.72	6.09	8.51	9.06
K	8.14	2.36	3.64	8.4		7.89	7.55
L	6.37	1.95	3.36	7.15		7.3	4.83
M	5.47	1.49	3.27	6.73		6.89	1.75
N	5.32		3.27	6.25		6.56	
O	4.97		3.03	5.14		6.38	
P	4.52		2.96	5.01		5.85	
Q	1.64		2.65	4.82		5.24	
R			2.58	4.28		5.02	
S			2.56	3.83		4.43	
T			**2.42** **	1.3		3.24	
U			**2.42** **	1.21		2.59	
V			2.37	0.95		2.39	
W			2.37			1.82	
X			2.21			1.69	
Y			2.14			1.63	

1.35
.67
.54
.43
.29
.08
0.8
Aa
100.
88.

all the tests performed, even the low stringency *Eco*RI Southern blot. They therefore maintain their previous status as distinct and separate genera within the Iridoviridae.

As shown by this and previous studies, iridoviruses naturally infect across host taxa, e.g., Lepidoptera and Coleoptera by Nelson IV, Lepidoptera and Hemiptera by Ntondwe IV, Crustacea and Nematoda by Riverside IV (Poinar et al., 1980). This has highlighted the inherent deficiencies of basing the nomenclature of the virus upon the host which can lead to much confusion, as is currently seen in the Baculoviridae. The use of geographical descriptors has precedence in a number of other virus families and, if adopted by other workers in iridoviruses, should prevent the problems of virus nomenclature seen elsewhere.

Recently, Stohwasser et al. (1993) used PCR techniques directed at highly conserved areas within the Aberystwyth IV and Plowden IV MSP genes to locate and characterize the homologous Dazaifu IV (IV6) gene. They identified two *Eco*RI fragments: X (2.85 kbp) and Q (5.9 kbp) as hybridizing to an RNA transcript of the gene. In the present study, only the X fragment hybridized strongly to the gene probe (at low stringency) (Table 3); presumably because approximately half of the 251 upstream bases in the Q fragment are not present on the Aberystwyth IV *Sal*I fragment which constituted the probe.

The MSP gene codes for a protein of ca. 50K, ubiquitous among the Iridoviridae. This gene has considerable value as a marker by which to orientate the circularly permutated genome characteristic of iridoviruses, and as a common gene for comparative investigations. The overall levels of DNA sequence identity to the Aberystwyth IV MSP gene are very similar for FLCDV and Dazaifu IV at 69.3% and 73.4% respectively. However, Dazaifu IV (IV6) hybridized to the gene probe, whereas FLCDV did not. This may be due to the length of the continuous base homology which is located more closely together for Dazaifu but dispersed over the entire gene in FLCDV. In addition, Schnitzler and Darai (1993) recently compared the deduced MSP amino acid sequences of Dazaifu IV, Aberystwyth IV, and Plowden IV with that of FLCDV. They reported overall amino acid identity/similarity values of 53%/29.5% for Dazaifu IV, 50.3%/33.8% for Plowden IV and 49.1%/34.2% for Aberystwyth IV when compared to the homologous FLCDV sequence. A diversity of other genes are now being identified from Dazaifu IV, including a putative helicase gene (Sonntag et al., 1994a, 1994b), zinc finger proteins, a non-histone chromosomal protein, and a polypeptide similar to GTP phosphohydrolase which is a known bacterial antimutator (Schnitzler et al., 1994).

The previously uncharacterized iridovirus isolate (San Miguel IV) from the Soya pest *Anticarsia gemmatalis* (Lep. Noctuidae) was found to be a new member of the Polyiridovirus complex by hybridization. This isolate, was distinct from previously described isolates in terms of restriction profiles and location of the MSP gene fragments. Originally from Tucumán province, Argentina (C.B. Moore pers. comm.), the isolate has been given the name San Miguel IV, this being the major town of the province, following the naming procedures of the new classification (Williams and Corv. 1994). It is one of a handful of IV isolates which have been

found to induce epizootics of patent (lethal) infection in the host population. This brings to a total of ten, the number of isolates confirmed as belonging within the Polyiridovirus complex.

A great number of iridovirus isolates have not been characterized (Table 1) but iridovirus isolates for which genetic information is available are from: *Simulium vittatum* (Diptera) (Erlandson and Mason, 1990), and *Scapteriscus aclectus* (Orthoptera) (Boucias et al., 1987). An isolate from *Nereis diversicolor* (Annelida) (Devauchelle, 1977) has been subject to detailed physical characterization. These isolates have yet to be assigned to complexes. This study further demonstrated the validity of the new system of classification for invertebrate iridoviruses, and showed how a novel isolate can be readily characterized and assigned to an iridovirus complex within the new system.

Acknowledgements

I am deeply grateful to the people who donated the material which made this study possible: Mark Allen, Anne-Marie Aubertin, Susan Avery, Martin Ayres, Brenda Ball, Peter Christian, Gholamreza Darai, Brian Federici, Rakesh Goorha, Barry Hill, Gary Kinard, Nigel McMillan, and Clyde Moore. I thank Chris Hatton for photography and Tim Carty for the *Galleria* supply. Jenny Cory and David Bishop provided helpful comments on the manuscript.

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