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THE IRIDOVIRUSES

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I. Introduction

In March 1954, on his first trip from the laboratory of Kenneth Smith in Cambridge, Claude Rivers went in search of crane fly larvae (*Tipula* spp.) infected with the recently discovered hemocyte polyhedrosis virus. He applied St. Ives fluid to pasture land in the grounds

of Plowden Hall, Shropshire, England. As the tipulid larvae wriggled to the surface to escape the irritating phenols, Rivers was amazed to see larvae with brilliant patches of iridescent blue color showing beneath the epidermis. Later, he returned with Nick Xeros and collected more infected material, which resulted in the note to *Nature* and which marked the beginning of research on the iridescent viruses (Xeros, 1954). At the time, the discovery caused great excitement and controversy because the structure and host range of the iridescent virus was in marked contrast to that of the polyhedrosis viruses discovered previously, and this brought into focus much of Smith's earlier work on the host specificity of insect viruses. Subsequently, a number of other arthropods were diagnosed with patent iridescent virus infections. Because of the occurrence of iridescent virus diseases in some important pest and vector species, the viruses have attracted attention as potential agents for biological control. For laboratory studies they have a number of advantages in that many can be grown in massive quantities in insect larvae and several isolates are highly amenable to cell culture.

Vertebrate iridoviruses are found in fish, amphibians, and reptiles. For the amphibian iridoviruses, initial interest was sparked by finding one isolate in association with a renal carcinoma of the leopard frog, *Rana pipiens* (Granoff *et al.*, 1966). The association was later shown to be coincidental. Much of the known biology of iridoviruses comes from work with this frog isolate (named frog virus 3), which has proved to be highly amenable to manipulation in cell culture, a more important reason for its popularity. For the fish iridoviruses, the wartlike lesions symptomatic of "lymphocystis disease" have been known for over a century although the disease-causing agent was not recognized until much later (Walker, 1962). Certain iridovirus infections of fish may be inapparent, whereas others produce overt, sometimes lethal disease, and thus have economic significance, but research has been hampered because of difficulties with cell culture, especially for the lymphocystis disease viruses.

Iridoviruses are large icosahedral viruses, 120 to 300 nm in diameter, that assemble in the cytoplasm of host cells (Fig. 1). The exact size measurement is highly dependent on the method of measurement and on the isolate; fish isolates tend to be larger (200–300 nm) than the amphibian or the invertebrate viruses (120–200 nm). Measurements made of particles in the hydrated state give significantly higher size values than measurements of dehydrated particles (e.g., Klug *et al.*, 1959). Particles comprise a capsid, intermediate lipid membrane, and core. Icosahedral form was demonstrated by double shadowing (Williams and Smith, 1958; Wrigley, 1969, 1970; Devauchelle, 1977). The

most abundant polypeptide is the major capsid protein (MCP) which is an important feature in comparative studies. The genome comprises double-stranded DNA (dsDNA), typically of 100–210 kbp. For all of the iridoviruses examined to date, the genome is circularly permuted and terminally redundant; that is, each virus particle contains a complete genome (plus about 10% redundant DNA), but the terminal sequences are different for each particle in a population (circular permutation).

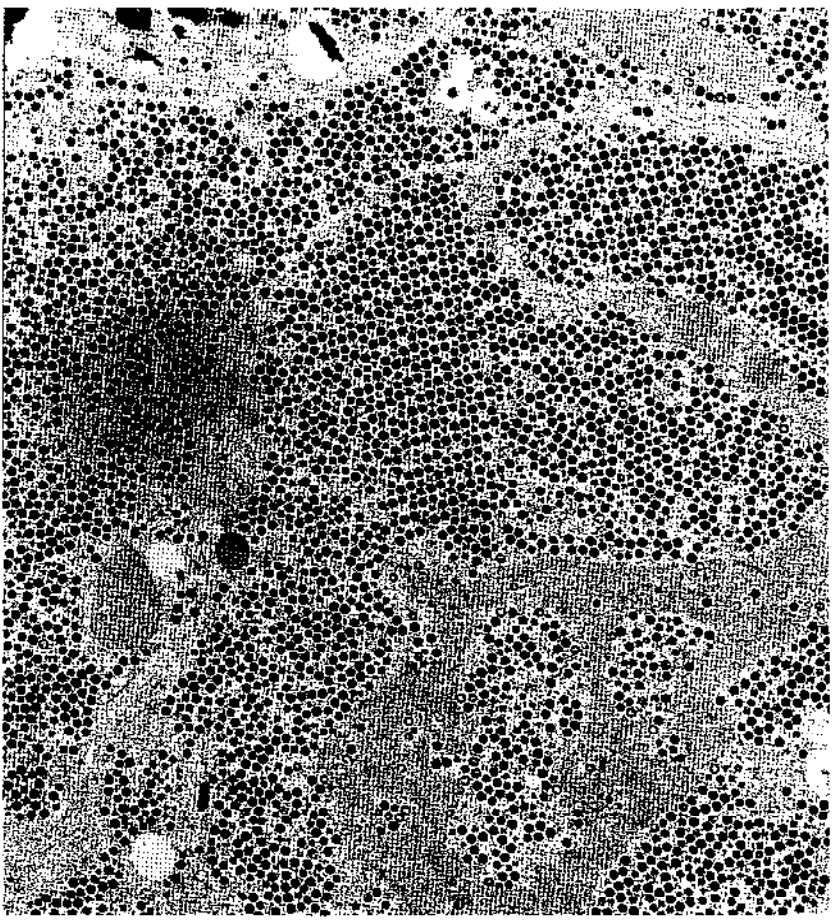


FIG. 1. Iridescent virus from a mosquito assembling in the cytoplasm of an infected host cell. Arrays of virus particles are surrounded by viroplasmic stroma (virus assembly sites) characteristic of infection by iridoviruses (× 14,000). (Photograph supplied by D. B. Stoltz.)

Vertebrate and invertebrate iridoviruses now attract little attention. There are probably no more than seven research groups worldwide active in this field, the majority of which comprise one or two individuals. This is probably set to change somewhat in the future as the relationships of the established viruses in the family are now becoming clearer and as it becomes feasible to study the impact of pathogens in natural animal populations following the development of powerful diagnostic and genetic analysis methods such as PCR (polymerase chain reaction) and DNA sequencing.

This review is general in nature, and I have tried to emphasize developments since the 1980s, particularly in comparative studies, molecular biology, and ecological aspects of iridoviruses. The standard abbreviation IV is used here for invertebrate iridescent virus. The word iridovirus is a coverall term for viruses of this family and may be qualified by reference to a particular host or group of hosts.

II. Classification

A. Current System

There are currently five recognized genera within the family (Table I). The iridescent viruses from invertebrates are named according to the host species and are given a type number according to the sequence of discovery following an interim system recommended when the number of IV isolates being reported was increasing rapidly (Tinsley and Kelly, 1970). The small iridescent viruses from invertebrates (genus *Iridovirus*) have been isolated from a diverse selection of invertebrate taxa, mostly insect orders. They are united by their size, some 120–140 nm in ultrathin sections. The type species for the genus is IV type 6, from *Chilo suppressalis*. This isolate has received the most attention probably because of the agricultural significance of the host from which it was isolated, a stem-boring lepidopteran, and for the ease with which it replicates in cell culture. The large iridescent viruses (genus *Chloriridovirus*) are some 180 nm in diameter in ultrathin sections. They have been isolated only from Diptera, and virtually all of these records are from mosquitoes. The type species for the genus is the first large IV to be discovered, IV type 3 from *Aedes taeniorhynchus*.

Nomenclature of the vertebrate isolates differs from that of the invertebrate isolates. There are many iridovirus isolates from fish and frogs, but attention has focused on just two isolates: frog virus 3 (FV3), the type virus for the genus *Ranavirus*, and lymphocystis virus type 1 (LCDV-1), the type virus of the *Lymphocystivirus* genus. The patho-

TABLE I

CURRENT CLASSIFICATION OF *Iridoviridae*

Genus	Vernacular name	Host species	Member of genus[a]
Iridovirus	Small iridescent insect virus	Diverse invertebrate taxa (mostly insects)	*Tipula paludosa* IV (IV1) *Sericesthis pruinosa* IV (IV2) *Chilo suppressalis* IV (IV6)**T** *Wiseana cervinata* IV (IV9) *Witlesia sabulosella* IV (IV10) *Costelytra zealandica* IV (IV16) *Pterosticus madidus* IV (IV17) *Opogonia* sp. IV (IV18) *Odontria striata* IV (IV19) *Simocephalus expinosus* IV (IV20) *Helicoverpa armigera* IV (IV21) *Simulium* sp. IV (IV22) *Heteronychus arator* IV (IV23) *Apis cerana* IV (IV24) *Tipula* sp. IV (IV25) Ephemopteran (IV26) *Nereis diversicolor* (IV27) *Lethocerus columbiae* IV (IV28) *Tenebrio molitor* IV (IV29) *Helicoverpa zea* IV (IV30) *Armadillidium vulgare* IV (IV31) *Porcellio scaber* IV (IV32)
Chloriridovirus	Large iridescent insect virus	Diptera (mosquitoes)	*Aedes taeniorhynchus* IV (IV3)**T** *Aedes cantans* IV (IV4) *Aedes annulipes* IV (IV5) *Simulium ornatum* IV (IV7) *Culicoides* sp. IV (IV8) *Aedes stimulans* (IV11) *Aedes cantans* (IV12) *Corethralla brakeleyi* IV (IV13) *Aedes detritus* IV (IV14) *Aedes detritus* IV (IV15) *Chironomus plumosus* IV (probable member)
Ranavirus	Frog virus	Amphibia	Frog Virus 1, 2 Frog Virus 3 (FV3) **T** Frog Virus 5-24 Frog Virus L2, L4, L5 Tadpole Edema Virus Lucké triturus virus LT1-LT4 Newt Virus T6-T20 *Xenopus* Virus T21
Lymphocystivirus	Lymphocystis disease virus	Many teleost (fish) families	Lymphocystivirus type 1 (LCDV-1) **T** Lymphocystivirus type 2 (LCDV-2) *Octopus vulgaris* disease virus (possible member)
Goldfish virus-like	Goldfish virus	Goldfish	Goldfish virus (GFV-1) **T** Goldfish virus (GFV-2)

[a] **T** denotes type species

genicity of the vertebrate viruses varies, but high levels of mortality have been attributed to iridovirus infections in natural and particularly in farmed fish populations (Langdon *et al.*, 1986, 1988; Wolf, 1988; Armstrong and Ferguson, 1989; Ahne *et al.*, 1989; Hendrick *et al.*, 1990; Pozet *et al.*, 1992). Most of the isolates from vertebrates have yet to be characterized to a level sufficient to assign them to genera. The International Committee for the Taxonomy of Viruses (ICTV) has recognized a new genus, described as "Goldfish virus-like," to account for two isolates detected in swimbladder cell cultures of the goldfish, *Carassius auratus* (Berry *et al.*, 1983). There are also reports of iridovirus-like agents from molluscs (Comps and Bonami, 1977; Barthe *et al.*, 1984), marine Crustacea (Montanie *et al.*, 1993; Lightner and Redman, 1993), and a reptile (Stebhens and Johnston, 1966), but those isolates have yet to be characterized and assigned to genera.

B. Comparative Studies of Iridoviruses

Throughout the late 1960s and 1970s, serological comparison with other established isolates was used to investigate the interrelationships among the iridoviruses, but it was only intermittently used as part of the characterization process when describing a novel isolate. Moreover, this method was not standardized, quantitative, or statistically robust, and vague terms such as "partially related" or "showing some relatedness" were used to describe various pairwise comparisons. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) polypeptide profiles were of use, especially when considering the differences between fairly similar isolates that had been grown in the same host, and indeed could even be used to indicate that apparently novel isolates were really variants of established viruses (Elliott *et al.*, 1977). Following a decline in interest in invertebrate IVs in the 1980s, knowledge of the interrelationships among these viruses benefited little from the dramatic advances in molecular biology that have since occurred.

The need for comparative genetic studies has been well recognized among those who maintained an interest in the family (Hall, 1985; Kelly, 1985; Willis, 1990; Ward and Kalmakoff, 1991; Stohwasser *et al.*, 1993; Schnitzler and Darai, 1993). Currently, taxonomic problems exist in all genera of the *Iridoviridae*, particularly in the invertebrate genera. This is mainly due to a lack of broad comparative studies among the various isolates and partly due to the interim system of classification and nomenclature, for invertebrate isolates, based on sequence of discovery and host. As will be explained, the system of naming isolates according to the host can be very misleading when

viruses naturally infect more than one species. In addition, previously, a number of uncharacterized and apparently unrelated isolates have been assigned to genera on the basis of common particle size or common host. This is not helpful to understanding the relationships among the various IV isolates. Proposals have now been put forward to change the system of classification and the system of nomenclature of invertebrate isolates in favor of a more revealing and less misleading alternative system.

The serological interrelationships among the various IV isolates that had been kept and studied was summarized by Kelly *et al.* (1979). For the small invertebrate IVs (genus *Iridovirus*) serologically, there appeared to be three distinct groups (Fig. 2). There was a large interrelated group of 11 isolates, some of which appear to be very closely related or even serologically indistinguishable from one another (e.g., IV21 and IV28). Conversely, IV6 and IV24 did not appear to be serologically related to any of the other small IVs or to one another. Finally, there was one isolate, IV29, which showed partial serological relatedness to some, but not all members of the large serogroup. The chloriridovirus, IV3, from mosquitoes showed no serological affinity to members of the genus *Iridovirus* (Cunningham and Tinsley, 1968). The FV3 and LCDV-1 isolates appear distinct from other members of the *Iridoviridae* and from one another (Bellett and Fenner, 1968; McAuslan and Armentrout, 1974; Darai *et al.*, 1983; Williams and Cory, 1994). Within the genus *Ranavirus*, the following isolates show high levels of serological interrlatedness: FV1, FV2, FV3, LT1, LT3, LT4, L4, L5, TEV, T6, T8, and T15 (Table I). The L2 virus from *Rana pipiens* was

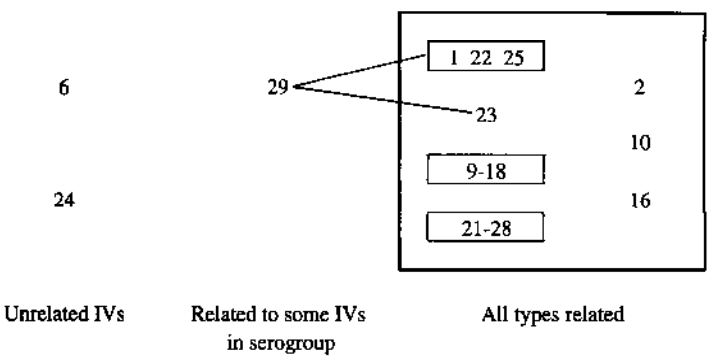


FIG. 2. Summary of serological relationships among invertebrate iridescent viruses. Viruses are identified by type number. Isolates showing the highest levels of interrelatedness are boxed together.

reported as serologically distinct from all others tested (Lehane *et al.*, 1968; Clark *et al.*, 1969; Kaminski *et al.*, 1969).

Sparked by the obvious need for comparative studies, recent and ongoing work has begun to reveal the interrelationships both within and among genera of the *Iridoviridae*. Comparative genetic studies have examined isolates from all the genera of the family (with the exception of the "Goldfish virus-like" genus). Standard molecular techniques have been used, namely, restriction endonuclease analysis, Southern blot analysis, PCR of the major capsid protein (MCP) gene region, and DNA–DNA hybridization. The results of these studies prompted the proposals for a new classification and nomenclature of invertebrate iridescent viruses (Williams and Cory, 1994; Williams, 1994). These studies are described in order to understand the reasons behind the new proposals.

Fourteen isolates were studied from a broad range of invertebrate hosts (IV1, IV2, IV9, IV10, IV18, IV21, IV22, IV24, IV28, IV29, IV30, IV31; see Table I for hosts) including the type species of the *Iridovirus* genus (IV6) and of the *Chloriridovirus* genus (IV3). In addition, the type species from the vertebrate genera were included, FV3 and LCDV-1. Restriction profiles (*Hin*dIII, *Eco*RI, and *Sal*I) immediately indicated that several isolates were actually variants of one another (Fig. 3a), namely, IV21 and IV28, with a separate group of variants being IV9, IV10, and IV18. A coefficient of similarity was calculated for all possible pairwise comparisons, representing the proportion of common-sized fragments which any two isolates shared, and this reinforced what was clear from the gels. Coefficients of similarity among IV9, IV10, and IV18 of up to 91.5%, depending on isolate and enzyme, and likewise between IV21 and IV28 coefficient values up to 94.3% were recorded. The mosquito IV (IV3) (*Chloriridovirus*) and the vertebrate iridoviruses, FV3 and LCDV-1, showed no restriction profile similarities to any other isolates or to one another.

Southern blots of the gels were probed with a MCP gene *Sal*I fragment (1.4 kb) from Aberystwyth IV (IV22), an isolate for which the entire MCP gene sequence is known. At high stringency (50% formamide, 37°C) the probe showed clear hybridization to a subset of the invertebrate IVs (IV1, IV2, IV9, IV10, IV18, IV24, IV29, IV30, and of course to IV22) (Fig. 3b). The MCP probe highlighted fragments of common size for the IV9, IV10, and IV18 isolates. At lower stringency (20% formamide, 37°C), in addition to the isolates above the probe hybridized to fragments of common size of IV6, IV21, and IV28 (Fig. 3c). This pattern was highly consistent for each of the blots tested. In no blots did the probe show affinity to the vertebrate isolates FV3 and LCDV-1. Probe hybridization to IV31 was notably weak but fairly

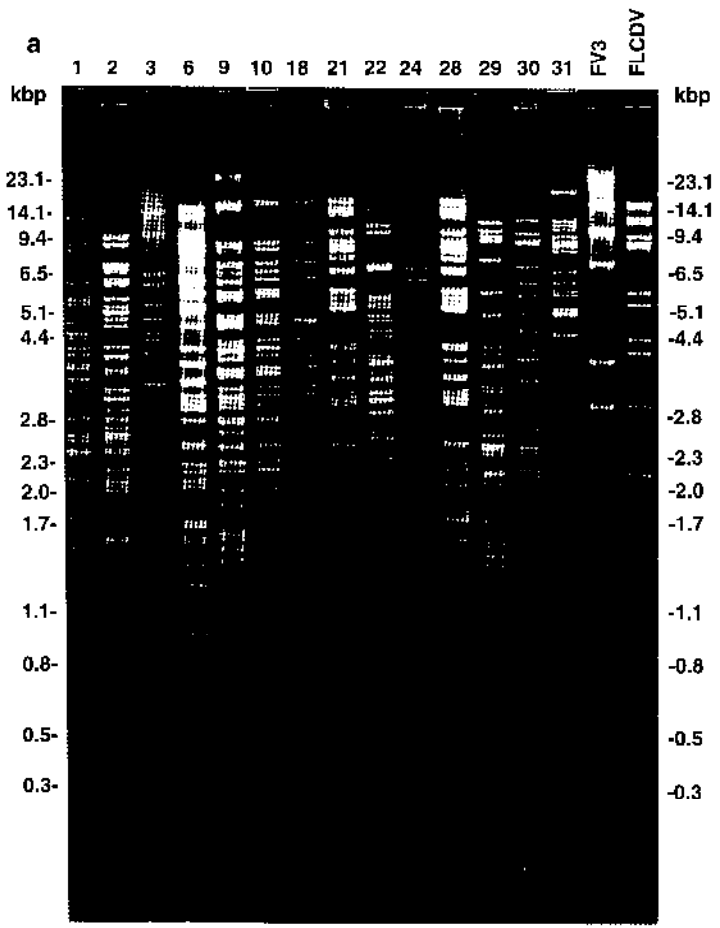
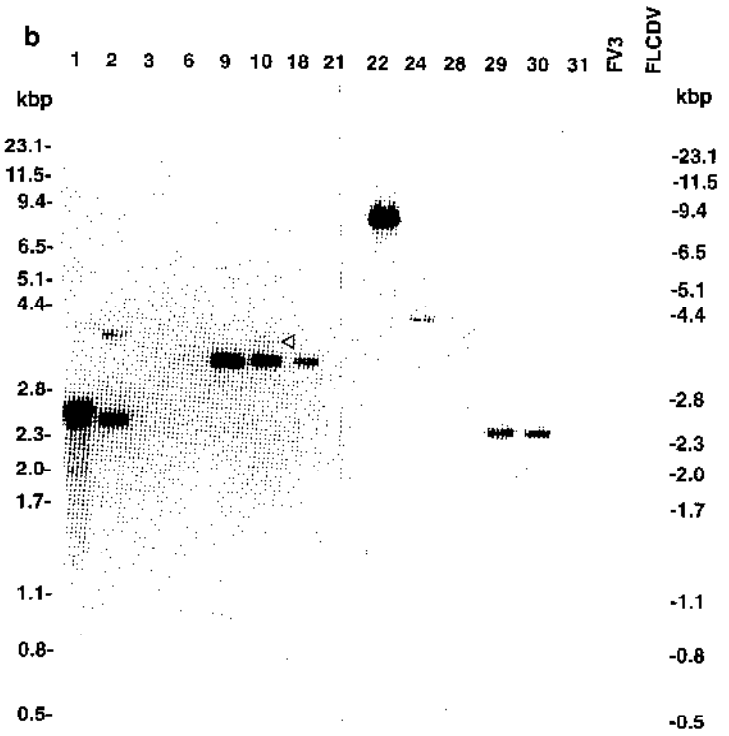


FIG. 3. (a) *Hin*dIII restriction endonuclease profiles of DNA from invertebrate iridoviruses (identified by type number) and isolates from vertebrates, namely, frog virus 3 (FV3) and flounder lymphocystis disease virus (FLCDV = LCDV-1). Note the similarities among IV9, IV10, and IV18 and between IV21 and IV28, as well as the distinct nature of profiles from FV3 and FLCDV. (b) Hybridization of a major capsid protein (MCP) gene fragment of IV22 at high stringency to a Southern blot of the *Hin*dIII gel. The gene probe shows hybridization to only a subset of the invertebrate iridoviruses and no hybridization to the vertebrate viruses (FV3 and FLCDV). (c) Hybridization of the MCP gene probe from IV22 at low stringency, showing additional hybridization to IV6, IV21, and IV28 and weak hybridization to IV31. (From Williams and Cory, 1994.)

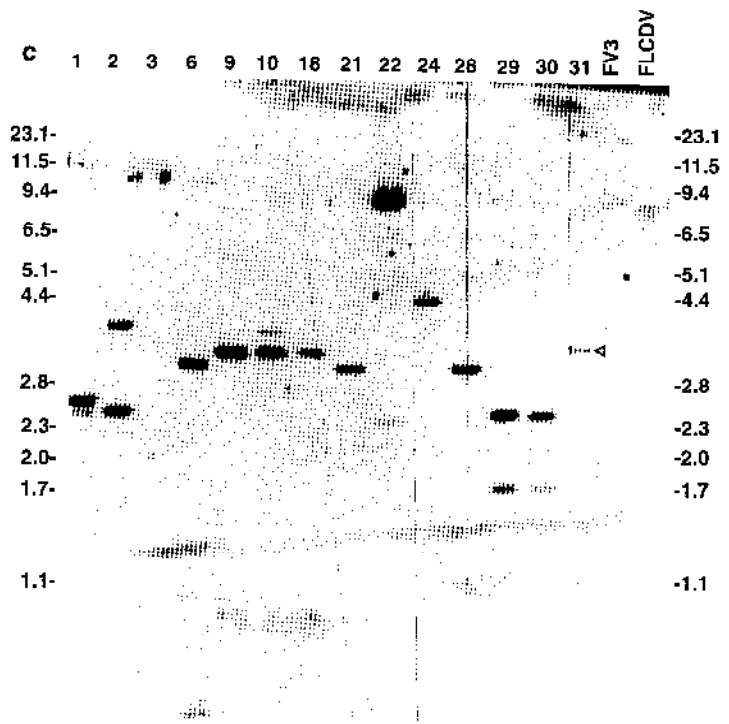
consistent, whereas probe hybridization to IV3 was only seen very weekly in a *Sal*I blot.

Under high-stringency conditions PCR amplification of a 719-bp re-



gion of the MCP gene using primers derived from the Aberystwyth IV sequence produced an amplicon only for IV1, IV2, IV9, IV10, IV22, and IV29, which were all members of the subset of isolates to which the Aberystwyth IV (IV22) MCP gene probe showed affinity at high stringency (IV18 and IV28 were not used in the PCR study).

DNA–DNA dot-blot hybridization results were very clear and were consistent with the Southern blot analysis. With hybridization conditions of 40% formamide, 37°C, and critical washes in 2 × SSC at 55°C, one large group of interrelated isolates was detected (Table II) which corresponded to the large serogroup of isolates described above (Fig. 2). The degree of relative hybridization within this group varied between 10 and 90%; the very high values coming from IV9, IV10, and IV18 confirm them to be variants of the same virus. There was a second, smaller group of closely related isolates that comprised IV21 and IV28, which are clearly variants of the same virus, and IV6, the type species



of the genus *Iridovirus*. The isolate from woodlice (IV31) appeared only distantly related to the other IVs in the study, and the relationship of IV3 from mosquitoes to other isolates appeared even more distant, confirming the placing of this isolate in a separate genus (*Chloriridovirus*). The vertebrate isolates consistently failed to show DNA hybridization to the invertebrate isolates or to one another under the conditions used.

Overall, it is possible to see a very clear picture emerging from these DNA studies that closely support the previous serological findings, albeit with a few differences involving the different placing of IV24 and IV21/IV28 to that given by serology. This prompted the proposal to assign isolates to discrete complexes based on DNA and serological characteristics (Fig. 4). Three complexes were identified within the genus *Iridovirus*. The main group of interrelated isolates were placed in a complex named *Polyiridovirus*. The second smaller group of isolates comprising IV6 and IV21/IV28 were assigned to a separate complex, the *Oligoiridovirus* complex. Finally, the isolate from woodlice

TABLE II

DNA–DNA Dot-Blot Hybridization Values Relative to Homologous DNA for Vertebrate and Invertebrate Iridoviruses[a]

	IV1	IV2	IV3	IV6	IV9	IV10	IV18	IV21	IV22	IV24	IV28	IV29	IV30	IV31	FV3	LCDV-1
IV1	100															
IV2	41	100														
IV3	0	<1	100													
IV6	0	<1	0	100												
IV9	20	46	0	0	100											
IV10	17	41	0	0	91	100										
IV18	17	55	0	0	100	89	100									
IV21	1.2	<1	0	42	0	0	<1	100								
IV22	33	22	0	0	28	27	20	0	100							
IV24	13	24	0	0	22	17	18	<1	10	100						
IV28	1	<1	0	44	0	0	<1	100	<1	1	100					
IV29	13	26	<1	1.1	32	31	28	0	15	27	0	100				
IV30	11	36	0	0	33	32	32	0	23	24	0	56	100			
IV31	<1	1.4	0	2.4	0	0	0	1.6	0	0	1.4	0	<1	100		
FV3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	
LCDV-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100

[a] Data from Williams and Cory (1994).

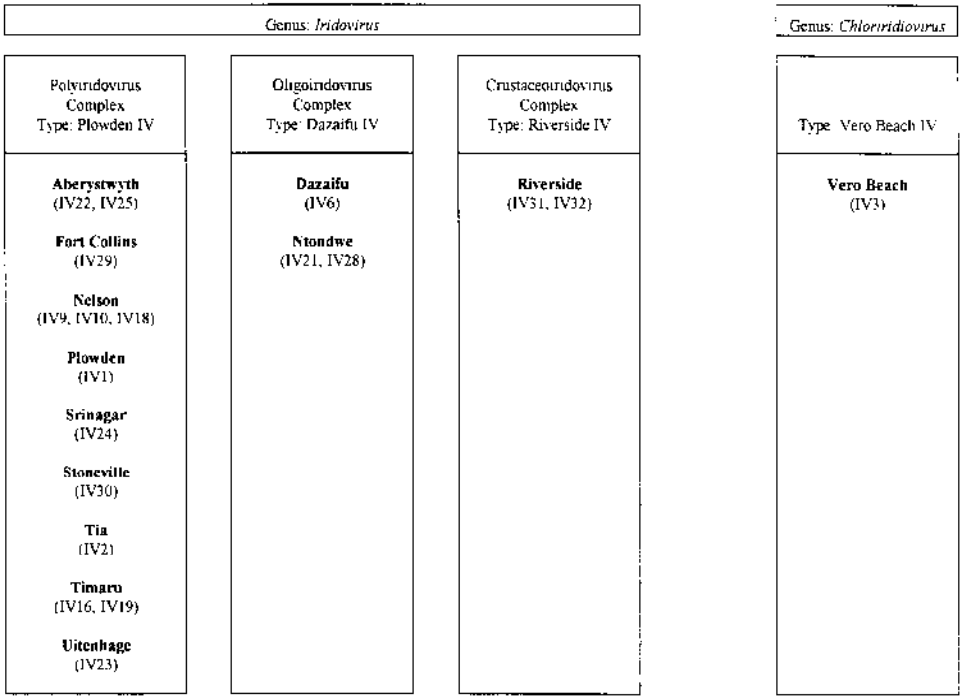


FIG. 4. Diagram of hybridization complexes in the genera *Iridovirus* and *Chloriridovirus* of invertebrate iridoviruses, detected by Williams and Cory (1994). Isolates that are strains of a common virus have been synonymized under new names based on geographical origin. See text for explanation.

(IV31) was assigned a separate complex, *Crustaceoiridovirus*. These findings did not change the situation regarding the mosquito isolate IV3, the sole representative of the genus *Chloriridovirus*, or that with respect to the vertebrate isolates FV3 or LCDV-1, as representatives of two separate vertebrate genera.

C. New Nomenclature for Iridescent Viruses

From these genetic studies and previous serology (Kelly *et al.*, 1979), it has become apparent that one IV may infect several host species, for example, IV9 from *Wiseana cervinata*, IV10 from *Witlesia sabulosella*, and IV18 from *Opogonia* sp. Other studies have reported that apparently different IVs may infect the same host population at the same place and the same time (see Section VI). Thus, using the name of the host to name the virus can be misleading. This system is causing much

confusion in other invertebrate virus families, for example, the *Baculoviridae*, where an increasing number of isolates from different host species appear to be strains of the same virus. Consequently, Williams and Cory (1994) proposed that the nomenclature of IV isolates should be changed to a neutral form which dissociates virus and host species. They suggested using the geographical origin (nearest large town) of the original isolate as a name. This system is currently used in a number of other virus families, for example, in the *Reoviridae, Bunyaviridae, Rhabdoviridae, Arenaviridae*, and others. Following this system, all the isolates used in these studies and existing characterized isolates were assigned new names according to their place of origin. Serological and genetic evidence from other studies indicated that IV16 and IV19 were strains of the same virus, as were IV22/IV25, and IV31/IV32, whereas IV23 appeared to be a distinct entity related to the main group (reviewed by Williams and Cory, 1994). Therefore, these viruses appear as tentative members of the polyiridovirus complex (Fig. 4).

It is important to note that using a geographical descriptor is in no way supposed to reflect information regarding the distribution or origin of a virus, for, as has been pointed out, diseases tend to travel with their hosts and so exotic hosts introduced to novel locations may harbor pathogens originating far away (J. Kalmakoff, 1995, personal communication). In addition, flexibility and common sense are required when adopting this system, and names should be selected that are essentially convenient to use. Thus, the type species of the *Iridovirus* genus was named Dazaifu IV (this being an earlier source of infected material mentioned in the original paper) rather than after the place where the authors themselves isolated infected stem borers: Tsukushinomachi (Fukaya and Nasu, 1966). This is discussed further in Section II,E. To demonstrate the effectiveness of this system of nomenclature, it is adopted here with the historical type numbers used alongside for maximum clarity.

D. Alternative Approaches

Schnitzler and Darai (1993) used a PCR technique developed for use with Dazaifu IV [IV6] (Stohwasser *et al.*, 1993) of selecting highly conserved regions of the MCP gene from published sequences (Tajbakhsh *et al.*, 1990a; Cameron, 1990) to construct primers for use in the amplification of the homologous LCDV-1 gene. Gene fragments amplified by PCR were used to probe an LCDV-1 gene library at high

stringency. Library fragments showing clear hybridization to the probes were sequenced and compared to the MCP gene sequences of the other isolates. Comparative sequence analysis revealed that the deduced amino acid sequences of LCDV-1 had a sequence identity of 49.1% with Aberystwyth IV [IV22], 50.3% with Plowden IV [IV1], and 53% with Dazaifu IV [IV6]. This pattern of interrelatedness is in agreement with the results of Williams and Cory (1994). In addition, MCP sequences were compared to the currently orphaned African swine fever virus (ASFV, previously a member of the *Iridoviridae*) and found to have a 16% amino acid sequence identity (38.1% similarity). ASFV has clear morphological similarities with iridoviruses but has a distinct genomic organization. The genome of ASFV is not circularly permuted or terminally redundant, but rather is cross-linked and unmethylated and appears more closely related to the poxviruses. ASFV is currently an orphan virus (Willis, 1990) but the sequence comparison results suggest a common ancestry.

Current work in progress is following the Schnitzler and Darai (1993) approach for a broad selection of invertebrate IVs. The use of primer sequences from the conserved MCP gene regions is proving to be a successful tool for the direct amplification and sequencing of gene fragments (R. Webby and J. Kalmakoff, 1995, personal communication). This approach has the advantage that it is highly reproducible both within and among different laboratories given standard sets of primers and cycling conditions and is more quantitative than, for example, DNA hybridization or serological comparisons. When completed, such studies should provide essential information on the phylogenetic relationships among a variety of isolates from different hosts.

Iridoviruses contain an internal lipid layer which usually differs in composition from that of the host cell. Williams and Thompson (1995) reasoned that the apparently unique lipid composition of these viruses could be used as an indicator for comparative studies. They compared the fatty acid profiles of eight IV species grown in the same lepidopteran host, *Galleria mellonella*. In all cases the virus fatty acid profiles were markedly different from that of host material. The isolates fell into two main groups of equal size: one containing Plowden IV, Tia IV, Nelson IV, and Riverside IV [IV1, IV2, IV9/10/18, and IV31, respectively] and the other group containing Ntondwe IV, Aberystwyth IV, Stoneville IV, and San Miguel IV [IV21/28, IV22, IV30, and *Anticarsia gemmatalis* IV, respectively]. Consequently, patterns of similarity in virus fatty acid profiles did not at all resemble the patterns of genetic or serological relatedness observed previously. Lipid composition does not seem to be a useful indicator of IV interrelationships.

E. Suggested Changes to Current Classification

The ICTV dictates that a polythetic system be employed for the classification of virus species. Strains of a polythetic species need not have a single defining characteristic in common. Rather, viral entities are united under the term species if they share a large proportion of predefined key features (Van Regenmortel, 1990). The problem then arises of precisely defining the set of characteristics on which the polythetic system is based. Without precise (preferably quantitative) definitions for each characteristic in the system, species definition would depend too heavily on individual interpretations of what should or should not be considered as separate species, leading to a lack of standardization and increased confusion. The flexible nature of polythetic classification would then work against a clearer understanding of virus taxonomic relationships. The definition of precise characteristics for iridoviruses is in its earliest stages. The ICTV recommends that characteristics be included from 10 different domains (structure, genetic properties, antigenic properties, ecology, replication, etc.), but the scarcity of information means that such complete definitions are not possible for iridoviruses. Many other lesser-studied virus families will probably experience the same problems.

There appears to be no reason at present to change the existing genera of the *Iridoviridae* or recognize additional ones. However, changes are necessary in the status of a number of isolates currently assigned to vertebrate and invertebrate genera and, I would argue, in the nomenclature of the invertebrate isolates.

1. Invertebrate Viruses

Many of the early isolates currently recognized by the ICTV seem no longer to exist. For these "ancient" isolates, data available are insufficient to recognize the moment of their reisolation because they have never been adequately characterized. As in any taxonomic system, a name cannot be assigned to an entity until the features which identify that entity have been determined. Consequently, many of the currently recognized isolates are simply records of IV-susceptible host species. The isolates themselves fall within the ranks of the "undescribed" to all intents and purposes and should not be recognized. The isolates for which no more than reports of an isolation exist should only be considered as records of host species susceptible to IV infection (listed in Williams, 1994).

In this respect it is remarkable to note, given the interest in insect vector control, that Vero Beach IV [IV3] from *Aedes taeniorhynchus* seems to be the sole surviving member (and thus type species) of the

genus *Chloriridovirus*. In the ICTV reports of Mathews (1982), Francki *et al.* (1991), and Murphy *et al.* (1995), a number of isolates were assigned to the *Chloriridovirus* genus: IV4, IV5, IV7, IV8, IV11, IV12, IV13, IV14, and IV15 (Table I). Such assignations are inappropriate because (i) there are insufficient characterization data to define these isolates as species (probably none of the original isolates are still in existence, so they cannot be characterized *a posteri*); (ii) large particle size (~180 nm) is the major criterion for assignation of isolates to the genus, but IV7, IV11, and IV13 are all "small" in size (~130 nm) and only IV12 has been reported to have serological similarity to IV3 (Tinsley *et al.*, 1971); (iii) these isolates are united only by the fact that they all come from Diptera, but host of origin is not one of the criteria for assigning isolates to any of the genera—likewise, there are dipteran isolates which are correctly placed in the genus *Iridovirus*, for example, Plowden IV [IV1] and Aberystwyth IV [IV22/IV25].

The correct placing of an isolate from *Chironomus plumosus* as a tentative member of the genus *Chloriridovirus* is also inappropriate because genetic or serological data on this isolate are not available and the size of the particle (145 nm) would place it in the genus *Iridovirus*. This isolate was unusual in the abundance of fibrillar structures originating from the viral capsid, and it did not show iridescence (Stoltz *et al.*, 1968). It seems doubtful that this isolate is still in existence (R. Webby, 1995, personal communication; D. B. Stoltz, 1995, personal communication).

The genus *Iridovirus* also currently contains isolates that no longer exist (Table I) and that should be reisolated and characterized in the appropriate manner before being recognized taxonomically. Of the isolates listed by type number in the Murphy *et al.* (1995) report, the following are not valid: IV17 from *Pterosticus madidus* (coleopteran), IV 19 from *Odontria striata* (coleopteran), IV20 from *Simocephalus expinosus* (daphnid), IV25 from *Tipula* sp. (dipteran), IV26 from a mayfly nymph (ephemopteran). The status of IV27 from *Nereis diversicolor* is not known; no genetic or serological data are available for IV27. (There is evidence that IV19 and IV25 are actually strains of other recognized viruses; see Section II,B.)

Limited characterization information is available for three isolates previously not recognized by the ICTV and not assigned type numbers: *Phylophaga anxia* IV, *Scapteriscus aclectus* IV, and *Simulium vittatum* IV (Poprawski and Yule, 1990; Boucias *et al.*, 1987; Erlandson and Mason, 1990). However, material for study of these isolates does not appear to be available. Consequently, no comparative studies are possible, and it is inappropriate to recognize them as distinct entities in the absence of suitable comparative data. One additional tentative

species has been characterized and found to belong to the main interrelated complex of IVs (polyiridovirus complex). This isolate from *Anticarsia gemmatalis* was reported to have no restriction profile similarities to previously characterized IVs. The MCP gene probe from Aberystwyth IV hybridized to a Southern blot of *Anticarsia gemmatalis* IV DNA, and the MCP gene fragment of this isolate was amplified by the PCR primers used previously (Williams and Cory, 1994). Genomic DNA showed significant hybridization only to members of the polyiridovirus complex. This isolate was assigned a geographical name in line with the new nomenclature: San Miguel IV.

2. Vertebrate Viruses

Similar to the invertebrate genera, the vertebrate genera currently include many viruses for which characterization data are not available. At best, there are some serological studies of relatedness (described earlier). The following members of the *Ranavirus* genus have not been adequately characterized to merit inclusion in the genus: frog virus 1, frog virus 2, frog virus 5-24, frog virus L2, frog virus L4, frog virus L5, Lucké triturus virus LT2-LT4, Newt virus T6-T20, *Xenopus* virus T21. Other members, namely, tadpole edema virus (TEV) and Lucké triturus virus 1 (LT1), have been demonstrated to be strains of frog virus 3 by restriction endonuclease analysis and DNA hybridization studies. The restriction profiles were nearly identical among these viruses (Essani and Granoff, 1989). As a consequence, FV3 should be the sole established member (and type species) of the genus *Ranavirus*.

Several studies have examined the relationships among vertebrate iridoviruses not yet recognized by the ICTV. An isolate of epizootic hematopoietic necrosis virus (EHNV), an iridovirus isolated from sheatfish (*Silurus glanis*), and an iridovirus isolated from catfish (*Ictalurus melas*) have been reported from fish showing similar symptoms of systemic disease. These isolates had near identical particle sizes when negatively stained and appeared to be strains of one species by cross indirect immunofluorescence assays and by comparison of polypeptide profiles (PAGE). Serologically and by PAGE analysis, the piscine isolates were shown to be related to the type species of the genus *Ranavirus*, frog virus 3 (FV3) (Hendrick *et al.*, 1992). It is appropriate to synonymize these isolates as one species on a tentative basis pending additional genetic studies. The name epizootic hematopoietic necrosis virus (EHNV) describes the disease and is a well-established name. A iridovirus from the burrowing frog *Lymnodynastes ornatus* has been characterized in a comparative study with EHNV isolates and compared to published data for FV3. This virus, Bohle virus, was shown to be closely related but distinct from EHNV in terms of particle

size, protein, antigenic and genomic characteristics (Hengstberger *et al.*, 1993). Both EHNV and Bohle virus showed characteristics which indicated their similarities to FV3. It is appropriate to assign these two species, Bohle virus and EHNV, to the *Ranavirus* genus on a tentative basis pending further comparative work with FV3. This change extends the known range of hosts for members of the genus *Ranavirus* to include piscine hosts.

An iridovirus-like agent has been reported from lymphocystis lesions of the common octopus (Runnger *et al.*, 1971). This was previously recognized as a possible member of the genus *Lymphocystivirus*. However, the isolate has not been characterized or compared genetically or serologically with other members of the genus or with members of other iridovirus genera. There is no basis for recognizing this entity as an iridovirus species, tentatively or otherwise. The genus *Lymphocystivirus* should comprise just two species, LCDV-1 (type species) and LCDV-2. Similarly, the genus "Goldfish virus-like" has two members (GFV-1 and GFV-2), but only GFV-1 has been characterized and included in comparative studies (Essani and Granoff, 1989). Nothing is known of GFV-2, and it is not appropriate to recognize this isolate as a distinct species.

3. New Classification Scheme

Recommendations for these changes are now being made. Incorporation of all the changes suggested above would result in a new classification scheme (Table III). The new scheme would greatly simplify the taxonomic situation across all the recognized genera and would permit novel isolates to be characterized and assigned to genera in a systematic way. The use of predescribed characteristics for defining iridovirus species will greatly assist in this.

There are now a number of options available for the nomenclature of invertebrate iridescent viruses. The most conservative measure would be to retain the current system unchanged and wait for additional reports to support or refute the studies described above. A second option would be to retain the current system with some changes: synonymize the type numbers of the isolates in line with recent findings and current work in progress. This would presumably be an interim measure while the ICTV decided the best course to take in IV nomenclature and would allow time for some other system to be proposed, perhaps based on comparative sequence data for the MCP gene (although phylogenetic systems of classification currently fall outside the remit of the ICTV). A third option would be to adopt the proposed system of geographical descriptors and rename the characterized isolates accordingly. This would be the boldest option and, I would argue, the best

TABLE III
PROPOSED NEW SYSTEM FOR IRIDOVIRUS TAXONOMY[a]

Genus	Recognized tentative species[b]	Tentative members
Iridovirus	Plowden IV [IV1] Tia IV [IV2] Dazaifu IV [IV6] Nelson IV [IV9/IV10/IV18] Timaru IV [IV16/IV19] Ntondwe IV [IV21/IV28] Aberystwyth IV [IV22/IV25] Uitenhage IV [IV23] Srinagar IV [IV24] Fort Collins IV [IV29] Stoneville IV [IV30] Riverside IV [IV31/IV32] San Miguel IV (*Anticarsia gemmatalis* IV)	None
Chloriridovirus	Vero Beach IV [IV3]	None
Ranavirus	Frog virus 3	Epizootic hematopoietic necrosis virus Bohle virus
Lymphocystivirus	Lymphocystivirus type 1 Lymphocystivirus type 2	None
"Goldfish virus-like"	Goldfish virus type 1	None

[a] Following changes outlined in text.
[b] Previous type numbers, where applicable, are given in brackets.

option. However, others have argued for a more cautious approach to nomenclature changes, pointing out that this is a major step and one that would be difficult to correct if found to be obstructive or unworkable in the future. Perhaps the main potential problem with geographical descriptors is the lack of internationality of place names, but this could be overcome with careful selection of a name from the available local options, as occurs in other virus families. Decisions on the above recommendations rest with the ICTV executive committee. Their report is expected in 1996.

III. STRUCTURE

A. Physicochemical Properties

The physicochemical properties of members of the family *Iridoviridae* are fairly well established (see reviews by Bellett, 1968;

Goorha and Granoff, 1979; Hall, 1985). Briefly, the molecular weight of intact virions is between 500 and 2000 million with a density of 1.26–1.6 g cm^{-3} (Aubertin, 1991; Cole and Morris, 1980). The small IVs all have an $s_{20,w}$ of approximately 2200 (Kelly and Robertson, 1973) or up to 4458 for the larger Vero Beach IV [IV3] chloriridovirus (Matta, 1970; Wagner *et al.*, 1973). Some 12–17% of the weight of the particle is dsDNA (Bellett and Inman, 1967; Kalmakoff and Tremaine, 1968; Glitz *et al.*, 1968; Matta, 1970; Stadelbacher *et al.*, 1978). The GC content is typically around 29–32% for the small invertebrate IVs (Glitz *et al.*, 1968; Black *et al.*, 1981), 54% in the chloriridovirus Vero Beach IV [IV3] (Wagner and Paschke, 1977), 30.7% for LCDV-1 (Darai *et al.*, 1983), and 53% in FV3 (Smith and McAusland, 1969; Houts *et al.*, 1970). The usual number of polypeptides resolved by one-dimensional PAGE is 20–32 (Krell and Lee, 1974; Barray and Devauchelle, 1979; Elliott *et al.*, 1980a; Cole and Morris, 1980; Black *et al.*, 1981; Flugel *et al.*, 1982; Tajbakhsh and Seligy, 1990), although two-dimensional PAGE has revealed additional polypeptide diversity (Cerutti and Devauchelle, 1985). Polypeptide sizes are usually in the range 11 to 200 kDa, although smaller (Tajbakhsh and Seligy, 1990) and larger proteins have been reported (Krell and Lee, 1974; Barray and Devauchelle, 1979; Cerutti and Devauchelle, 1990). Several virion-associated enzymes have been detected, the functions of which are discussed later. The structure of the capsid, lipid membrane, and core are considered in turn.

B. Capsid

The capsid comprises an icosahedral lattice of closely packed hexagonal subunits of 7–9 nm diameter and about 7–9 nm height (Wrigley, 1969, 1970; Stoltz, 1971, 1973; Murti *et al.*, 1984). The total number of subunits is probably 1472 for Plowden IV [IV1] and Tia IV [IV2]. These subunits are arranged in 20 trisymmetrons (each of 55 subunits) and 12 pentasymmetrons (each of 31 subunits). The corners of the trisymmetrons are not perfectly aligned with those of the pentasymmetron but are offset (skewed) about the pentasymmetron by 3 subunits, at least in Plowden IV [IV1] (Manyakov, 1977). The larger viruses (e.g., an isolate from the midge, *Chironomus plumosus*, ~165 nm diameter) have larger trisymmetrons, with 78 subunits, giving a total of 1560 subunits per particle (Stoltz, 1971, 1973).

The capsid subunits are comprised of a single polypeptide of 48 to 55 kDa (typically 50 kDa), the major capsid protein (MCP), which comprises some 40–45% of the total particle polypeptide (Willis *et al.*, 1977; Moore and Kelly, 1980; Aubertin *et al.*, 1981; Black *et al.*, 1981; Flugel *et al.*, 1982; Davison *et al.*, 1992; Schnitzler and Darai, 1993;

Stohwasser *et al.*, 1993). The MCP protein is highly conserved and has proved to be a valuable indicator in comparative studies. The length of the MCP varies from 459 amino acid residues in LCDV-1 (Schnitzler and Darai, 1993) to 472 amino acids in Aberystwyth IV [IV22] (Cameron, 1990), with intermediate values in Plowden IV [IV1] and Dazaifu IV [IV6] (Tajbakhsh *et al.*, 1990a; Stohwasser *et al.*, 1993). A series of SDS–PAGE and surface labeling studies under different conditions has indicated that the MCP is the basis for two different structures in the capsid of Dazaifu IV. A trimeric form of the MCP, held together (presumably) by hydrogen bonding, is located on the outer surface of the capsid, whereas a covalently bonded trimer of the same protein lies beneath the surface layer (reviewed by Cerutti and Devauchelle, 1990). Tia IV [IV2] is alone in being suspected of having two MCPs of different sizes; one of 53 and one of 55 kDa (Elliott *et al.*, 1977). Whether the two proteins are structurally different or the smaller is produced by cleavage or degradation of the larger is not known. There is also evidence that an infected cell protein (ICP 38) is located externally on the FV3 particle (Chinchar *et al.*, 1984).

For a number of isolates, from both vertebrate and invertebrate hosts, the presence of fibrils attached to capsid subunits has been reported (Fig. 5). Fibrils were particularly evident in an isolate from the midge (*Chironomus plumosus* (Stoltz *et al.*, 1968; Stoltz, 1971) but also in LCDV (Zwillenberg and Wolf, 1968; Midlige and Malsberger, 1968; Yamamoto *et al.*, 1976). Neutron scattering measurements of FV3 particles indicated that half the volume of the capsid was water, a level of hydration expected in the presence of fibrillar structures (see also Lunger and Came, 1966). In LCDV, the fibrils were measured at 200 nm length and 4 nm width (Zwillenberg and Wolf, 1968), but in other iridoviruses the fibrils are far shorter (~2.5 nm) and appear as a fringe around the edge of the capsid (Willison and Cocking, 1972; Cole and Morris, 1980; Black *et al.*, 1981; Devauchelle *et al.*, 1985a; Flugel, 1985). Short fibrils may have terminal knobs (Stoltz, 1971, 1973).

C. Lipid Membrane

The lipid layer is intimately associated with the capsid (Stoltz, 1973; Cuillel *et al.*, 1979; Klump *et al.*, 1983). This layer is an essential component for infectivity in the vertebrate iridoviruses (Wolf *et al.*, 1968; Willis and Granoff, 1974; Berry *et al.*, 1983; Langdon *et al.*, 1986; Speare and Smith, 1992). It has become generally accepted in the literature that invertebrate IVs are ether-resistant. Day and Mercer (1964) stated that the infectivity of Tia IV [IV2] was unaffected by treatment with ether or chloroform, although no data were presented in support

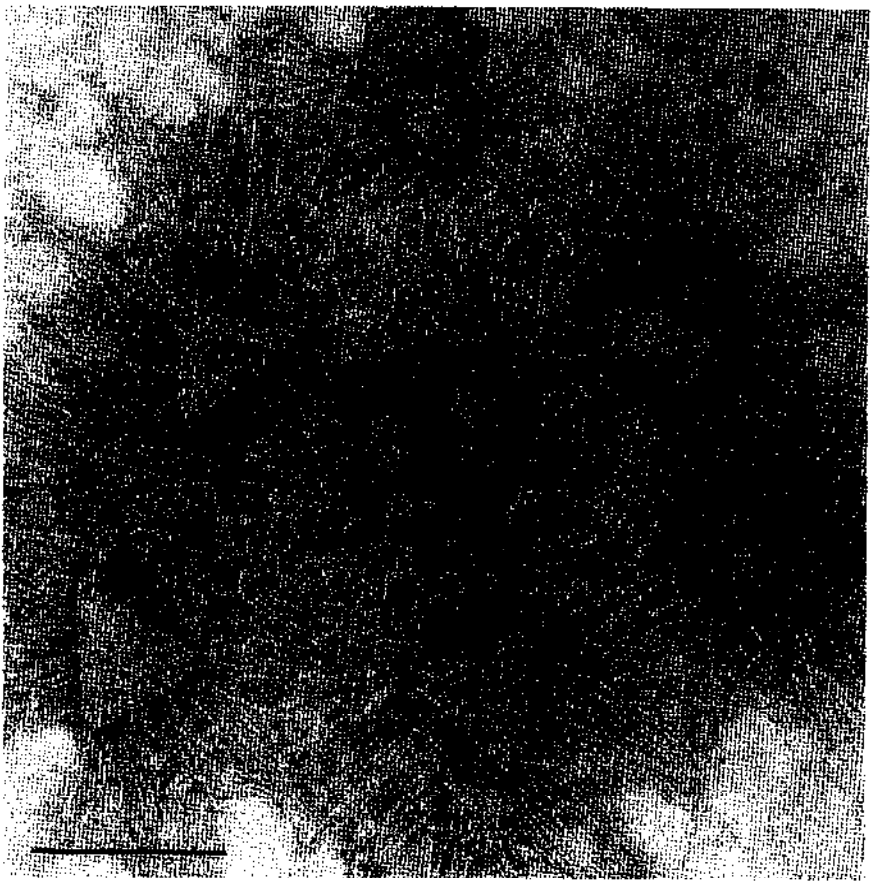


FIG. 5. Trisymmetrons from the capsid of an iridovirus of *Chironomus plumosus* (Diptera) showing abundant fibrillar structures attached. Bar: 100 nm. (Photograph supplied by D. B. Stoltz.)

of this assertion and, given the biological activity of membrane extracts during the early stages on infection (described later), such statements must be viewed with suspicion. This basic characteristic of the invertebrate viruses clearly requires confirmation.

The lipid layer is about 4 nm thick (Cuillel *et al.*, 1979; Kelly, 1985). Complexes of protein pass through the intermediate lipid layer and appear to connect capsid and core polypeptides (Aubertin *et al.*, 1980; Cerutti and Devauchelle, 1982; Tripier-Darcy *et al.*, 1982; Klump *et al.*, 1983). The total lipid content of the particle has been reported as 5.2% (dry weight) of Plowden IV [IV1] (Kalmakoff and Tremaine, 1968), 7%

for Dazaifu IV [IV6] (Balange-Orange and Devauchelle, 1982), 9% of Dazaifu IV, Tia IV [IV2], and FV3 (Kelly and Vance, 1973; Willis and Granoff, 1974), and 12.5% for purified cores of Vero Beach IV [IV3] (Wagner *et al.*, 1975). Higher values (17%) have been reported for a lymphocystis disease virus isolate, although the assayed material appeared to be a heterogeneous mixture of filled and unfilled virions (Robin *et al.*, 1993).

The phospholipid content has been reported as 90% of total lipid for FV3 (Willis and Granoff, 1974), 75% for Dazaifu IV (Balange-Orange and Devauchelle, 1982), and 44% for Dazaifu IV and Tia IV (Kelly and Vance, 1973). The fatty acid composition of these lipids has been reported as nearly identical to the host cell in FV3 (Willis and Granoff, 1974) but markedly different in the invertebrate viruses, Dazaifu IV (Balange-Orange and Devauchelle, 1982), and eight other IVs (Williams and Thompson, 1995). In Dazaifu IV [IV6] the phospholipid composition was not sensitive to the type of host: lymantrid cell line or noctuid larvae (Balange-Orange and Devauchelle, 1982). Such findings and the observation that iridovirus particles do not obviously bud through a particular organelle membrane during synthesis have led to the assumption that iridoviruses acquire their lipid component by *de novo* synthesis, a situation also believed to exist in poxviruses. However, Schmelz *et al.* (1994) have shown that vaccinia virus inner lipid envelopes are derived from an intracellular compartment between rough endoplasmic reticulum (ER) and the Golgi apparatus. Virally encoded proteins appear in the trans Golgi network shortly after infection and appear to facilitate the sequential wrapping events of vaccinia. The relevance of these observations for iridoviruses is not known. Viruses released by budding may possess an outer envelope (Yule and Lee, 1973; Webb *et al.*, 1976; Braunwald *et al.*, 1979), but this is not an essential component for infectivity (Granoff *et al.*, 1966). Some fish iridoviruses also appear to have an outer envelope in ultrathin sections, which may be double layered (Flugel, 1985).

D. Core

The particle core is an electron-dense, highly hydrated entity containing about 80% water in FV3 (Cuillel *et al.*, 1979). Thermodynamic studies were consistent with the presence of a nucleosomal structure in Dazaifu IV [IV6] (Klump *et al.*, 1983), and ultrasonic adsorption studies have supported the concept that there is structural organization among the DNA and protein components of the core (Robach *et al.*, 1983). Freeze-etching of FV3 particles in cells showed randomly oriented rods some 10 nm width to be evident in the core. These rods

appear to be part of the DNA–protein complex arranged so as to form a long coiled filament (Tripier-Darcy and Nermut, 1983). Lipid-enveloped cores of Vero Beach IV [IV3] were reported to have a diameter of 176 nm when negatively stained compared to 224 nm for intact particles (Wagner *et al.*, 1973). Cores were not infectious to mosquito cells or larvae; apparent failure of the cores to attach to cells may have been the cause.

Polypeptide profiles of cores were remarkably similar to those of whole particles, the main difference being the absence of the 55-kDa capsid protein (Wagner *et al.*, 1975). This suggests that much of the polypeptide (structural and enzymatic) complexity of IVs is associated with the core and membrane. Cerutti and Devauchelle (1985) reported that at least six DNA-associated polypeptides are localized within the core of Dazaifu IV [IV6], ranging from 12.5 to 81 kDa, of which the 12.5-kDa protein was the major species. A number of other IVs also have a major polypeptide species close to this mass (Elliott *et al.*, 1977; Carey *et al.*, 1978; Kelly *et al.*, 1979; Cole and Morris, 1980; Tajbakhsh and Seligy, 1990).

E. Iridescence Phenomenon

Most, but not all, of the invertebrate iridescent viruses iridesce. The vertebrate isolates do not iridesce in host tissues or as purified pellets of virus, although there is one report to the contrary (R. Walker, personal communication in Stoltz, 1973). It is easy to get transfixed by the iridescence of invertebrate isolates, and many authors, myself included, find it hard to resist a few choice adjectives concerning the eye-catching nature of patent IV infections.

The iridescence occurs because, at high densities, IV particles self-assemble and crystallize in host cells following the laws of entropy, that is, crystalline organization is the arrangement with the lowest energy. The spacing between the particles is such that light reflected from the surface of the viral arrays interferes with newly arriving light, resulting in "Bragg" reflections (Klug *et al.*, 1959). For such events to occur, a superabundance of virus particles must be present in the host tissues, so that iridescence is not seen until fairly late in the infection cycle. The colors commonly seen in patent IV infections include lavender, blue, or turquoise for the small IVs (genus *Iridovirus*) and green-yellow, orange, or red for the large IVs of mosquitoes (genus *Chloriridovirus*). These are of course the colors of the rainbow, and the Greek word *iridos* translates as "shining like a rainbow" (Aubertin, 1991). There is a direct relationship between particle size and iridescent color as described in the formula:

$$\lambda = 2m\{D^2 - [(D/2)\cos 30°]^2\}$$

where λ is the wavelength of the iridescent color, m is the refractive index of the material, and D is the particle center–center spacing, which is the same as particle diameter in a close-packed tetrahedral arrangement of particles.

Although most of the measurements of iridoviruses are made in ultrathin sections, negative staining may actually yield more realistic results. Negatively stained particle size measurements have been reported at 170 nm for Plowden IV [IV1] (*Iridovirus*) and 224 nm for Vero Beach IV [IV3] (*Chloriridovirus*) (DeBlois *et al.*, 1978; Wagner *et al.*, 1973). Using the above measurements in this formula and assuming an average refractive index for biological matrices of 1.52, iridescent colors of blue and orange are correctly predicted for Plowden IV and Vero Beach IV, respectively (Hemsley *et al.*, 1994).

Certain isolates (e.g., FV3, LCDV-1, and an isolate from the midge, *Chironomous plumosus*) appear to assemble into paracrystaline structures in the cytoplasm but show no iridescence. This could be due to the presence of the fibrillar structures described earlier which may increase the interparticular spacing and prevent the occurrence of Bragg reflections, etc. The relationship between fibrils and iridescence is not clear.

IV. Replication

The pathology of invertebrate IVs in host cells is characterized by cell enlargement, rounding and detachment, cell–cell fusion, vesicle production from protrusions at the cell surface, increased vacuolization, nuclear hypertrophy, decondensation of nuclear chromatin, and, most characteristically, the formation of electron-dense viroplasmic centers (virus assembly sites) in the cytoplasm. Not all effects are seen simultaneously, and details depend on the virus and host under study (Hall and Anthony, 1971; Webb *et al.*, 1973; Lee and Brownrigg, 1982; Lea, 1985; Czuba *et al.*, 1994).

Kelly and Tinsley (1974) noticed that infection of cells with Tia IV [IV2] or Dazaifu IV [IV6] caused a marked contraction of mosquito and lepidopteran cells (*in vitro*) with 72 hours. The cellular changes elicited by components of the Dazaifu IV [IV6] virion have been found to be dramatic both physically, in the formation of syncytia, and physiologically, as the shutdown of host cell macromolecular synthesis (Cerutti and Devauchelle, 1979, 1980). Viral particles caused extensive and very rapid cell fusion in permissive and nonpermissive cell lines

alike. UV-inactivated virus elicited the same effect. The incidence of cell fusion was sensitive to the multiplicity of infection. The syncytic effect of virions was even observed in vertebrate (monkey) cells, albeit in a less spectacular fashion. Host hormones can have a profound effect on patterns of replication of IVs (Kloc *et al.*, 1984). In general, the cytopathology of FV3 shows similar features to those above, although the effects on gross pathology at the tissue level are markedly different (e.g., Wolf *et al.*, 1968).

A. Cell Penetration and Uncoating

Patterns of replication and the time course of replicative events vary with temperature, cell type, virus, and multiplicity of infection, among other things. Frog virus 3 replicates at temperatures between 12° and 32°C (Gravell and Granoff, 1970). Replication of invertebrate IVs is also inhibited at temperatures over about 30°C, which no doubt reflects the damp habitats and cool body temperature of the majority of their poikilothermic hosts (Tanada and Tanabe, 1965; Day and Dudzinski, 1966; Carter, 1975; Witt and Stairs, 1976). In cell culture or in the insect hemocoel (following injection of inocula) virus particles appear to be adsorbed onto the plasma membrane of the host cell and enter by pinocytosis. Direct penetration of cells has also been suspected for FV3 following the observation of particles lying free in the cytoplasm (Houts *et al.*, 1974). Host macromolecular synthesis is rapidly inactivated.

In the cytoplasm, virions may be enclosed by a membrane or may exist as free particles during uncoating (Kelly and Tinsley, 1974; Webb *et al.*, 1976). In cells infected by Plowden IV [IV1], pinocytotic vesicles containing virus aggregate and fuse to form lysosomes full of virus particles, which have been reported as the site of viral uncoating (Mathiesen and Lee, 1981). Uncoating renders the virus sensitive to DNase treatment. Following cell penetration by FV3, approximately 50% of viral DNA became DNase-sensitive within 1 hr. Uncoating appears not to require any form of protein synthesis, as the rate of uncoating was similar in the presence or absence of a protein synthesis inhibitor (Smith and McAusland, 1969).

B. Shutdown of Host Macromolecular Synthesis

Whole virions of FV3 have been shown to elicit the shutdown of cellular macromolecular synthesis in permissive and nonpermissive host cells alike. This occurs even if the virions are inactivated by heat or UV light (Maes and Granoff, 1967a; Guir *et al.*, 1970; Willis and

Granoff, 1976) or if a soluble extract of the virions is applied to cells (Aubertin *et al.*, 1973, 1976). Goorha and Granoff (1974) superinfected cells with active FV3 following macromolecular shutdown caused by 4-hr exposure to heat-inactivated FV3; they observed a normal cycle of replication with normal kinetics and a normal yield of progeny virus. Studies by Drillien *et al.* (1977) have indicated that a single FV3 particle can elicit the inhibition of DNA, RNA, and polypeptide synthesis in a cell, which results in cell inactivation (defined as lack of ability to form colonies). The kinetics of cell inactivation were very similar when cells were treated with a soluble extract of FV3. This was interpreted as evidence that single entities (a single polypeptide or group of polypeptides) were responsible for cell macromolecular synthesis shutdown. This polypeptide must be heat stable. Cordier *et al.* (1981) treated mammalian cells with heat-inactivated FV3 or soluble FV3 extracts and then assayed the rate of protein synthesis in cell-free systems. Exposure to virus or viral extracts greatly diminished the rate of protein synthesis *in vitro*, and the effect was not reversed by altering the sodium ion concentration (which usually affects the initiation of intracellular protein synthesis). The cause of the inhibition was traced to impaired activity in the ribosomal fraction of the cell lysate. Ribosomal preparations from the livers of mice treated with FV3 displayed similarly reduced transcriptional activity (Elharrar and Kim, 1977).

As pointed out previously, this shutdown phenomenon has been extremely useful in the study of viral replication, as host cell synthetic pathways can be completely disrupted by brief exposure to inactivated virus. Subsequent incorporation of labeled compounds following superinfection with viable virus will be due to viral macromolecular synthesis alone (Willis *et al.*, 1985).

Similar shutdown properties have been studied in Dazaifu IV [IV6]. Shutoff of all macromolecular synthesis in mosquito cells was achieved within 1 hr of exposure to virions, and the rate of shutoff was dependent on the multiplicity of infection. A soluble extract of Dazaifu IV [IV6] was shown to have the same properties as intact particles when tested on invertebrate or vertebrate cells under permissive and nonpermissive conditions (Cerutti and Devauchelle, 1980). The lipid content of the virus was extracted and reconstituted as vesicles of trilayered lipid membrane, 50–200 nm in diameter. These vesicles had the same cell fusion and macromolecular shut-down properties as whole virions (Cerutti and Devauchelle, 1982). The vesicles were found to comprise 86% of the viral phospholipid and 4% of total viral protein. The proteins were identified as five entities up to 53 kDa in size, the major species being of 11 kDa (Cerutti and Devauchelle, 1990). Inci-

dentally, populations of subgenomic viral DNA fragments (~75 kbp) contaminating the vesicle preparations were later found to be infectious (at very low levels), indicating that recombination had occurred among coinfecting fragments to form viable viral genomes. Viral DNA alone was not infectious (Cerutti *et al.*, 1989).

C. DNA Replication

Descriptions of DNA replication can be found in Murti *et al.* (1985a) and Willis *et al.* (1985). Most detailed studies on iridovirus replication have used FV3. In this species, replication of virus DNA occurs in two phases: a nuclear phase and a cytoplasmic phase. This two-site DNA replication is unique for animal viruses. A functional nucleus is an essential cellular component for virus replication (Goorha *et al.*, 1977). Following shutdown of host cell macromolecular synthesis by treatment with inactivated FV3, viral DNA synthesis was detected in the nucleus by autoradiography. Purified nuclei were shown to contain viral DNA by hybridization (Goorha *et al.*, 1978). During the first 3 hours postinfection, viral DNA is synthesized solely in the cell nucleus. DNA produced here is genomic or up to twice genomic size. Pulse–chase experiments showed that the majority of viral DNA synthesized in the nucleus moved to the cytoplasm (Goorha, 1982). In the cytoplasm viral DNA exists as long concatamers (more than 10 times genomic length).

A temperature-sensitive FV3 mutant held at nonpermissive temperatures showed low levels of DNA production in the nucleus, but concatamers did not appear in the cytoplasm. When shifted to a permissive temperature, large concatamers quickly appeared in the cytoplasm (within 30 minutes), indicating that concatamers are formed as a result of recombination among the lengths of viral DNA produced in the nucleus (as observed in the bacteriophage T4) rather than, as an alternative hypothesis, that concatamers arise by a rolling circle process. The mutant FV3 appears to encode a protein involved in the second stage of DNA replication. The activity of this protein, possibly some 31 kDa in mass (Martin *et al.*, 1984), was not affected by the addition of protein synthesis inhibitor at the moment of switch between nonpermissive and permissive temperatures, indicating that it is the protein itself which is thermosensitive rather than the mechanism of *de novo* synthesis (Goorha and Dixit, 1984).

The similarities between FV3 and phage T4 in DNA replication may extend to the packaging of DNA into the viral capsid. In T4, concatameric DNA is packaged until the head of the phage is full, and this leads to a genome that is circularly permuted and terminally redun-

dant. Because these are features of the FV3 genome (and several other members of the *Iridoviridae*), it is assumed that "headful" packaging of DNA also occurs in these viruses (Goorha and Murti, 1982; Darai *et al.*, 1983; Soltau *et al.*, 1987; Ward and Kalmakoff, 1987). The evidence for genomic circular permution and terminal redundancy are outlined in Section V.

D. Methylation of Viral DNA

High levels of methylation of cytosine at CpG residues are only seen in iridoviruses from vertebrate hosts, namely, FV3 (Willis and Granoff, 1980), LCDV-1 (Wagner *et al.*, 1985), Goldfish iridovirus (Essani and Granoff, 1989), and epizootic hematopoietic necrosis virus (EHNV) (Eaton *et al.*, 1991), as indicated by treatment of genomic DNA with the endonuclease *Hpa*II, which recognizes CCGG sequences. DNA methylated at CpG residues is not cleaved by *Hpa*II but is cleaved by *Msp*I, which recognizes identical sequences but which is not sensitive to methylation.

There are conflicting reports as to the site of DNA methylation in FV3. Eukaryotic DNA methylases are found in the nucleus, whereas pulse–chase studies using radiolabeled uridine (a cytosine precursor) indicated that cytosine of FV3 was methylated in the cytoplasm. Endonuclease studies have supported this; FV3 DNA isolated from the cytoplasm was cleaved by *Msp*I but not *Hpa*II, whereas viral DNA isolated from the nucleus was cleaved by both enzymes. Host DNA methylase activity in the nucleus appears to be inhibited by the infecting virus, and there is evidence of differences in substrate specificities for host and viral DNA methylases. The FV3 DNA methylase showed an affinity for dsDNA and an ability to methylate a broad range of natural and synthetic DNAs *in vitro* (Willis *et al.*, 1984a). However, Schetter *et al.* (1993) reported that DNA methylase activity was detected only in the nucleus, not in the cytoplasm of FV3-infected cells, which suggests that DNA is methylated prior to export to the cytoplasm.

The FV3 DNA methylase comprises two polypeptides of 18 and 26 kDa, and there is also some evidence for endonuclease activity (possibly related to a polypeptide of 30 kDa) (reviewed by Essani, 1990). Methylation has been speculated as a mechanism to prevent nicking of viral DNA by viral endonucleases. In the T4 bacteriophage, DNA that has been nicked is not packaged. A DNA methylase inhibitor does not appear to influence the rate of macromolecular synthesis but dramatically reduces the rate of production of virions in FV3-infected cells (Goorha *et al.*, 1984). Methylation appears to be important in the packaging of DNA into virions. Apparently, FV3 mutants that lack DNA

methylase appear able to survive only because they do not show any viral endonuclease activity (Essani, 1990). The goldfish iridovirus is unique in that cytosines appear to be methylated at CpT as well as CpG sites (Essani and Granoff, 1989), perhaps reflecting a requirement for additional protection against endonucleases in this virus.

E. Transcription

Transcription and control of transcription of iridoviruses appear complex and have only been studied in detail in FV3. A clear review has been provided by Willis *et al.* (1990). Studies of transcription have been assisted by the host macromolecular synthesis shutdown immediately following infection and the fact that most FV3 mRNAs do not have poly(A) tails to protect against degradation (Willis and Granoff, 1976). This allows the resolution of up to 47 mRNA species on denaturing acrylamide or agarose gels (Willis *et al.*, 1977). Transcription of viral DNA in FV3-infected cells is a coordinated sequential process involving the production and regulation of mRNAs that can be classified according to their temporal sequence of synthesis. These classes are "immediate early," "delayed early," and "late," although finer divisions have also been defined by the use of various temperature-sensitive mutants (Goorha and Granoff, 1979). Analysis of such mutants has indicated that transcription is not dependent on successful DNA synthesis for any but the very last mRNAs to appear (~15% of the genome) (Goorha and Granoff, 1979; Goorha *et al.*, 1981). Other studies produced different findings. The presence of various DNA synthesis inhibitors has markedly reduced late gene expression in some studies (Elliott and Kelly, 1980; Elliott *et al.*, 1980b; Chinchar and Granoff, 1984). One explanation may be that template DNA undergoes modifications during the course of infection that permit the stepwise sequence of transcriptional events to progress.

1. Transcription and Nongenetic Reactivation

Immediate-early mRNAs are those transcribed in the presence of protein synthesis inhibitor (e.g., cycloheximide). The immediate-early mRNAs comprise 10 species, of which 7 with masses of 600 kDa or less can be clearly resolved on polyacrylamide gels (Willis and Granoff, 1978). These sequences represent about 32% of the genome, although probing of Northern blots with radiolabeled viral restriction fragments has suggested that rather more than one-third of the FV3 genome may be transcribed in the presence of cycloheximide (Mesnard *et al.*, 1988). The presence of host RNA polymerase II is necessary for early transcription (Goorha, 1981), but this enzyme must be modified

by a trans-acting viral protein(s) to overcome methylation of immediate early promoters.

Virions of both FV3 and Dazaifu IV [IV6] possess the property of nongenetic reactivation (Gravell and Naegele, 1970; Gravell and Cromeans, 1971; Cerutti *et al.*, 1989). UV-irradiated or heat-treated particles alone possess no infectivity but show cytocidal effects. However, when in combination, infection occurs and viable virus is produced. The UV-irradiated virus has a nonfunctional genome but possesses functional polypeptides. The heat-treated virus has denatured proteins but possesses a normal genome. The genome of the progeny virus in such coinfections is always derived from the heat-treated inoculum, whereas the UV-irradiated partner provides essential components to initiate replication. Naked "cores" or purified viral DNA can also be rendered infectious in the presence of UV-inactivated (genetically nonfunctional) virus. The titer of progeny virus was proportional to the concentration of transfecting DNA except at very high concentrations, where inhibition was observed (Willis *et al.*, 1979a). In Dazaifu IV [IV6] the entity responsible for nongenetic reactivation was identified as one or more structural viral polypeptide(s) that could be solubilized with detergent (octylglucoside) and reconstituted with lipid vesicles derived from the virus intermediate membrane (Cerutti *et al.*, 1989).

Evidence from transcription studies suggests that the solubilized protein(s) is the first of a series of trans-acting proteins which facilitate the temporal sequence of viral transcription. Transcription studies have focused on an immediate early FV3 gene (ICR 169). The ICR 169 (infected cell RNA of 169 kDa) codes for a putative protein of 18 kDa of unknown function. This RNA is produced in large quantities throughout the course of the infection cycle. Fathead minnow cells were transfected with a plasmid containing a 78-bp promoter region of the ICR 169 gene inserted upstream of a chloramphenicol acetyltransferase (CAT) reporter gene. The CAT activity was observed only when the cells were subsequently challenged by untreated or UV-irradiated FV3 but not by heat-treated FV3 (Willis and Granoff, 1985). Production of CAT-specific RNA occurred when transfected cells were infected with FV3 in the presence of cycloheximide, demonstrating a viral protein, a trans-acting factor, to be responsible, and that protein synthesis is apparently not required for mRNA synthesis from this early gene. Synthesis of CAT induced by FV3 showed marked inhibition in Chinese hamster ovary cells (CHO) cells in the presence of the polymerase inhibitor, α-amanitin, but inhibition did not occur under identical conditions in CHO cells with an α-amanitin-resistant RNA polymerase II (Goorha, 1981). These studies suggest the viral transactivating factor to have one or two possible modes of action. Either the

factor interacts with the host polymerase and modifies the enzyme to facilitate the transcription of virus-specific sequences, or the factor binds to a DNA template to enhance the attachment or processing of the sequences by host polymerase (Willis and Granoff, 1985). The latter hypothesis has some support by the fact that two viral proteins in the reconstituted membrane vesicles studied by Cerutti *et al.* (1989) showed marked DNA-binding properties.

The activity of the ICR 169 promoter was further investigated by S1 nuclease mapping and deletion studies using the CAT reporter system. The start site of transcription for the ICR 169 gene was located 29 bases upstream of an (A–T)-rich motif: TATTTTA. Deletion of this motif reduced CAT synthesis by 85%. However, a region of 14 bases 3′ of the A–T motif was identified as of even greater importance in the function of this promoter. Point mutations or deletions in the sequence CAGGGGAATTGAAA dramatically reduced CAT activity (Willis *et al.*, 1984b; Willis, 1987). The central GGGGAAT motif of this region is seen in a number of other viral and cellular enhancers and was demonstrated to bind strongly to a nuclear protein (Willis *et al.*, 1990).

A promoter region with significant sequence similarity to the 14-base sequence above has been found in another immediate early gene (ICR 489), although at a greater distance from the 5′ end of the message. Transcription of the ICR 489 gene contrasts with that of ICR 169. As with ICR 169, a trans-acting viral protein(s) is required to elicit transcription. However, in the presence of cycloheximide a massive overproduction of ICR 489 is observed, indicating that transcription is normally downregulated by an early synthesized viral protein (not produced in the presence of cycloheximide). The key regions for the ICR 489 promoter are different from those of ICR 169. Transcription of ICR 489 is dependent on the presence of a CCGCCC and a CCAAT motif, which have been reported to bind cellular transcription factors. The marked differences between the promoter regions of these two immediate early genes indicates that transcription of the two genes is not directed by common signals but is under the control of different processes and different cellular transcription factors (Beckman *et al.*, 1988).

There are three delayed early mRNAs. Studies with a temperature-sensitive FV3 mutant and a phenylalanine analog (fluorophenylalanine) indicated that a virus-induced protein(s) was required for expression of delayed early mRNAs (Willis and Granoff, 1978; Goorha *et al.*, 1979). This protein(s) is probably another trans-acting factor for switch on of delayed early mRNA synthesis, and it appears not to contain phenylalanine at any key sites.

The remaining species of mRNA are classed as late. These mRNAs

are usually synthesized after DNA replication has occurred in the nucleus. However, DNA replication is not a prerequisite for production of late proteins (Goorha *et al.*, 1979), and temperature-sensitive mutants have been analyzed that synthesize DNA but do not produce late mRNAs (Willis *et al*, 1979b). Again, a trans-acting viral-induced protein appears necessary for transcription of late sequences. A 95-bp region 5′ to the transcriptional start site has been studied using the CAT reporter system. The appearance of CAT activity in transfected cells, subsequently infected by FV3, was consistent with the "late" nature of this mRNA. No CAT mRNA was detected in the presence of cycloheximide. A motif of TATTTTA identical to that of the ICR 169 promoter was seen, but the upstream sequences (5′) were completely different, indicating that the late properties of the promoter reside 5′ to the (A–T)-rich region (Willis *et al.*, 1990). In general, the TATA and CAAT motifs are found upstream of genes in a number of other iridoviruses that have been studied, wherein their function is presumed to follow that found in FV3 (Tajbakhsh *et al.*, 1990a; Home *et al.*, 1990; Cameron, 1990; Schnitzler and Darai, 1993; Sonntag *et al.*, 1994; Schnitzler *et al.*, 1994b).

2. Transcription of Methylated DNA

A CAT reporter gene under the control of a methylated adenovirus promoter was not activated when transfected mammalian cells were subsequently infected by FV3 in the presence of cycloheximide or by UV-inactivated FV3 (Gorman *et al.*, 1982). This indicates that a virally induced protein is required to permit transcription of methylated viral DNA. This is a different situation from the trans-activating factor for the immediate-early ICRs 169/489, which are virion-associated rather than virally induced. If methylated DNA is not transcribed without prior protein synthesis, then immediate early genes must be unmethylated, or the methylation must be restricted to regions which were not crucial for transcription. Mutant promoters were produced in which each CG site was changed to methylated CCGG or CGCG by site-directed mutagenesis followed by treatment with bacterial methylases. Transcription of the methylated mutants was observed following infection by UV-inactivated FV3 or by normal FV3 in the presence of protein synthesis inhibitor. It appears that methylation of immediate early promoters does not affect transcription, suggesting that the CG motifs appear in regions that are not important for transcription (Thompson *et al.*, 1988).

3. Methylation and Stability of mRNA

The degree of methylation of FV3 mRNAs is dependent on the sequence in which they are synthesized. All viral mRNAs were termi-

nally blocked and methylated with three common types of nuclear RNA cap structures: $m^7GpppN^1mpN^2p$ and $m^7GpppN^1mpN^2mpN^3p$ comprised more than 80% of the total cap types and m^7GpppN^1p the rest. Between four and seven m^6A residues were detected in each early viral mRNA transcript, whereas methylation was not detected in late gene transcripts (Raghow and Granoff, 1980). This may reflect the fact that transcription of early genes occurs in the nucleus, whereas later genes are transcribed in the cytoplasm (possibly from concatameric DNA) by a virally induced RNA polymerase. Evidence for the existence of such a polymerase has come from sequencing studies of Dazaifu IV [IV6]. Schnitzler *et al.* (1994b) reported the presence of an open reading frame (ORF) encoding a putative protein of 1051 amino acids with very significant sequence similarity to the largest subunit of DNA-dependent RNA polymerase II, although with the C-terminal domain missing.

All FV3 mRNAs observed to date have terminal hairpin structures (dyad symmetry) (Willis *et al.*, 1984b; Beckman *et al.*, 1988; Schmitt *et al.*, 1990; Rohozinski and Goorha, 1992). Chinchar *et al.* (1994) examined the stability of transcripts from early and late genes with and without the transcription inhibitor actinomycin D. The level of viral transcripts remained high or increased in untreated cells infected by FV3. In the presence of actinomycin D, however, the level of early and late gene transcripts declined rapidly. Half-lives were estimated to be between 1.75 and 3.25 hours (although these values are affected by the experimental methods used). The mRNA-degrading mechanism is not known although synthesis of a virally encoded RNase is the most appealing option. These results led Chinchar *et al.* (1994) to suggest that the terminal hairpin structures of FV3 mRNAs play a role in signaling transcript termination rather than affecting transcript stability, as suspected previously (Aubertin *et al.*, 1990; Rohozinski and Goorha, 1992).

F. Translation

The mechanisms by which translation is regulated in iridovirus-infected cells is not well understood. Early mRNAs continue to be synthesized but are not translated late in infection (Willis *et al.*, 1977). For example, the immediate early transcript ICR 489 (described above) can be detected long after production of the protein has peaked and declined to undetectable levels. Mesnard *et al.* (1988) reported the abundant production of two RNAs complementary to the 5′ region of the ICR 489. If the "antisense" RNA hybridizes to ICR 489 to prevent translation, this may be an effective way of limiting production of the early protein later in the replication cycle. Another suspected mecha-

nism for translational control appears to reside in the structure of the 5′ region of the transcript. The length of an immediate early mRNA was found to change from 1.3 kb early in infection to 1.35 kb late in infection. This difference arises from an additional 50-base sequence at the 5′ end of the late transcript (Aubertin *et al.*, 1990). Translation was observed only for the early (unmodified) species (Tondre *et al.*, 1988). The mode of action of 5′ alterations on message viability is not known.

AUG codons are signals for the initiation of translation that are detected by the 40S ribosomal subunit. The number and context of AUG codons are different in early and late mRNAs of FV3. Translation of early gene mRNAs starts at the first AUG encountered, in a context for which the consensus sequence is A/GCCAUGGG. However, for the major capsid protein mRNA (late gene), translation starts at the fourth AUG codon encountered, and none of the codons have more than one base in common with the consensus sequence (apart from the AUG codon) (Aubertin *et al.*, 1990). Post-translational modifications appear limited to phosphorylation of some core polypeptides (Aubertin *et al.*, 1980). No evidence of glycosylation has been obtained (Krell and Lee, 1974).

G. Packaging of Virions

Different hypotheses have been advanced regarding the sequence of events leading to production of the mature virion. These ideas have arisen from electron microscope observations of particles in various stages of formation in tissue sections. Bird (1961, 1962) studied Plowden IV [IV1] and observed that the viral DNA and nucleoprotein condensed to form the core, which was then enveloped by the capsid. This interpretation found support in studies of Tia IV [IV2] and Dazaifu IV [IV6] in cultured insect cells (Kelly and Tinsley, 1974). Yule and Lee (1973) described the capsid of Plowden IV [IV1] assembling face by face and then being filled by DNA and fibrillar structures through a hole left in the shell. Studies with Riverside IV [IV31/32] supported this view (Hess and Poinar, 1985). Similar scenarios were previously proposed to explain the common observation of empty capsids (Smith, 1958; Xeros, 1964), but without explicit reference to DNA entry through a capsid pore. Others have interpreted the sequence of events being more simultaneous than the stepwise mechanisms above. There is coordinated assembly of inner membrane and capsid. As the capsid enlarges it sequesters core material from the assembly site (viroplasmic center). The DNA condenses to form the core proper once capsid construction is complete (Devauchelle, 1977; Federici, 1980; Devauchelle *et al.*, 1985a).

There is a striking similarity in the patterns of particle assembly in some of the vertebrate iridoviruses. In these viruses capsids initially appear as numerous angular crescent-shaped bodies in the assembly sites. As the crescents develop into icosahedrons, the early core structure becomes apparent. In some iridoviruses the degree of condensation of core material is clearly heterogeneous ("patchy" in appearance). The electron opacity of the core remains heterogeneous after apparent completion of the capsid, but the core subsequently develops into the even electron-dense structure typical of all iridovirus cores. These observations apply to the frog viruses studied by Lunger and Came (1966), a putative reptilian iridovirus from erythrocytes of a gecko (Stebhens and Johnson, 1966), goldfish iridovirus (Berry *et al.*, 1983), and epizootic hematopoietic necrosis virus (Eaton *et al.*, 1991). The mechanism of particle development is not well understood in FV3 (Goorha and Granoff, 1979). In tadpole edema virus, a strain of FV3 (Essani and Granoff, 1989), crescent-shaped capsid precursors develop into fully formed capsids, but condensation of the core elements does not occur until after completion of the capsid (Wolf *et al.*, 1968). A similar situation appears to exist in assembly of LCDV virions (Zwillenberg and Wolf, 1968) although production and assembly of LCDV may be seasonally affected as indicated by the large proportion of "empty" capsids and low virion production in lesions from flounders sampled in winter months compared to abundant mature virions in lesions of fish caught in the spring (Flugel, 1985).

H. Cytoskeletal Manipulation

Frog virus 3 manipulates the host cytoskeleton to assist in the production and release of virions. The three major cytoskeletal components are subject to different forms of manipulation and play different roles at different sites in the infected host cell. Three types of elements are involved: microtubules, intermediate filaments, and microfilaments. Microtubules are tubes of 22–26 nm diameter composed of tubulin. They appear to play no role in virus replication or release, and between 6 and 10 hours postinfection they disappear. This is due to severe inhibition of synthesis of tubulin (along with other host macromolecules) in infected cells (Murti and Goorha, 1983; Murti *et al.*, 1985b). The disappearance of microtubules is concurrent with the appearance of virus assembly sites. The morphology of virus assembly sites is different for FV3 in vertebrate cells compared to iridescent viruses in invertebrate cells, wherein they are dense entities often referred to as virogenic stroma or viroplasmic centers (Maes and Granoff, 1967b; Yule and Lee, 1973; Devauchelle *et al.*, 1985a; Ward and Kalmakoff, 1991). The assembly sites in cell infected by FV3, epizootic he-

matopoietic necrosis virus, and goldfish iridovirus are reported as being less electron dense than the surrounding cytoplasm and devoid of cellular components such as organelles and ribosomes (Maes and Granoff, 1967b; Murti *et al.*, 1984; Berry *et al.*, 1983; Eaton *et al.*, 1991).

Intermediate filaments are 7–11 nm in diameter composed of vimentin (for cells of mesenchymal origin). These filaments surround and radiate from the perinuclear region into the cytoplasm. Their usual function is uncertain. In FV3-infected cells, at 6–8 hours postinfection filaments retract from the periphery and cluster around the newly forming viral assembly sites. A marked increase in the degree of phosphorylation of the vimentin protein in infected cells is required to trigger this event (Willis *et al.*, 1979b). Inhibition of intermediate filament function by a drug (taxol) results in poorly defined assembly sites and a reduction in virion production of about 80% (Murti *et al.*, 1988). Although not strictly essential for virion assembly, the intermediate filaments are important in the structural integrity of assembly sites and in the efficient production of particles. Early but not late FV3-induced proteins also seem to be involved in the formation of assembly sites (Chinchar *et al.*, 1984).

Microfilaments are filaments of 4–8 nm diameter comprising subunits of actin. Concurrent with other major changes in the cytoskeleton, at 6 hours postinfection existing bundles of microfilaments disappear and the actin subunits disperse through the cytoplasm. Existing individual microfilaments persist unchanged. At 7–10 hours postinfection the microfilaments re-form at the cell surface and facilitate the budding of virus particles through the cell membrane. At this time the normally smooth cell surface is transformed into numerous microvilli-like projections. Actin continues to be synthesized during this period, although it appears in a biochemically different form. Whether this is a result of postranslational processing or is due to the synthesis of a different isoform of the protein is not known. The presence of microfilament disrupters did not affect the rate of FV3 production, but almost all the virus remained cell-associated, as large accumulations of particles beneath the cell membrane. As a result, the yield of budded virus fell by 80–99% compared to untreated controls (Murti and Goorha, 1990). In a remarkably similar manner to the FV3 studies above, an intimate association between cytoskeletal elements and epizootic hematopoietic necrosis virus has been observed. Intermediate filaments were clearly associated with the periphery of the virus assembly sites, and particles were observed enmeshed within the cytoskeleton of critical point dried cells. The role of the cytoskeletal elements in budding of this virus is not certain; 99% of the virus produced in bluegill cell culture remained cell-associated (Eaton *et al.*, 1991).

Kelly and Tinsley (1974) reported a marked reorganization of microtubules in lepidopteran cells infected by Tia IV [IV2]. In contrast, for Plowden IV [IV1] replicating in lepidopteran and mosquito cells, immunofluorescence studies indicated that microtubules and microfilaments were not involved in the formation and maintenance of assembly sites (Seagull *et al.*, 1985). Moreover, cytoskeletal disrupting drugs did not prevent the formation of viral assembly sites of Plowden IV [IV1] in insect cells (Bertin *et al.*, 1987). However, the presence of nuclear matrix or nuclear matrix-associated proteins within the structure of assembly sites has been reported for this system. Antibodies raised against mammalian lymphocyte nuclear matrix bound to Plowden IV [IV1] assembly sites *in vivo* and to purified (fractionated) assembly sites *in vitro* (Bladon *et al.*, 1986).

I. Enzymatic Activities

A variety of enzymatic activities have been detected in association with the purified virions of Dazaifu IV [IV6], FV3, and LCDV-1. The enzymes are similar for each of the viruses, possibly reflecting a common set of requirements during the initial stages of infection; however, not in all cases have the enzymes been shown to be virally encoded, so caution is necessary in the interpretation of some of these results. A nucleotide phosphohydrolase with high affinity for ATP has been reported for all three viruses (Monnier and Devauchelle, 1976; Vilagines and McAuslan, 1971; Flugel *et al.*, 1982). This enzyme hydrolyzed ATP to ADP and in the case of FV3 was localized in the core (Vilagines and McAuslan, 1971), whereas enzymatic activity was detected between the core and envelope of LCDV-1 (Flugel, 1985). A protein kinase (PK) is also common to these viruses. The PK of Dazaifu IF [IV6] phosphorylated low molecular weight proteins (*in vitro*) and showed a high affinity for basic substrates, especially a 12.5 K core polypeptide with DNA-binding properties (Monnier and Devauchelle, 1980). Phosphorylation changes the solubility of this protein, and it is presumed that endogenous PK activity within the core may result in the decondensation of DNA as a preliminary step toward release of the virus genome during virion uncoating early in infection (Cerutti and Devauchelle, 1990). The PK of FV3 appears different. The enzyme is external (i.e., can be solubilized), of mass 44 kDa, and is virally encoded (Silberstein and August, 1973, 1976). Little is known of the PK of LCDV-1 (Flugel, 1985). A third common enzyme is a DNase. In FV3, pH optima were reported as pH 5 (Aubertin *et al.*, 1971) and pH 7.5 (Kang and McAuslan, 1972). The DNase of Dazaifu IV was apparently able to cleave DNAs from various origins including

homologous viral DNA (unpublished data in Devauchelle *et al.*, 1985b; Cerutti and Devauchelle, 1990). DNase activity was also shown associated with LCDV-1 virions (Flugel, 1985). RNase activity has been reported only for LCDV-1 (Flugel, 1985) and FV3 (Kang and McAuslan, 1972).

Thymidine kinase (TK) activity has been detected in purified LCDV-1 (Flugel, 1985) and in TK-negative mouse cells following infection by FV3 (Aubertin and Longchamps, 1974). The TK of LCDV-1 has been shown to be virally encoded. Transformation of 3T3 TK^- cells into TK^+ cells was observed following transfection of cells with LCDV-1 DNA restriction fragments (Scholz *et al.*, 1988). The viral TK gene was mapped and sequenced and found to encode a polypeptide of 318 amino acids (Schnitzler *et al.*, 1991). A protein phosphatase has been reported from FV3 particles (Silberstein and August, 1973). The presence of a virion-associated RNA polymerase has been reported for Tia IV [IV2] and Dazaifu IV [IV6] but has not been supported by the work of others (Monnier and Devauchelle, 1976; Goorha and Granoff, 1979).

V. Molecular Biology

Advances in molecular biology of iridoviruses through the 1980s have been summarized by Darai (1990). Developments since that time include the identification of a number of iridovirus genes. A unique feature of iridoviruses is the organization of the genome. The evidence that the genome is circularly permuted and terminally redundant (for all iridoviruses examined to date) comes from the following observations, which are based mainly on FV3 but also on LCDV-1 and Dazaifu IV [IV6].

1. Treatment with λ 5′-exonuclease exposes single-stranded ends to the DNA molecule. Subsequent annealing of FV3 DNA results in the formation of duplex circles with two gaps of single-stranded DNA, indicating that the single-stranded ends are complementary (the gaps result from digestion proceeding beyond the terminal repeats). Circles were not observed in FV3 DNA not pretreated with exonuclease, indicating terminal DNA to be normally blunt. The length of these terminal repeat sections was estimated by measurement of photomicrographs at approximately 4% of the length of the FV3 genome (Goorha and Murti, 1982).

2. Fully denatured DNA reanneals as duplex circles, each with a pair of single-stranded tails at different positions along the length of the molecule. The tails occur where terminal repeats fail to find com-

plementary single-stranded regions available for hybridization (Goorha and Murti, 1982; Darai *et al.*, 1983; Delius *et al.*, 1984).

3. End labeling of FV3 DNA failed to show specific labeled fragments following restriction endonuclease treatment because there are no fixed DNA termini in a population of virions with a circularly permuted genome (Goorha and Murti, 1982).

4. The DNA molecule is linear, yet restriction maps for all iridoviruses mapped to date are circular (Lee and Willis, 1983; Darai *et al.*, 1985; Ward and Kalmakoff, 1987; Soltau *et al.*, 1987; Schnitzler *et al.*, 1987; Davison *et al.*, 1992).

5. In LCDV-1 and Dazaifu IV [IV6], treatment of genomic DNA with 3′- or 5′-exonuclease followed by restriction endonuclease treatment results in the gradual disappearance of all restriction fragments, indicating that the distribution of termini is random across the length of the genome (Delius *et al.*, 1984; Darai *et al.*, 1983). However in FV3, each molecule in a population had a common region comprising about 75% of the total genome; the location of terminal sequences was limited to the remaining 25% (Murti *et al.*, 1982).

Estimates for the degree of terminal redundancy in iridoviruses range from 4 to 6% in FV3 (Goorha and Murti, 1982), 12% in Dazaifu IV [IV6] (Delius *et al.*, 1984), and up to a remarkable 50% in LCDV-1 (Darai *et al.*, 1983). It is to be expected that circularly permuted genomes should have a number of origins of replication. To date, six origins of replication have been reported scattered across the genome of Dazaifu IV [IV6]. Each origin was detected by plasmid rescue of genomic library fragments that were amplified following transfection into insect cells infected by Dazaifu IV [IV6]. Three of these origins of replication have been sequenced and found to comprise a 12- to 16-base inverted repeat. The degree of sequence similarity among the three entities was 55–77%, and all were predicted to form a hairpin structure (Sonntag and Darai, 1992).

A Plowden IV [IV1] isolate from North America appears unusual in that it has been reported to comprise a number of genomic components: a large component (176–247 kbp) and a small component (10.8 kbp) (Tajbakhsh *et al.*, 1986). The relative abundances of the two components were dependent on the stage of virion assembly. The small component was found in much greater abundance in partially filled capsids. Transcription from the small component was detected in permissive and semipermissive cells (Tajbakhsh *et al.*, 1990b). How common such genomic components are in the *Iridoviridae* is not known. An Irish strain of Plowden IV [IV1] studied in the same laboratory showed only one component (see review by Tajbakhsh and Seligy, 1990).

A. Virus Genes

In addition to the transcription-regulating genes of FV3 described previously, sequencing work has provided a small but intriguing catalog of putative viral genes. The approach has been either to sequence genomic fragments from established iridovirus gene libraries or to construct oligonucleotide primers using available information on conserved and variable regions of viral genes. The latter approach has been highly successful in the identification and sequencing of the major capsid protein (MCP) gene, and it appears set to become a standard technique for comparative studies and characterization of novel isolates (R. Webby and J. Kalmakoff, 1995, unpublished data). The MCP gene was first sequenced from Plowden IV [IV1] (Tajbakhsh *et al.*, 1990a) and subsequently from Aberystwyth IV [IV22] (Cameron, 1990), Dazaifu IV [IV6] (Stohwasser *et al.*, 1993), and LCDV-1 (Schnitzler and Darai, 1993). Sequence information generated from these studies has been used for comparative studies, described previously (Schnitzler and Darai, 1993).

Other identified genes code for putative proteins with nucleic acid replication or transcription functions. Schnitzler *et al.*, (1994a) detected eight ORFs in a 5.7-kb *Eco*RI fragment of the Dazaifu IV [IV6] genome. Of these eight, relationships were found to exist with sequences in databases for three of the ORFs. The putative proteins for these genes were as follows: (i) a homolog of eukaryotic nonhistone chromosomal protein (221 amino acids) with a DNA-binding region some 70 amino acids in length; (ii) a polypeptide of 145 amino acids with a single putative zinc finger motif similar to that known as the RING motif, a motif known for its DNA-binding properties and involvement in transcription and repair of DNA damage (another RING motif was also found in second larger polypeptide on another *Eco*RI fragment in the Dazaifu IV [IV6] genome); and (iii) a polypeptide of 127 amino acids with a highly conserved region common to the bacterial antimutator, GTP phosphohydrolase (Mut-T). This enzyme hydrolyzes the highly mutagenic substrate 8-oxo-GTP to prevent transversions during replication. Another viral antimutator enzyme, uracil DNA glycosylate, has been reported from poxviruses, wherein it is essential for replication (Stuart *et al.*, 1993).

In Dazaifu IV [IV6], comparison of sequence data has led to the suggestion that certain iridovirus genes were more recently acquired from eukaryotic hosts than the homologous genes of other cytoplasmic DNA viruses. A putative helicase gene coding for a polypeptide of 606 amino acids and a gene coding for a putative DNA-dependent RNA polymerase II enzyme subunit (1051 amino acids) have been reported

from different fragments of the gene library of this virus (Sonntag *et al.*, 1994; Schnitzler *et al.*, 1994b). These enzymes appeared more closely related to eukaryotic polymerases and helicases than to the homologous enzymes from vaccinia virus or African swine fever virus. However, these comparisons make important assumptions regarding the rate of evolution of iridoviruses compared to other viral and eukaryotic systems, assumptions for which data do not exist.

A putative apoptosis inhibiting gene has been found in Dazaifu IV [IV6] (Birnbaum *et al.*, 1994). The ability for cells to undergo programmed death (apoptosis) in response to viral infection may be blocked by viral gene products in a number of different ways. In other invertebrate DNA viruses such as baculoviruses, the mechanism for blocking cell apoptosis appears different from those found in mammalian viruses (adenoviruses or herpesviruses). Several baculoviruses have been found to have a gene (*iap*, inhibition of apoptosis) for a product of 30 kDa that showed apoptosis blocking activity when cotransfected with DNA from an apoptosis-negative baculovirus (*Ac*-NPV *p35*). The putative IAP protein has a central tandemly repeated motif containing cysteines and histidine, probably a zinc finger with nucleic acid-binding properties, as well as a more widely recognized cysteine/histidine motif; C3HC4, found in approximately 30 other proteins and described from Dazaifu IV [IV6] as being a zinc finger structure (Handermann *et al.*, 1992; Sonntag *et al.*, 1994). The *iap* gene of Dazaifu IV [IV6] is not complete compared to baculovirus homologs because it has only one repeat motif and one C3HC4 motif. Whether this apparent difference is biologically important is not known because the activity of this gene has not been tested (Birnbaum *et al.*, 1994).

Home *et al.* (1990) reported the sequence and transcription map for a late gene of Plowden IV [IV1]. The putative protein was 867 amino acids (96 kDa), rich in serine, proline, and basic residues, hydrophilic, and showed similarities to proteins with known DNA-binding properties, in particular with GAG polyproteins of vertebrate viruses. A role in DNA packaging and core formation was suggested. The gene was unusual in having 3′ polyadenylation signals, structures that are not commonly seen in FV3 transcripts (Willis *et al.*, 1990). Polyadenylation signals were also reported for the major capsid protein gene of Plowden IV [IV1] (Tajbakhsh *et al.*, 1990a), and for genes of unknown function in LCDV-1 (Schnitzler *et al.*, 1990), but the relatively sharp appearance of bands in Northern blots of other iridescent virus mRNAs suggests that poly(A) tails are not ubiquitous.

The gene sequence for a putative integrase–recombinase enzyme of 275 amino acids has been reported from FV3. This group of enzymes is responsible for catalyzing strand exchange between DNA molecules

and plays an important role in DNA replication of bacteriophages. The mRNA for this gene was delayed early in nature. The enzyme is believed to be important in the formation of concatameric DNA intermediates or resolution of such entities prior to virion packaging. This finding once more emphasizes the similarities between the replication of FV3 and these bacterial viruses (Rohozinski and Goorha, 1992).

B. Repetitive DNA

Restriction endonuclease mapping of FV3, LCDV-1, and several invertebrate IVs has revealed extensive repeat sequences in certain regions of the genome. In the IV9 strain of Nelson IV [IV9/10/18] the repetitive DNA was found to occur in over 25% of the genome (Ward and Kalmakoff, 1987; Kalmakoff *et al.*, 1990). These repeat sequences are distinct from the complementary terminal sequences that arise from the terminal redundancy of iridovirus genomes. Transcription of the repetitive sequences of Nelson IV [IV9/10/18] was clearly restricted to late times postinfection (McMillan and Kalmakoff, 1994), and the coding function of these regions was obscure. The repetitive sequences of LCDV-1, however, were less extensive, comprising two nearly identical *Eco*RI fragments of some 1400 bp in length. One fragment had strong promoter function when inserted into a plasmid with a CAT reporter gene and transfected into *Escherichia coli*. Virtually identical repeat sequences were found in LCDV-2, a genetically distinct isolate from dabs (94.9–99.9% homology) (Schnitzler *et al.*, 1990).

By sequence analysis the pattern of repetitive DNA in Dazaifu IV [IV6] has been shown to be unusually complex. Initially two boxes (A and B) were found in *Eco*RI fragment. Box A was 91 bp in length and was complementary to nine tandemly repeated regions of a *Pvu*II fragment that mapped to coordinates distant from the *Eco*RI fragment. Box B was 46 bp in length and was complementary to one region of the same *Pvu*II fragment. In addition, a cluster of imperfect but somewhat regular repeat sequences spanning a region of over 4 kb was detected in the *Pvu*II fragment. Four distinct repetitive elements were identified, three of which appeared duplicated with 80–87% homology. The fourth element, some 240 bp, appeared as 12 repeats in three segments (3–5 boxes per segment). The degree of homology among these boxes of the fourth element was 90–98%, although three of the boxes were not complete. In general, the arrangement of boxes was complex and interdigitated. The coding function of these regions was not clear, although a number of ORFs were detected (Fischer *et al.*, 1988a,b, 1990). Inverted repeats were also responsible for a stem–loop structure that was detected in the sequence of one *Eco*RI fragment. The structure was

confirmed by electron microscopy of reannealing DNA, forming a stem of 62 bp and a loop of 692 bp (Fischer *et al.*, 1988a).

VI. Ecology

Knowledge of the ecology of both vertebrate and invertebrate iridoviruses lags far behind biochemical and molecular laboratory studies. The search for effective agents for the biological control of insect pests has meant that the ecology of iridescent viruses has received more attention than the ecology of the vertebrate isolates. Sadly, the amount of data on the ecology of vertebrate isolates barely merits a small paragraph here. There have been some studies on experimental transmission by cohabitation of infected and uninfected fish (e.g., Hendrick *et al.*, 1990) and some tests of pathogenicity to different amphibian life stages (Tweedell and Granoff, 1968; Wolf *et al.*, 1968; Clark *et al.*, 1968; Granoff *et al.*, 1969), but quantitative studies of iridovirus ecology in vertebrate populations do not appear to exist.

What information is available on IV ecology is plagued by using the iridescent phenomenon as the sole criterion for infection; in all but a few cases the possibility of covert IV infections have been ignored. Actually, iridescence may be a trivial characteristic (Kelly, 1985) with no selective advantage to the virus in terms of enhanced virus transmission, for example. Previous authors have speculated on unifying features in the ecology of these viruses, in the mechanisms of transmission and persistence in host populations (Hall, 1985; Kelly, 1985; Ward and Kalmakoff, 1991). As yet, however, there is no evidence to indicate that the strategies of transmission, persistence, or dispersal of iridoviruses show any obvious unification at all. Rather, a spectrum of different strategies may be involved dictated by the biology and life history of the host(s) and the environment that it inhabits. Consequently, the opportunities for transmission and replication available to the virus will differ in each system, and generality in statements concerning the ecology of these viruses may not be possible. The true incidence of transmission and the infectivity of iridescent viruses remain particularly vague, as the importance of covert infection by IVs in host populations is largely unknown.

Key parameter values necessary for the development of models of host–iridovirus population dynamics, for example, the rate of transmission, are not known. It is possible to make crude estimates of certain parameters by scanning the available literature. Survival time (the interval between infection and death) is dependent on temperature, route of infection (Carter, 1973b, 1975), and stage of host infected

(Carter, 1974; Sieburth and Carner, 1987). Survival times seem to be fairly independent of dose (Carter, 1974), although when very high doses are administered larvae may become paralyzed and die without developing patent disease (Sieburth and Carner, 1987; Ward and Kalmakoff 1991). This may be related to the cytotoxic effects of iridoviruses. Survival times following *per os* doses range from 45 days for Plowden IV in *Tipula oleracae*, about 35 days for Riverside IV [IV31] in terrestrial isopods, approximately 28 days for Stoneville IV [IV30] in *Helicoverpa zea*, and 21 days for an IV of the cricket *Scapteriscus borellii* (Carter, 1973a; Grosholz, 1992, 1993; Sikorowski and Tyson, 1984; Fowler, 1989), although survival times as short as 6 days have been reported for late instar *Anticarsia gemmatalis* larvae orally infected with the homologous virus, San Miguel IV (Sieburth and Carner, 1987). Compared to feeding, survival times were reduced by 30–50% when the inoculum was injected; a reduction in the variability of survival times was also seen (Carter, 1973a,b).

The yield of infectious particles per host is massive for arthropods with patent IV infections. Remarkably, 25% (dry weight) of the body of a dead insect may be virus (Williams and Smith, 1957). Using this figure and previously published values for IV particles, namely, weights of approximately 2×10^{-15} g (Thomas, 1961; Glitz *et al.*, 1968), Carter (1973b) calculated that an infected tipulid larva of 200 mg should contain some 2.5×10^{12} particles of Plowden IV [IV1]. Day and Mercer (1964) obtained a similar figure of 2×10^{12} particles per pupa for Tia IV [IV2] in *Galleria mellonella*. Following these same assumptions, yields for Riverside IV [IV31/32] can be crudely estimated in different isopod species at approximately 2.6×10^{10} particles released from *Armadillidium vulgare* and 3.1×10^{10} particles from the larger *Porcellio scaber*. Likewise, a typical *Tenebrio molitor* pupa would be expected to yield some 1.5×10^{10} particles of Fort Collins IV [IV29] [estimates from data given in Cole and Morris (1980) and Black *et al.* (1981), respectively]. Clearly, these calculations are open to criticism, but they provide a starting point for the development of models of epizootiology of these diseases.

Most of the work on the ecology of IVs comes from four systems for which the hosts are mosquitoes, crane flies (*Tipula* spp.), terrestrial isopods (woodlice), and blackflies (*Simulium* spp). With one exception, described later, nothing is known about the rate or mechanisms of IV dispersal, although, as will become apparent, autodispersal via the infected host may be one likely mechanism. Consequently this section is divided into two main parts: transmission and persistence. The persistence section also includes examples of the limited knowledge on the natural host range of IVs.

A. Transmission

Previous authors have looked for a common transmission strategy for all the known invertebrate isolates and have postulated about possibilities such as the role of cannibalism of infected conspecifics, cuticular lesions, and parasitic vectors (e.g., nematodes) as possible routes of horizontal transmission. Riverside IV (IV31/IV32) is transmitted horizontally via cannibalism of infected conspecifics or by predation of infected individuals of other woodlouse species. Enzyme-linked immunosorbent assays (ELISA) indicated that covert IV infections do not occur in this system (<1% of total); all infections appear to be patent and lethal. Interspecific competition between two woodlouse species (*Porcellio laevis* and *P. scaber*) had more effect on the incidence of disease in *P. scaber* than did intraspecific competition: the prevalence of infection was doubled in the presence of the competitor. This was not a direct effect of competition for food, but probably arose from increased levels of aggression when the other species was present. This result would be expected for a pathogen directly transmitted by predation/cannibalism. An increase in the frequency of aggressive encounters and wounding may also increase the probability of virus entry through cuticular lesions (Grosholz, 1992).

In a mosquito IV system, Vero Beach IV [IV3], horizontal transmission was demonstrated via cannibalism of patently infected mosquito cadavers (Linley and Nielsen, 1968b; Hall and Anthony, 1971). In this system, it was evident that inapparent IV infections could also occur but at unknown frequencies. Vertical transmission occurred when mosquito larvae were challenged with IV inoculum shortly prior to pupation. The progeny of 19–47% of IV-challenged females developed patent infections (Woodard and Chapman, 1968). Vertical transmission may be an all-or-nothing response. The progeny from 5 egg batches produced by females exposed to IV showed patent infection, whereas the remaining 58 egg batches did not give rise to patently infected larvae (Linley and Nielsen, 1968a). The all-or-nothing theory has found only partial support (Fukuda and Clark, 1975). However, mosquito progeny that failed to develop patent infections in these studies were not assayed for the presence of covert infections, so the true incidence of vertical transmission in this system remains unclear. When wild or cultured mosquito larvae were challenged with increasing doses of IV, it was evident that progeny from the wild mosquitoes developed notably higher frequencies of patent disease (19.6% overall) than their laboratory cultured conspecifics (5.7% overall). The possibility that exposure to IV provoked patent infections in wild mosquitoes that were already covertly infected is an appealing concept and

finds parallel in the latent infections of lepidopteran baculoviruses (Hughes *et al.*, 1993).

The transmission of pathway of infection of Plowden IV [IV1] in the crane fly, *Tipula oleracae,* were studied using iridescence as the criterion for infection. The frequency of inapparent infections in this system is unknown, although when pupae were injected with virus, 20% of the resulting adults developed patent infections while the remaining 80% appeared to be covertly infected as shown by serology (Carter, 1973c). In another assay, the feces of infected larvae were demonstrated to contain viable virus, but in insufficient quantities to cause patent disease in healthy larvae when ingested. Larvae, pupae, and adults that died following the dose of feces were tested for inapparent infections and proved negative; however, the remaining apparently healthy insects were apparently not tested, so the ability of contaminated feces to induce covert infections in *Tipula* is not known. Transmission of virus by cannibalism of infected cadavers was an efficient mechanism of transmission and resulted in high levels of patent infection in susceptible conspecifics; the doses of virus ingested during cannibalism are massive (Carter, 1973a). The route of infection was also investigated by injecting virus, applying virus to cuticular lesions, applying virus to the spiracles, or by allowing larvae to drink a virus suspension. The most efficient routes of transmission were first infection, followed by abrasion, feeding, and lastly via spiracles. Larvae did not become patently infected when tipulid eggs were allowed to hatch in agar containing virus (Carter, 1973b). Covert infections have also been reported in host range studies where Nelson IV [IV9/IV10/IV18] was injected into various lepidopteran species; the larvae did not develop iridescence, but IV DNA could be detected in tissue extracts by dot-blot hybridization (Ward and Kalmakoff, 1991).

We may predict that vertical transmission should be common in many invertebrate IV systems due to the rarity of patent IV infections, which suggests a low virulence of these viruses. The majority of infections may be inapparent and somewhat benign in nature (Kelly, 1985; Poprawski and Yule, 1990; Williams, 1993). However, pathogens cannot be sustained in host populations by vertical transmission alone, and they must effect horizontal transmission to some extent (Anderson and May, 1981). How this is achieved in covert infections by IVs is not known. The possibility of prolonged low-level excretion of virus particles by covertly infected hosts appears an appealing concept and finds comparison in nonlethal cytoplasmic polyhedrosis infections of Lepidoptera in which virus particles are continually produced from gut cells and excreted in the feces. Such a method of horizontal transmission would require that IVs display a far higher *per os* infectivity than

previously recognized. Such infectivity is yet to be demonstrated. A microscopic study by Stoltz and Summers (1971) demonstrated that large doses of IV were degraded in the gut of mosquito larvae; the observation of intimate contact between gut cell and virus was not seen, a finding which appears not to support a theory of high infectivity of these viruses. The possibility that subparticle units are infectious (e.g., viral DNA and some viral proteins) was noted by these authors. Given the highly infectious nature of FV3 DNA in the presence of soluble viral proteins (nongenetic reactivation), this possibility merits consideration.

If a unifying feature to IV ecology exists, it may be related to transmission of these viruses. The fact that IV infections are mostly limited to hosts in damp or aquatic environments along with the stability of the particles in water might be indicative of transmission of "free-living" infective stages (*sensu* Anderson and May, 1981) as being important in the ecology of the viruses. In contrast, if cannibalism/predation of infected hosts were a major transmission route for most of the IVs, we may expect these viruses to be far more common than they appear to be among species in which cannibalism was common (lepidopteran larvae, grasshoppers, etc.) or in predatory arthropods (predatory beetles, wasps, ants, spiders, etc.). This is probably an oversimplification of a potentially complex area. The matter remains wide open.

B. Persistence

1. Physical Persistence

The ability of IV particles to physically persist outside of the host is virtually unknown. Temperature may be an important factor in this respect, as IVs are rapidly inactivated at temperatures over 50°C (Day and Mercer, 1964). Humidity may be more important still, given the moist or aquatic host habitats. The ability of crude preparations of Vero Beach IV [IV3] to elicit patent infections in mosquito larvae has been reported to decline by 50% in 0.8 days in fresh water at 27°C. In brackish water, virus retained infectivity for 2 days, after which infectivity declined rapidly; on soil, the virus was inactivated even more rapidly (estimated from data in Linley and Nielsen, 1968b). Riverside IV [IV31/32] in the cadaver of a woodlouse apparently remained infective for up to 5 days at ambient laboratory temperatures (Grosholz, 1993). The ability of purified Tia IV [IV2] at 4°C to elicit patent infections of *Galleria mellonella* larvae fell by 50% after 32 days in a laboratory refrigerator (estimated from data in Day and Gilbert, 1967).

2. Persistence in Host Populations

For one putative iridovirus from the water snail *Lymnaea truncatula*, the incidence of infection in each of two annual generations was monitored between 1983 and 1984 and again in 1987–1989. Snails were sampled from 11 sites. Virus-induced hypertrophy of blood cells and the presence of an intracytoplasmic inclusion were used as the diagnostic features of infection. The incidence of infection was between 1.6 and 87%, depending on site and time of year. The incidence of infection was reasonably consistent and specific to each site. The site where the incidence of infection was highest, in beds of rushes, consistently had infection frequencies greater than 70%, whereas infection frequencies at certain meadow sites did not exceed 15%. There was no obvious pattern in the incidence of infection in spring versus autumn generations. Data on the density of snail populations at each site were not given (Rondelaud and Barthe, 1992).

A handful of IV isolates have been reported as causing epizootics of patent (lethal) disease. One such report comes from Ricou (1975), who sampled a population of *Tipula* larvae wherein the incidence of disease differed according to host population density and soil moisture. Larval densities were twice as great in damp soil compared to wet or dry soil. The incidence of disease was highest in wet and damp soils, which may indicate moisture to be of great importance in IV persistence. In this study the epizootic developed over the course of a year to 90% infection levels, with a clear resulting impact on the *Tipula* population density in the following years.

In the woodlouse–Riverside IV (IV31/32) system, Grosholz (1993) presented data to show how changes in the incidence of IV infections were affected by the patchiness of the host population and the within-patch density of hosts. These factors changed seasonally. During the dry months of late summer, the woodlouse populations were highly aggregated, the within-patch host density was high, and the distance between adjacent patches was large (July mean, 5.75 m). The incidence of IV infection at this time was approximately 1–2%. During the wet winter and spring months, the patchy structure of the population became more homogeneous, the within patch host density declined, and the distance between patches fell (January mean, 2.0 m). These conditions were more suitable for the transmission of the virus, and the incidence of infection rose to approximately 13%. No significant differences were found in the incidence of infection in different sexes of host or in different size classes of host. However, significant species effects were detected: *Porcellio laevis* suffered the highest frequency of infection (5.7% overall), *Armadillidium vulgare* had the lowest incidence

(1.6%), and *P. dilatatus* and *P. scaber* were intermediate. The effect of interpatch spacing on the prevalence of IV infection was supported by field experiments in which the distance between patches was manipulated. The distance between adjacent patches was also shown to be negatively correlated with the probability of isopod dispersal, although the effect of virus infection on the dispersal abilities of isopods was not tested. Clearly, in this system, both population structure and the presence of competing species (see above) are important factors affecting the prevalence of the disease. Moreover, the overall population density appeared vital in determining the persistence of the disease. Viral infections were endemic at high host densities (>2000 isopods m^{-2}) but completely absent in low-density host populations nearby (Grosholz, 1992). With four different hosts involved in the ecology of the virus, this system is a clear demonstration of why IV nomenclature should be uncoupled from that of the host species.

Studies of blackfly populations in the River Ystwyth, Wales (UK), have addressed the problem of detecting and describing the incidence of covert IV infections in insects. These studies have demonstrated that the incidence of covert IV infection can be measured using sensitive bioassay and PCR techniques. Larvae of the lepidopteran *G. mellonella* developed patent IV infections following intrahemocoelomic injections of blackfly homogenates derived from covertly infected blackfly larvae. This assay is extremely sensitive and may have detection limits of below 10 particles as reported for another IV isolate (Day and Mercer, 1964; Day and Gilbert, 1967). The bioassay results were supported by results of PCR analysis using primer sequences targeted at the MCP gene of Aberystwyth IV (IV22), an isolate from blackflies found in the River Ystwyth previously (Batson *et al.*, 1976). Nested sets of primers were designed for high specificity (high GC content and therefore high annealing temperature) rather than to target particularly conserved regions of the MCP gene. Likewise, cycling conditions were highly stringent. An outer set of primers were designed to yield an amplicon of 816 bp. This amplicon was not visible by gel electrophoresis. A sample of this reaction mixture was subjected to further amplification using a second set of primers to amplify a fragment of 719 bp from within the 816-bp fragment. The 719-bp fragment was visible by electrophoresis. Cameron (1990) reported a *Xho*I site in this region of the MCP gene, and so the identity of the amplicon was confirmed by treatment with *Xho*I to yield fragments predicted to be 472 and 247 bases in length. Using the bioassay and PCR techniques, the presence of abundant covert infections in the springtime populations of *Simulium* larvae in the River Ystwyth was detected (Williams, 1993). The frequency of covert infection was highest in the central

section of the river. The nature of the covert infection in blackflies is not known. Certainly the infection does not appear to be latent in the sense usually used for insect viruses (Hughes *et al.*, 1993), because it can be transmitted to other insects by injection. Probably the infection exists as particles within host cells but at very low densities.

Following this finding, the bioassay technique was used for screening larval blackfly samples from a monthly sampling program from sites along the River Ystwyth over the reproductive season of the blackflies. This has revealed a complicated interaction involving one blackfly species and three tentative virus species in the river. The incidence of covert infection in blackfly larvae sampled in March (overwintering populations) varied between 17 and 37% depending on site. All of these infections were different variants of the original Aberystwyth IV. In April, the incidence of covert infection declined (0–20% depending on site), and a seemingly new (second) virus was detected. In May, a number of patent infections were observed albeit at an extremely low density, all of which were different variants of Aberystwyth IV (Williams and Cory, 1993). However, at this time, no covert infections could be detected in the blackfly populations. Throughout the remainder of the summer period the incidence of covert infections remained at virtually undetectable levels (<1%). However, in September, covert infections from a third virus species appeared in the populations at levels of up to 20%. This third virus had been isolated from a patently infected larva in September of the previous year. High frequencies of covert infection occurred when host population densities were low. Coefficients of similarity were calculated from restriction profiles following treatment with *Hin*dIII or *Eco*RI, for both the Aberystwyth IV and the new (September) isolates. Coefficient values within each tentative virus species ranged from 70 to greater than 95%, whereas comparison of isolates from different "species" consistently gave coefficient values of less than 66%. The genetic diversity of variants isolated from covert or patent infections was remarkable. In no instance were identical virus variants found infecting different individual larvae. Just what the adaptive significance of such variation is remains obscure. The marked fluctuations in the incidence of covert IV infections in the blackfly populations and the fact that three apparently different viruses were involved at different times of the year led to speculation about the potential role of other aquatic organisms in the transmission and persistence of these viruses (Williams, 1995).

3. Role of "Alternative" Hosts

Certainly one-virus-multihost systems evidently exist, judging from the observation of patent infections elsewhere. Examples from sym-

patric species include Nelson IV [IV9/10/18] which infects two lepidopterans and a coleopteran, Riverside IV [IV31/32] which infects *Armadillidium vulgare, Porcellio scaber, P. laevis, P. dilatatus,* and possibly several other isopods (Grosholz, 1993; Schultz *et al.*, 1982), and probably Timaru IV [IV16/19] which infects two scarabid species, *Costelytra zealandica* and *Odontria striata* (N. McMillan, 1990, personal communication). A possible example from nonsympatric species may be Aberystwyth IV (IV22/25) from a blackfly (*Simulium* sp.) which has also been isolated from a crane fly larva (*Tipula* sp.) in the United Kingdom (Elliott *et al.*, 1977).

Aquatic organisms of overtly different taxa than arthropods may be involved in the transmission and persistence of IVs, it has been speculated. Such speculation arises mainly from the observation of iridovirus-like particles in other aquatic organisms. In *Chlorella*-like algae symbiotic with *Hydra* and *Paramecium,* icosahedral particles have often been reported that are 150–190 nm diameter in negatively stained preparations, contain 5–10% lipid, possess a major structural protein of approximately 54 kDa, and have a dsDNA genome (Van Etten *et al.*, 1982). However, it is now clear that these algal viruses are not closely related to iridoviruses. The most obvious differences lie in the structure of the genome (which is linear and nonpermuted with hairpin ends) and the fact that some isolates showed high levels of methylation at cytosine and adenine bases, which is not seen in any iridovirus (Schuster *et al.*, 1986; Van Etten *et al.*, 1991). The *Chlorella* viruses have now been assigned to a new family, the *Phycodnaviridae.* The observation of IV-like particles in a daphnid, *Cerodaphnia dubia,* lead to speculation that this may be an alternative host for IVs of two mosquito species found patently infected in the same pools of water (Ward and Kalmakoff, 1991). The role of "alternative" hosts in persistence of IVs is clearly yet another facet of the ecology of these viruses that requires attention.

VII. Future Directions for Iridoviruses

Looking to the future, it is clear that two important issues require study: the taxonomy and the ecology of iridoviruses. The taxonomic organization within and among the various genera of the *Iridoviridae* requires revision. Genetic studies have begun to clarify the interrelationships of the invertebrate IVs for which distinct complexes have been detected using hybridization and other techniques. These studies require support from studies using complementary techniques. Work is in progress to sequence regions of the major capsid protein gene from a broad range of iridoviruses, from both vertebrate and inverte-

brate genera. This should become the basis for defining a new characteristic by which iridoviruses can be characterized and classified in a rigorous standardized manner. The polythetic system of classification requires that a number of other characteristics would be included in the definition, but the quantitative nature of sequence data puts this character at the top of the list for comparative studies. The discarding of a considerable number of previously recognized but uncharacterized viruses from the family should permit a fresh start with a new set of criteria for iridovirus classification. Characterization of novel isolates should always be made with reference to existing characterized material, and the rationalization of recognized iridovirus species should assist in the selection of key species for use in comparative studies.

Possibly, a virologist would perceive a different set of priorities for future research with iridoviruses, but 40 years after IV discovery, knowledge of the ecology of these viruses is still in its earliest stages. This is because most work with iridoviruses has been done by virologists interested in questions different from those that interest the ecologist. Studies of host–pathogen ecology may also be neglected because it involves learning new and rather esoteric techniques from those used by the traditional field ecologist. This situation is now changing as PCR and other molecular techniques have been developed that are routine and simple to master. For the invertebrate IVs, the recognition of widespread covert infection raises a number of intriguing questions. Within individual hosts, the nature of covert infection remains unknown. Are covert infections localized to specific tissue types? Are the gonads infected, and is vertical transmission resulting in covertly infected host progeny a common occurrence? Preliminary evidence suggests that IVs may have a far higher infectivity than previously believed because challenging hosts with IV inocula may lead to few patent infections, yet abundant covert infections (T. Williams, C. Tiley, and J. Cory, 1993, unpublished data). Thus, the incidence of transmission of IVs may previously have been grossly underestimated due to the rarity of patent infections in most host–IV systems.

Likewise, the involvement of alternative host species in the transmission and persistence of IVs deserves far more attention than received to date. The involvement of alternative hosts may also be reflected in the high levels of genetic diversity observed in patent and covert IV isolates from blackfly larvae. Here, too, studies are required to understand the distribution of variable regions within particular isolates, the diversity of isolates found in a particular host blackfly, and the diversity of genotypes within the IV population. Are blackflies infected with a similar diversity of IV strains that are selected out during the process of bioassay in *Galleria* larvae, or does each blackfly

harbor distinct, near clonal isolates? The population consequences of such diversity touches on major theories of host–pathogen evolution: Red Queen, arms-race hypotheses (e.g., Dawkins and Krebs, 1979; Anderson and Gordon, 1982; Hamilton, 1982).

Basic studies of the ecology of vertebrate iridoviruses are crying out to be done. With animals as easy to culture as frogs/tadpoles and a virus that is as amenable to cell culture as FV3, the system seems perfect for experiments on transmission and persistence. Most of the fish viruses also require quantitative studies of basic aspects of their ecology. An appreciation of the major impact that iridoviruses can have on fish populations will no doubt elicit the required studies in due course.

The iridoviruses are a fascinating but neglected family, clearly united by physical and genetic features but showing diverse patterns of pathogenicity, host range, and host exploitation. Advances in understanding the taxonomy and ecology of certain iridoviruses should permit a diversity of further studies. Although IVs have previously been likened to Cinderella-like characters for their striking iridescent hues, a major advance will come from recognizing that invertebrate iridoviruses are more "Jekyll and Hyde" in nature, and the majority of their existence may be as covert infections that must be searched for. This probably also applies to many of the isolates from vertebrates.

Note Added in Proof

At the time of going to press, reports of extensive mortality in the frog populations of Britain are making the national newspapers (*The Times*, July 17, 1995). Up to 2000 deaths per site have been reported (Drury *et al.*, 1995; Cunningham *et al.*, 1995).

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