

Invertebrate Iridescent Viruses

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I. INTRODUCTION

Invertebrate iridescent viruses (IVs) belong to the family *Iridoviridae*, which are large icosahedral viruses with a large genome of double-stranded DNA. These viruses are not occluded in a protective protein matrix, unlike the well-known nucleopolyhedroviruses (NPVs), granuloviruses (GVs), cytoplasmic polyhedrosis viruses (CPVs), and entomopoxviruses (EPVs). Members of the *Iridoviridae*, are structurally complex, however, and show a number of unique characteristics in terms of their genomic organization, structure, and mechanisms of replication, which unite the family.

A common feature is that IV hosts generally occur in aquatic or damp habitats such as the soil or leaf litter. Geographically, IVs appear widely distributed. The first IV was discovered in larvae of the crane fly, *Tipula paludosa*, in Great Britain (Xeros, 1954); since then, an additional 73 species of hosts have been reported with naturally occurring IV infections. Of these, the majority are insects from the orders Diptera (40 spp.), Coleoptera (8 spp.), and Lepidoptera (7 spp.). Most of the dipteran records are mosquitoes. A number of noninsect species are also infected by IVs, particularly terrestrial isopods (8 spp.) and daphnids.

The IVs have attracted interest as pathogens of insect vectors of medical importance, such as mosquitoes and *Simulium* spp. Few of the viruses isolated from infected hosts, however, have been characterized, probably because of the low incidence of lethal infections in host populations and a resultant decline of interest in IVs as biocontrol agents. In spite of that, the past 5 years have seen initial changes in our perception of these viruses in terms of their relationships

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with their hosts, their molecular biology, and the taxonomic relationships within the family.

II. CLASSIFICATION

The family *Iridoviridae* was created in 1976 to encompass a growing number of icosahedral DNA viruses that had been reported from both invertebrate and vertebrate animals and that characteristically assembled in the cytoplasm of host cells (Fenner, 1976). The term *iridovirus* was then adopted to describe isolates from vertebrates; inasmuch as it is a coverall term, it may be applied equally to invertebrate isolates, although for historical reasons the term *iridescent virus* continues to be used.

The invertebrate isolates were divided between two genera based principally on particle size. The small IVs, with a diameter of approximately 120 nm, were assigned to the genus *Iridovirus*, whereas the larger IVs, with a diameter of approximately 180 nm, reported only from mosquitoes, midges, and a *Simulium* sp. were assigned to the genus *Chloriridovirus* (although strangely, this genus also contained members that should have been assigned to the *Iridovirus* genus on grounds of particle size) (Mathews, 1982). The prefix *chlor* was taken from the Greek *chloros* (green), reflecting the yellow-green coloration that the large IVs display in infected host tissues and as purified pellets of virus.

Isolates from vertebrates were assigned to three genera: *Ranavirus*, representing viruses from amphibians; *Lymphocystivirus*, for isolates from flat fish (dab and flounder); and African swine fever virus, which was initially incorporated into the family as a separate genus but was later removed in light of genomic organization data. Following this, the taxonomy of the family remained little changed, with 22 recognized small IVs (*Iridovirus*), 10 recognized large IVs (*Chloriridovirus*), and 50 isolates distributed among the vertebrate genera, mostly in the *Ranavirus* genus. The creation of an additional vertebrate genus was also sanctioned to accommodate two viruses isolated from a goldfish cell line (Francki *et al.*, 1991).

The type species for the whole *Iridoviridae* family is frog virus 3 (FV3) of the *Ranavirus* genus. This virus is highly amenable to cell culture and more is known about its composition, structure, replication, and molecular biology than any other iridovirus. Consequently, FV3 is considered to be the model virus for this family, and I will make reference to studies of FV3 periodically throughout this chapter.

A. Problems with the Established Classification of Iridescent Viruses

There are three principal problems with the classification of IVs devised in the 1970s: (1) the criteria used to recognize isolates as separate virus entities; (2) a grave lack of information on the interrelationships among IVs from differ-

ent hosts; and (3) the nomenclature system employed to name IVs. Let us consider each problem in turn.

As the number of reports of arthropod species with patent IV infections grew, lists were constructed of those hosts from which IV infections had been reported in the literature or were known to the authors from personal communications (Tinsley and Kelly, 1970; Tinsley and Harrap, 1978). The issue of whether or not the virus had been isolated and had undergone some degree of characterization was not taken into account when producing these lists. However, the lists were accepted by the International Committee on Taxonomy of Viruses (ICTV), presumably because of the desire to give some structure and order to the classification of the group and on the assumption that the status of each isolate would become apparent with the passage of time. Thus, viruses that lacked any degree of characterization achieved undeserved taxonomic status by publication in the ICTV reports (Mathews, 1982; Murphy *et al.*, 1995).

Within the IVs, the data concerning taxonomic interrelationships among the 32 recognized IV types were extremely limited. What data existed were primarily serological in nature. This was useful in confirming that most of the small IVs (*Iridovirus* genus) were interrelated (Kelly *et al.*, 1979). However, the absence of comparative genetic data prevented any real advance in understanding IV interrelationships, a situation that remained unchanged throughout the 1980s.

Under an interim system of nomenclature for IVs, each record of a virus isolated from a different host was given the name of the host followed by the words "iridescent virus," and a type number was assigned that indicated the sequence of discovery (Kelly and Tinsley, 1970). Hence, the first IV reported was named *Tipula paludosa* iridescent virus (IV type 1). The type number system was not applied universally, however, and after 1980, the assigning of type numbers ceased altogether, although applying the host name to the isolate continued. This scheme of nomenclature has one primary failing: It ties the identity of the virus to a particular host. This becomes a problem when viruses are not highly host specific.

B. New Changes in the Classification of Iridescent Viruses

Today, the picture is different. Recommendations were accepted by the ICTV regarding the status of previously recognized IV types and a new list of tentative IV species was established (Williams *et al.*, 1996). In summary, 16 of the 32 previously recognized IV types were removed from the classification, as no characterization data were available and stocks of the viruses did not exist. Additional IV types were identified as strains of recognized IVs bearing different type numbers, for example, IV9, IV10, and IV18 are actually variants of a single virus (see Section VI).

Consequently, following the inclusion of one new tentative species, an IV from the lepidopteran *Anticarsia gemmatalis* (Williams, 1994; Kinard *et al.*,

TABLE I. Iridescent Virus Classification
following Recent Changes Approved by the ICTV

Genus	Particle diameter	Tentative species identified by type number(s)	Host(s)
<i>Iridovirus</i>	120 nm	IV1	<i>Tipula paludosa</i> (Dip.)
		IV2	<i>Sericesthis pruinosa</i> (Col.)
		IV6 ^a	<i>Chilo suppressalis</i> (Lep.)
		IV9/10/18	<i>Wiseana cervinata</i> (Lep.)
			<i>Witlesia sabulosella</i> (Lep.)
			<i>Opogonia</i> sp. (Col.)
			<i>Costelytra zealandica</i> (Col.)
		IV21/28	<i>Helicoverpa armigera</i> (Lep.)
			<i>Lethocerus colombiae</i> (Hom.)
		IV22	<i>Simulium variegatum</i> (Dip.)
		IV23	<i>Heteronychus arator</i> (Col.)
		IV24	<i>Apis cerana</i> (Hym.)
		IV29	<i>Tenebrio molitor</i> (Col.)
		IV30	<i>Helicoverpa zea</i> (Lep.)
		IV31/32	<i>Armadillidium vulgare</i> (Isop.)
			<i>Procellio dilatatus</i> (Isop.)
<i>Chloriridovirus</i>	180 nm	No number assigned	<i>Anticarsia gemmatilis</i> (Lep.)
		IV3 ^a	<i>Aedes taeniorhynchus</i> (Dip.)

^aType species of the genus.

1995), the revised list of tentative IV species now includes just 13 viruses in the *Iridovirus* genus and one virus, IV3, from *Aedes taeniorhynchus* (Clark *et al.*, 1965) as the sole member and type species of the *Chloriridovirus* genus (Table I). This species list is tentative because of a continuing lack of detailed information for the majority of member viruses. Arguments supporting the classification changes have already been described in detail and were applied as much to the vertebrate genera as to the invertebrate genera (Williams, 1996).

C. The Nomenclature Problem

The existing nomenclature of IVs is no longer useful and should be replaced with a more appropriate system (Ward and Kalmakoff, 1991; Williams and Cory, 1994). In particular, the name of the virus should not depend on the host, because the same virus may naturally infect more than one host species; likewise, a single host species may be infected by several different IVs.

Thus, an alternative nomenclature system for IVs has recently been put forward in which the viruses are given geographical descriptors; the name of a town or city close to the site of discovery of the virus (Williams and Cory, 1994). Under this system, the name of the IV9/IV10/IV18 example given above would be changed to "Nelson iridescent virus"; Nelson being the town in New Zealand close to the site of isolation of IV9. Similarly, IV31/IV32 becomes "River-

side IV''; this being the point of discovery of the virus. This system is subject to criticism on the grounds that viruses may be assigned names that are difficult to pronounce by English users, although with careful selection of names from the local options, this should not be a major problem.

However, nomenclature changes need to be taken up by the majority of scientists working in the field, and changes should be accepted only cautiously as they can be difficult to rectify if subsequently found to be unworkable. Consequently, this nomenclature has not yet been adopted by the ICTV, and in this chapter I will continue to use the established system of type numbers and host names.

III. STRUCTURE AND COMPOSITION

IV particles comprise an electron-dense core of DNA and associated proteins, surrounded by a lipid membrane, which in turn is closely associated with the exterior protein capsid (Fig. 1). IVs released by budding may have an additional outer envelope, most commonly observed in virus grown in cell culture (Hukuhara and Hashimoto, 1967; Yule and Lee, 1973; Webb *et al.*, 1976). The structure and composition of each component are considered in turn.

A. Capsid

The IV capsid comprises a lattice of identical hexagonally packed subunits of a single polypeptide of 48–55 kDa, the major capsid protein (MCP). The MCP comprises about 470 amino acids and represents 40–45% of the total particle polypeptide (Moore and Kelly, 1980; Black *et al.*, 1981; Davison *et al.*, 1992; Stohwasser *et al.*, 1993). This protein is highly conserved and is distantly related to the MCP of icosahedral viruses from other virus families, that is, African swine fever virus and an algal virus from the *Phycodnaviridae* (Stohwasser *et al.*, 1993; Mao *et al.*, 1996). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and surface labeling studies on IV6 have indicated that the MCP is the basis for two different structures: a weakly united trimeric form held together seemingly by hydrogen bonding located on the exterior surface of the capsid, whereas a covalently bonded MCP trimer lies beneath the surface layer (Cerutti and Devauchelle, 1990).

The capsid subunits are tubular in shape, 7–9 nm in diameter, and 7–9 nm in length, with a central hole. The subunits are the building blocks for 20 trisymetrons (each comprising 55 subunits) and 12 pentasymetrons (each of 31 subunits) that are needed to form the icosahedral particle. Thus, each IV particle probably comprises a total of 1472 subunits (Wrigley, 1969, 1970; Stoltz, 1971, 1973). Five trisymetrons are attached to the sides of each pentasymetron but with the corners out of alignment with the pentasymetron by three subunits, shown clearly in IV1 (Manyakov, 1977). Larger IVs, such as IV3 and an isolate from *Chironomus plumosus*, have larger trisymetrons, probably com-

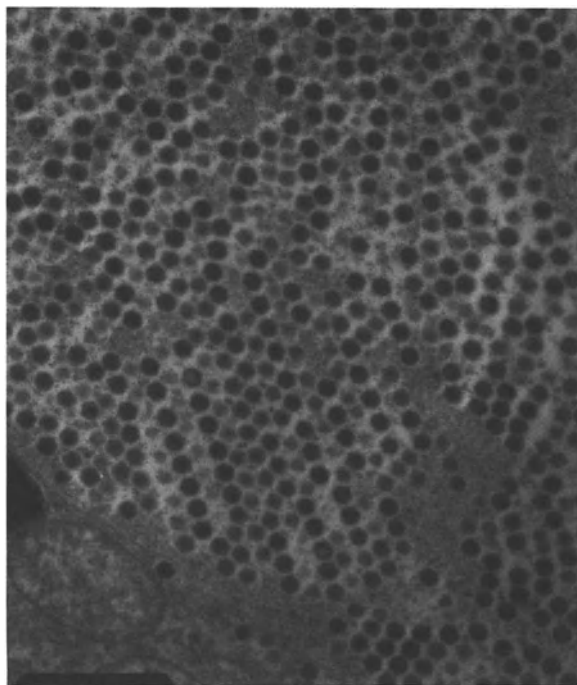


FIGURE 1. Particles of IV3 in a mosquito cell showing regular paracrystalline packing and the presence of virogenic stroma. Scale bar, 1 μm . (Photo courtesy of J. Becnel.)

prising 78 subunits, giving a likely 1560 subunits per particle (Stoltz, 1971, 1973).

An external fringe of small fibrils about 2.5 nm in length has been reported in certain IVs (Cole and Morris, 1980; Black *et al.*, 1981; Devauchelle *et al.*, 1985b). Fibrils with terminal knobs have also been observed (Stoltz, 1971, 1973). Some isolates have extremely long fibrils; these are most obvious in an isolate from the midge, *Chironomus plumosus* (Stoltz *et al.*, 1968) and in an iridovirus from fish (Zwillenberg and Wolf, 1968). In such cases, each capsid subunit appears to bear a single fibril.

B. Lipid Layer

The lipid layer is about 4 nm thick (Kelly, 1985), is intimately associated with the icosahedral capsid, and has been reported to make up 5.2–9% of the particle dry weight. Much of the lipid is phospholipid (Kalmakoff and Tremaine, 1968; Kelly and Vance, 1973; Balange-Orange and Devauchelle, 1982). The fatty acid and phospholipid composition of IVs differ from that of the host cell for all IVs assayed to date (Balange-Orange and Devauchelle, 1982; Williams and Thompson, 1995).

The lipid layer is essential for infectivity of vertebrate iridoviruses (Willis

and Granoff, 1974). In contrast, IVs are recognized to be resistant to ether treatment (Murphy *et al.*, 1995), although the evidence for this is somewhat anecdotal; ether-treated IV3 produced patent infections in mosquito larvae (Matta and Lowe, 1970). Likewise, Day and Mercer (1964) mentioned that patent infections were observed following injection of *Galleria mellonella* larvae with ether- or chloroform-treated IV2. However, lipid membrane extracts show biological activity of importance during the initial stages of infection. This property of IVs requires confirmation.

Differences in lipid composition between host and virus, and the observation that IVs do not appear to obtain their lipid component by budding through a cell organelle membrane, have led to the assumption that the lipid layer is acquired *de novo* during particle maturation; this situation also is believed to occur in poxviruses. However, recent work has shown that vaccinia virus particles acquire their internal lipid membrane from an intracellular compartment between the endoplasmic reticulum and the Golgi apparatus. Virally encoded proteins appear to facilitate this process (Schmelz *et al.*, 1994). The relevance of these findings to IVs is not known.

C. Core

The core is a highly hydrated electron dense entity. The core components appear to be uniformly distributed through the core but show a degree of structural organization. There is some evidence for the interaction of core structures with the lipid membrane, and it is suspected that core and capsid polypeptides are interconnected by way of complexes of protein passing through the lipid layer (Cuillel *et al.*, 1979; Cerutti and Devauchelle, 1982; Klump *et al.*, 1983; Robach *et al.*, 1983). In FV3, freeze etching revealed the presence of randomly oriented rods, some 10 nm in diameter, which are possibly part of the DNA-protein complex arranged in a long coiled filament (Tripier-Darcy and Nermut, 1983). Core structures also have been likened to bundles of parallel threads in an isolate from the mosquito *Culiseta annulata* (Buchatsky, 1977).

Much of the polypeptide diversity of IVs appears to be associated with the core and lipid membrane. At least six polypeptide species are associated with the DNA within the core, the major component being a 12.5-kDa species in IV6 (Cerutti and Devauchelle, 1985). Lipid-enveloped cores of IV3 were not found to be infectious to mosquito cells or larvae, possibly because of the failure of cores to attach to cells (Wagner *et al.*, 1973).

D. Physicochemical Characteristics and Composition

Size measurements among IVs tend to vary because of side-to-side or vertex-to-vertex measurements being reported. Measurements of dehydrated particles in ultrathin sections are most common and range from about 120 to 130 nm for the small IVs and 180 to 200 nm for large IVs, although several isolates have been reported with intermediate sizes (Stoltz *et al.*, 1968; Popel-

kova, 1982), indicating that size alone is not an infallible criterion by which isolates can be assigned to genera. Measurements from negatively stained material give particle diameters some 20% larger than dehydrated sections (Wagner *et al.*, 1973; Fukaya and Nasu, 1966; Cole and Morris, 1980; Poprawski and Yule, 1990; Lacey and Adams, 1994). A smaller variant of IV3 showing turquoise iridescence has been detected in laboratory cultures (Wagner *et al.*, 1973) and was termed "turquoise mosquito iridescent virus." However, all the studies described in this chapter refer to the original "regular" strain, unless otherwise stated.

The weight of intact virions is 1.28×10^6 kDa for small IVs and 2.49×10^6 kDa for the larger chloriridovirus IV3 (Glitz *et al.*, 1968; Lowe *et al.*, 1970). Particles of IV3 have a density of 1.35 g cm^{-3} compared to 1.30 g cm^{-3} or slightly more for IV1 and IV2 (Matta, 1970; Robertson and Longworth, 1973; Glitz *et al.*, 1968). Small IVs have an $S_{20,w}$ of approximately 2200 compared to up to 4458 for IV3 (Kelly and Robertson, 1973; Matta, 1970; Wagner *et al.*, 1973; Kinard *et al.*, 1995). The IV particle contains a linear dsDNA molecule representing 12–17% of the particle weight (Bellett and Inman, 1967; Kalmakoff and Tremaine, 1968; Matta, 1970; Stadelbacher *et al.*, 1978). Reported genome sizes for small IVs range from 140 kilobasepair (kbp) in IV24 to 209 kbp in IV6 (Williams and Cory, 1994; Fisher *et al.*, 1990). There are conflicting reports of the genome size of IV3, which was calculated to be 135 kbp by restriction endonuclease analysis (Williams and Cory, 1994) compared to a remarkable 383 kbp by sucrose gradient centrifugation (Wagner and Paschke, 1977). The genomes of small IVs (*Iridovirus* genus) have a characteristic GC content of 29–32% compared to 54% for IV3 (Glitz *et al.*, 1968; Black *et al.*, 1981; Wagner and Paschke, 1977).

One-dimensional PAGE analysis of intact virions usually resolves 20–32 polypeptides, although two-dimensional techniques have indicated greater diversity indicating several viral proteins to be oligomeric structures composed of subunits linked by disulfide bridges (Cerutti and Devauchelle, 1985). Polypeptide weights typically range from 11 to 200 kDa, although more extreme values have also been reported (Krell and Lee, 1974; Moore and Kelly, 1980; Cerutti and Devauchelle, 1990; Tajbakhsh and Seligy, 1990). Despite their larger size, IVs from mosquitoes have been reported to be structurally less complex than the small IVs with just 9 or 12 polypeptides detected by SDS-PAGE (Wagner *et al.*, 1974; Buchatsky and Sherban, 1976).

A number of virion-associated enzymes have been detected in IV6, including a nucleotide phosphohydrolase that hydrolyzed ATP to ADP; a protein kinase possibly responsible for phosphorylation of a DNA-binding protein; an alkaline protease possibly involved in virus uncoating; and a nonspecific DNase (Monnier and Devauchelle, 1976, 1980; Farara and Attias, 1983, 1986; Devauchelle *et al.*, 1985a).

IV. REPLICATION AND MOLECULAR BIOLOGY

Replication of IVs is sensitive to cell type (Tajbakhsh *et al.*, 1990b) and temperature. Replication of IV22 in mosquito cells, measured by the produc-

tion of viral antigen, was abundant at 20°C, minimal at 28°C, and absent at >30°C (Tesh and Andreadis, 1992). The same trend is observed *in vivo*; most IVs do not replicate at high temperatures (Tanada and Tanabe, 1965; Carter, 1975; Witt and Stairs, 1976). In contrast, in mosquito cells infected with IV3, cytopathic effects were observed only at >30°C (Webb *et al.*, 1974).

Replication of iridoviruses at the cellular and molecular level has been most extensively studied in FV3 and to a lesser extent in IV6. Consequently, the following account focuses mainly on FV3, with additional information from IVs where applicable.

A. Penetration and Shutdown of Macromolecular Synthesis

IV particles appear to be adsorbed onto the cell membrane and enter cells by pinocytosis. In the cytoplasm, particles may be surrounded by a membrane or may appear as free virions that undergo uncoating. Vesicles containing IV1 were reported to aggregate and fuse into lysosomes in which virus uncoating takes place (Kelly and Tinsley, 1974; Webb *et al.*, 1976; Mathiesen and Lee, 1981). Uncoating of FV3 does not require protein synthesis.

There follows a rapid shutdown in host cell macromolecular synthesis, a characteristic of the infection process in all iridoviruses studies to date. FV3 particles can produce this effect in permissive and nonpermissive cells and the effect is not destroyed by treatment with heat or UV light (Willis and Granoff, 1976). This shutdown property is very potent; a single particle or possibly a single protein molecule may be able to elicit shutdown (Drillien *et al.*, 1977). A heat-stable protein that affects ribosomal transcription activity is believed to be responsible (Elharrar and Kirn, 1977).

Host cell shutdown in mosquito cells was seen within 1 hr of infection with IV6, and the rate at which this occurred was dependent on the multiplicity of infection. IV6 also provoked shutdown in permissive and nonpermissive cells including vertebrate cells. Lipid vesicles extracted from IV6 particles made up 86% of the viral phospholipid and 4% of total viral protein and caused cell-cell fusion and shutdown in the same fashion as intact particles. Five protein components were detected, the major component being 11 kDa (Cerutti and Devauchelle, 1980, 1982, 1990; Cerutti *et al.*, 1989). Production of viral macromolecules is not affected by the shutdown process.

B. DNA Replication

Iridoviruses are unusual in that replication occurs in both the nucleus and the cytoplasm, although virus particle assembly is strictly cytoplasmic. During the first 3 hr postinfection, FV3 DNA is synthesized only in the nucleus in fragments of genomic or up to twice the genomic size. DNA then moves to the cytoplasm where it exists as concatamers of more than 10 times the genomic length (Goorha, 1982). Formation of concatamers probably occurs by direct joining of the DNA lengths present in the nucleus (Goorha and Dixit, 1984;

Martin *et al.*, 1984). It is assumed that concatameric DNA is packaged into virions using a "headfull" mechanism.

The iridovirus genome is circularly permuted and terminally redundant. This means that within each particle the complete genome is present plus a little extra DNA from the next genome copy in the concatamer (terminal redundancy), and this leads to a population of particles with different terminal sequences (circular permutation) (Goorha and Murti, 1982; Willis *et al.*, 1985). Consequently, although each genome is a linear DNA molecule, when restriction maps are made, they appear circular for all IVs mapped to date (Ward and Kalkmakoff, 1987; Soltau *et al.*, 1987; Schnitzler *et al.*, 1987; Davison *et al.*, 1992). In IV6, the degree of terminal redundancy has been estimated as 12% and a total of six origins of replication have been detected (Delius *et al.*, 1984; Handermann *et al.*, 1992). The IV genome is not methylated, whereas high levels of methylation of cytosine are seen in vertebrate iridoviruses (Willis and Granoff, 1980; Essani and Granoff, 1989; Eaton *et al.*, 1991).

Extensive regions of repetitive DNA have been mapped from the genomes of IVs. In IV9, the repetitive sequences were found to make up over 25% of the genome. These sequences are distinct from the DNA that accounts for the terminal redundancy of the genome. The coding function of these regions is unknown, although transcription of these regions was detected but only late in the infection cycle (Ward and Kalkmakoff, 1987; Kalkmakoff *et al.*, 1990; McMillan and Kalkmakoff, 1994). The pattern of repetitive DNA in the genome of IV6 is complex and involves boxes of tandem repeat sequences and others with a number of different interdigitated repeat sequences of variable size and homology. Within this region a number of open reading frames (ORFs) of unknown function were detected (Fischer *et al.*, 1988a,b, 1990).

The only exception to the genomic organization described above is a North American isolate of IV1 in which the genome was reported to comprise two components, one of approximately 200 kbp and one of 10.8 kbp. The relative abundance of the two components was dependent on the stage of particle assembly and on the host (Tajbakhsh *et al.*, 1990b; Czuba *et al.*, 1994). Other isolates of IV1 show only one component (Tajbakhsh and Seligy, 1990, personal observation).

C. Transcription, Translation, and IV Genes

Transcription and transcriptional control have been studied only in FV3, wherein it resembles a coordinated sequential process that is not dependent on DNA synthesis for any but the last 15% of the viral genome. Three classes of mRNAs have been recognized, depending on the sequence in which they appear. There are ten "immediate-early" mRNAs representing about one third of the FV3 genome, or possibly more (Goorha and Granoff, 1979; Willis and Granoff, 1978; Mesnard *et al.*, 1988).

A virus-associated protein appears to be the first in a series of *trans*-acting factors that facilitate the temporal sequence of viral transcription. This protein

is suspected to interact with the host RNA polymerase to facilitate virus transcription, possibly by binding to viral template DNA to enhance attachment or processing of immediate-early sequences by the host polymerase (Willis and Granoff, 1985). The promoter sequence that regulates transcription of a major immediate-early gene was identified as a 23-bp region 5' to a TATA-like box that signaled the start of transcription (Willis, 1987). Regulation of transcription of other immediate-early mRNAs appears to depend on inhibition by an early synthesized gene product(s) (Beckman *et al.*, 1988), whereas synthesis of another *trans*-acting factor is required for the switch on of the next class of transcription products—"delayed-early mRNAs"—of which there are three in FV3 (Willis and Granoff, 1978; Goorha *et al.*, 1979).

Transcription of late genes usually occurs after the nuclear phase of DNA replication, and again viral-induced *trans*-acting factors are required (Willis *et al.*, 1979, 1990). There is a rapid turnover of FV3 mRNAs and continual transcription is required to maintain messages at a steady state (Chinchar *et al.*, 1994).

Early genes appear to be transcribed in the nucleus, whereas late genes may be transcribed in the cytoplasm, possibly from the concatameric DNA, by way of a virally encoded DNA-dependent RNA polymerase. The gene for such an enzyme has been reported in IV6, although the C-terminal domain was missing from the ORF detected by Schnitzler *et al.* (1994b).

The mechanisms that control translation are poorly understood. Certain FV3 mRNAs continue to be produced after production of the relevant protein has finished. The production of antisense RNA that binds to mRNAs from early genes to prevent translation has been proposed as a way of limiting the production of early proteins (Mesnard *et al.*, 1988; Willis *et al.*, 1990). Similarly, modification of 5' termini of early mRNAs has been detected to occur later in the replication cycle. Such modifications prevented subsequent translation (Tondre *et al.*, 1988; Aubertin *et al.*, 1990). The number and context of AUG codons that signal the initiation of translation also differ among early and late mRNAs and are likely to play a role in translational control (Aubertin *et al.*, 1990).

In IV9, many immediate-early transcripts were detected originating from seven discrete locations scattered over the genome. Certain proteins were produced for the entire replication cycle; among these was probably the major capsid protein, which appeared at 4 hr postinfection together with two other possibly structural proteins. Three suspected structural proteins appeared as late as 36 hr postinfection. Overall, translation of 20 proteins was detected, of which 4 appeared and were shut off within 18 hr postinfection and 16 were still being produced at 48 hr postinfection (McMillan and Kalmakoff, 1994).

A putative apoptosis-inhibiting gene has been identified in IV6, which may be necessary to inhibit programmed cell death following viral infection. In baculoviruses, a similar gene produced a product of 30 kDa with a central zinc fingerlike structure composed of cysteines and histidine, probably with nucleic acid-binding properties. This protein effectively blocked host cell apoptosis. The homologous protein of IV6 lacked certain repeat motifs, but it has not

been tested for biological activity (Birnbaum *et al.*, 1994; Handermann *et al.*, 1992; Sonntag *et al.*, 1994).

Additional ORFs with high homology to genes with established functions have been detected in IV6, including a putative antimutator enzyme GTP phosphohydrolase, a nonhistone DNA-binding protein, another DNA-binding protein possibly involved with transcription or DNA repair, and a putative DNA or RNA helicase (Schnitzler *et al.*, 1994a; Sonntag *et al.*, 1994). A gene (*L96*) for a large, basic, and mostly hydrophilic protein has been identified in IV1. The protein is possibly involved in DNA packaging in the core. As in FV3, putative TATA and CAAT boxes were detected upstream of ATG start codons of this gene and are suspected to be involved in gene expression (Home *et al.*, 1990).

D. Particle Assembly

The sequence of particle formation has been subject to different interpretations, some stepwise, involving the sequential assembly of individual components, and others a coordinated and simultaneous process of particle organization and construction. In the stepwise process, viral DNA and nucleoprotein condensed to form the core, which was then enveloped by the capsid (Bird, 1961, 1962; Kelly and Tinsley, 1974). Others have reported face-by-face assembly of the capsid followed by entry of the core components through a hole left in the shell that was subsequently capped (Yule and Lee, 1973; Hess and Poinar, 1985). In contrast, the coordinated process involves the concurrent assembly of capsid and internal lipid membrane and the sequestering of core material from the surrounding area. Once the capsid is complete, DNA condenses to form the core proper (Devauchelle, 1977; Federici, 1980; Devauchelle *et al.*, 1985b). It seems likely that the pattern of particle assembly will be affected by a number of host- and virus-related factors, including the type, age, and condition of cell infected; stage of infection; temperature; permissiveness of host; and so forth.

Following infection by FV3, the host cytoskeleton undergoes dramatic changes in which microtubules (22–26 nm in diameter) breakdown concomitant with the appearance of large areas of granular, organelle-free, virus assembly sites referred to as *virogenic stroma* or *vioplasmic centers*. The structural integrity of these sites is maintained by intermediate filaments (7–11 nm in diameter) that reorganize from their normal position, radiating from the nucleus, and cluster around virogenic stroma. Fine microfilaments (4–8 nm in diameter) composed of actin break up and reform at the cell surface where they assist in budding and release of virus (Murti *et al.*, 1985; Murti and Goorha, 1990).

The role of the cytoskeleton in IV replication in insect cells is less clear. Cytoskeleton-disrupting drugs did not prevent the formation of virogenic stroma following infection by IV1 and immunofluorescent studies directed at microtubules and microfilaments have not indicated that these elements are associated with virogenic centers (Bertin *et al.*, 1987; Seagull *et al.*, 1985). However, nuclear matrix or associated proteins have been detected serologi-

cally in assembly sites *in vivo* in cells infected by IV1 and in assembly sites extracted and fractionated *in vitro* (Bladon *et al.*, 1986).

V. SIGNS AND CHARACTERISTICS OF PATENT DISEASE

The principal sign of patent IV infection is the iridescent hue that arises from the paracrystalline arrangement of virus particles in host cells. Light is reflected from the surface of close-packed particles and causes interference with incident light (so-called "Bragg reflections"), resulting in a range of colors, most commonly lavender, blue, turquoise, and green. The viruses from mosquitoes and chironomids commonly display colors such as yellow-green, orange, and red by virtue of their larger particle size and greater interparticle spacing (Klug *et al.*, 1959; Hemsley *et al.*, 1994). In fact, the family name, *Iridoviridae* has its origin in the Greek *iridos*, meaning "shining like a rainbow," which is an appropriate way to describe the striking appearance of patently infected individuals. Purified pellets of IV also iridesce.

An isolate from the midge, *Chironomus plumosus*, did not show iridescence in host tissues, possibly because of the presence of fibrillar structures attached to the virus capsid that may increase interparticular spacing and prevent the occurrence of Bragg reflections (Stoltz *et al.*, 1968). Iridescence has been dismissed as a trivial characteristic due solely to the physical structure of IVs (Stoltz, 1971; Kelly, 1985). There is no known adaptive advantage arising from iridescence in terms of enhanced virus transmission, for example. Iridoviruses from vertebrates do not iridesce.

Replication of IVs following injection of inoculum is not sensitive to the host life stage (larva, pupa, adult), unlike baculoviruses, for example, although natural patent infections are mainly observed in juvenile stages. However, adults are prone to lethal infections in bees, isopods, and *Tenebrio* beetles. In bees, IV infection appears to be the cause of "clustering disease" in *Apis cerana* from Kashmir and northern India. Diseased colonies become uncharacteristically inactive and form small detached groups of flightless individuals that crawl on the ground. Infected colonies perish rapidly (Bailey *et al.*, 1976; Bailey and Ball, 1978; Singh, 1979; Mishra, *et al.*, 1980).

A. Pathology

Most IVs are catholic in their tissue tropisms. Almost invariably, extensive replication occurs in the fat body and epidermis. For example, IV31 replicates in most isopod tissues, especially the epidermis, muscles and fat body, nerves, hemocytes, and in patches along the gut (Federici, 1980; Cole and Morris, 1980). The same virus appears to infect extensive tissues, including the gonads of *Thaumamermis cosgrovei*, a mermithid nematode parasite of the isopod (Poinar *et al.*, 1980). IV1 provoked the formation of epidermal tumors in *Bombyx mori*, but such pathology is not seen in other IV infections of other hosts (Hukuhara, 1964). In *Aedes taeniorhynchus* infected by IV3, the fat body, epi-

dermis, imaginal disks, hemocytes, trachea, muscle, visceral nerves, gonads, and esophagus were infected but not the remaining gut or Malpighian tubules (Hall and Anthony, 1971). A red IV from *Aedes punctor* caused the fat body to take on the appearance of grape clusters and was easily broken apart (Weiser and Zizka, 1985). Individuals with patent infections that survive to pupate characteristically show marked deformations of the pupa, particularly of the wing buds (Smith *et al.*, 1961; Carter, 1974; Stadelbacher *et al.*, 1978).

The principal pathological changes at the cellular level include cell rounding and the appearance of extensive areas of finely granulated material devoid of cell organelles—the virogenic stroma. Large numbers of particles form in close association but not generally within this zone. Most studies of the cellular pathology of IVs have focused on cell culture systems where the pathology of infection may be different from the equivalent *in vivo* changes.

Infection of mosquito and lepidopteran cells with IV2 or IV6 resulted in marked contraction of the cells after 72 hr followed by cell detachment at 96 hr, effects that were not sensitive to the multiplicity of infection and that may be caused by toxic viral proteins (Kelly and Tinsley, 1974). Rapid cell–cell fusion, however, was observed to be a function of the multiplicity of infection in cells infected by IV6. A virus protein inactivated by heat or antiserum (described in Section IVa) appears to be responsible (Cerutti and Devauchelle, 1979). The formation of numerous vesicles arising from blebbing of cell membranes followed by loss of cell adhesion and cell–cell fusion was reported in *Spodoptera* cells infected by IV1. Changes in the position and morphology of mitochondria were noted in this system and in infected isopods (Czuba *et al.*, 1994; Hess and Poinar, 1985).

B. IV Production and Quantification

Purely in terms of numbers, IVs are among the most efficient insect viruses in turning host resources into virus particles. Around 25% of the dry weight of a dead insect may be virus (Williams and Smith, 1957). With a particle weight of approximately 2×10^{-15} g (Thomas, 1961; Glitz *et al.*, 1968), predicted yields are 2.5×10^{12} particles of IV1 in a *Tipula oleracea* larva and 2×10^{12} particles of IV2 in a *Galleria mellonella* pupa (Carter, 1973b; Day and Mercer, 1964). Following the same reasoning, a *Costelytra zealandica* third instar larvae may yield 5.1×10^{12} particles of IV16, a *Phyllophaga anxia* larva may yield 2.5×10^{12} particles, a *Heteronychus arator* larva may yield 1.75×10^{12} particles, a *Tenebrio molitor* pupa may yield 1.5×10^{10} particles, and field-collected *Armadillidium vulgare* and *Porcellio scaber* are predicted to yield 2.6×10^{10} particles and 3.1×10^{10} particles, respectively (estimates from data in Moore, 1973; Poprawski and Yule, 1990; Longworth *et al.*, 1979; Black *et al.*, 1981; Cole and Morris, 1980). Obviously these calculations make unsupported assumptions about the efficiency of virus replication and the composition of insect and virus samples. These estimates, however, do emphasize the great productivity of IVs within invertebrate hosts.

IVs may be quantified by plaque assay, insect bioassay, optical density, direct counting, and, to a degree, by enzyme-linked immunosorbent assay (ELISA). Czuba *et al.* (1994) reported reliable quantification of IV1 by plaque assay in *Spodoptera* cells. There was a linear relationship between the production of plaque-forming units (PFU) and the DNA content of viral inoculum at multiplicities of infection between 0.1 and 1000. Optical density measurements at 260 nm indicated that from one A_{260} unit it was possible to obtain 10^5 PFU or 11 μg of DNA which would be equivalent to approximately 4.6×10^6 IV particles (assuming a mean genome size of 200 kbp). Direct counting using an electron microscope indicated that four to six times more particles were actually present per A_{260} , probably because many particles in these preparations contained little or no DNA. The efficiency of infection was low; 0.1–0.3% of IV1 particles produced virogenic stroma in host cells, and of these less than 1% went on to produce a visible plaque. From these data, the formation of one PFU requires 4×10^5 filled particles of IV1. However, values as low as 75 particles per PFU were reported for IV22 in *Spodoptera* cells using virus freshly extracted from *G. mellonella* (Brown *et al.*, 1977). The reason for such differences may reside in the different isolates used, or in the passage history of each virus.

IV2 was quantified by optical density and direct counting was used to calculate the number of particles required to produce a patent infection in *G. mellonella* larvae. One A_{260} unit of IV2 was equivalent to 1.8×10^9 particles by direct counting. Lethal infections were achieved by injection of between 1.7 and 6.9 particles into final instar *G. mellonella*. In these studies, empty particles lacking DNA were rare (Day and Mercer, 1964; Day and Gilbert, 1967). The insect bioassay, involving calculation of a median lethal dose or infective dose value (LD_{50} or ID_{50}) following injection of *G. mellonella* larvae, may be one of the most sensitive methods of IV quantification (Carter, 1973c; Witt and Stairs, 1976; Ohba and Aizawa, 1978).

Ward and Kalmakoff (1991) suggested that ELISA could be used to give a semiquantitative estimate of the amount of virus present in a sample. Purified virus was used to raise polyvalent antiserum in rabbits. Field-collected mosquitoes were individually homogenized, fixed into microtiter plate wells, reacted with rabbit antiserum followed by horseradish peroxidase-labeled sheep anti-rabbit antibody, and read in an ELISA reader. The virus concentration was then estimated by reference to a standard curve.

Virus purification by sucrose gradient centrifugation resulted in disruption of virion structure and subsequent degradation of an orthopteran IV. Use of Ficoll in place of sucrose avoided this problem (Boucias *et al.*, 1987).

C. Dose–Response Relationships and Survival Time

Study of the dose–response relationships of IVs generally has not been performed in a highly quantitative manner, and the “response” criterion has always been patent iridescence or mortality of the host. Many studies report that large doses are required to achieve patent infection by *per os* inoculation

(Carter, 1973a; Linley and Nielsen, 1968a; Federici, 1980; Sieburth and Carner, 1987) although high doses may also result in rapid paralysis and death prior to extensive virus proliferation (Stadelbacher *et al.*, 1978), probably due to the action of cytotoxic viral proteins (Aubertin *et al.*, 1977; Lorbacher de Ruiz, 1990).

The dose-response relationship has been shown to be sensitive to temperature: The LD₅₀ value of IV1 injected into *G. mellonella* larvae was three orders of magnitude higher at 30°C compared to the LD₅₀ value at 20°C (Witt and Stairs, 1976). The oral LD₅₀ of IV6 in the boll weevil *Anthonomus grandis* was 3.61×10^{-6} larval equivalents of *G. mellonella* (McLaughlin *et al.*, 1972), which, assuming a similar productivity to IV2 in the same host (Day and Mercer, 1964), would be roughly 7×10^6 particles. The LD₆₃ of IV1 and IV2 by injection into lepidopteran and dipteran larvae ranged between 6.0 and 5×10^7 particles, depending on virus and host species (Glitz *et al.*, 1968).

Survival times reported following *per os* doses range from about 45 days for IV1 in *Tipula oleraceae*, about 35 days for IV31/IV32 in isopods, about 28 days for IV30 in *Helicoverpa zea*, about 21 days for an IV of the mole cricket *Scapteriscus borellii*, and as little as 6 days for an IV of *Anticarsia gemmatalis* (Carter, 1973a; Grosholz, 1992; Sikorowski and Tyson, 1984; Fowler, 1989; Sieburth and Carner, 1987). Late instar larvae developed disease faster than younger conspecifics (Carter, 1974; Sieburth and Carner, 1987), which contrasts with the pattern shown by other insect viruses that tend to kill younger, smaller hosts more quickly than older, larger ones. Compared to feeding, injection of inoculum may result in a 30–50% reduction in survival time and a reduction in the variability of survival times (Carter, 1973a,b; Federici, 1980; Cole and Morris, 1980).

Infected hosts are reported to be more sluggish than healthy conspecifics but may continue to live several weeks after developing iridescent coloration (Linley and Nielsen, 1968a; Poprawski and Yule, 1990). IV-infected soil-dwelling insects have been reported to move to near the surface prior to death where the probability of transmission by cannibalism or predation may be enhanced (Fowler and Robertson, 1972).

VI. RELATIONSHIPS AMONG IVs

Relationships among the members of the *Iridoviridae* are not well understood currently and the need for comparative studies of these viruses has been stated repeatedly (Hall, 1985; Kelly, 1985; Willis, 1990; Ward and Kalmakoff, 1991; Schnitzler and Darai, 1993; Stohwasser *et al.*, 1993).

A. Serology

The principal tools for comparative studies in the 1960s and 1970s were serologically based analyses involving a range of different IVs, although DNA renaturation and SDS-PAGE also were used occasionally (Kelly and Avery,

1974; Carey *et al.*, 1978). Serological relationships were summarized by Kelly *et al.* (1979), who classified isolates into two main groups. One large group comprised the majority of interrelated isolates (IV1, IV2, IV9, IV10, IV16, IV18, IV21, IV22, IV23, IV25, IV28), some of which were almost indistinguishable from one another by serology (e.g., IV9 and IV18, IV21 and IV28). In contrast, IV6 and IV24 were classified as serologically unrelated to other IVs or to one another. IV29 was placed in between these groups as being related to some but not all of the principal serogroup. The isopod virus IV31 showed a low cross-reactivity to the antiserum of IV1 and was considered to be distantly related to the main serogroup (Cole and Morris, 1980). Viruses from other genera, IV3, FV3, and lymphocystis disease virus type 1 (LCDV-1), showed no serological relatedness to IVs in the *Iridovirus* genus or to one another (Cunningham and Tinsley, 1968; McAuslan and Armentrout, 1974).

B. Genetic

Fueled by the need to clarify the interrelationships among these viruses, genetic analyses have recently been published. Comparative studies were performed involving 14 IV isolates plus FV3 and LCDV-1, the type species from the two vertebrate genera (Williams and Cory, 1994; Williams, 1994). Each isolate was subject to restriction enzyme analysis and the resulting gels were Southern blotted and probed with a fragment of the MCP gene of IV22 under different stringencies of hybridization.

From the restriction profiles it was immediately apparent that several isolates were strains of the same virus (Fig. 2a). Coefficients of similarity were calculated by pairwise comparison of the proportion of similar-sized restriction fragments shared by isolates. Coefficient values were particularly high among IV9, IV10, and IV18 (up to 91.5%) and for comparison of IV21 and IV28 (up to 94.3%). In contrast, IV3, FV3, and LCDV-1 showed no restriction profile similarities to any other isolates or to each other.

By Southern blot analysis at high stringency, the IV22 MCP gene probe consistently hybridized to a subset of the isolates, namely IV1, IV2, IV9, IV10, IV18, IV22, IV24, IV29, and IV30 (Fig. 2b). When blots were probed at lower stringency, hybridization to comigrating fragments was evident for three additional isolates: IV6, IV21, and IV28 (Fig. 2c). Hybridization of the probe to IV31 was weak but fairly consistent at low stringency. Only in one *Sa*II blot did the probe show affinity to IV3. Probe hybridization to FV3 or LCDV-1 was not detected. Polymerase chain reaction (PCR) amplification of an MCP gene fragment using primers derived from the IV22 MCP gene sequence provided additional support for the results of the Southern blot analysis.

DNA-DNA dot-blot hybridization measurements were also consistent with the results of restriction endonuclease and Southern blot analyses. One large group of interrelated isolates was detected showing relative hybridization values of between 10 and 91% at the intermediate stringency conditions employed (Fig. 3). The highest levels of hybridization were detected among IV9, IV10, and IV18; so clearly they are strains of the same virus. A second, smaller

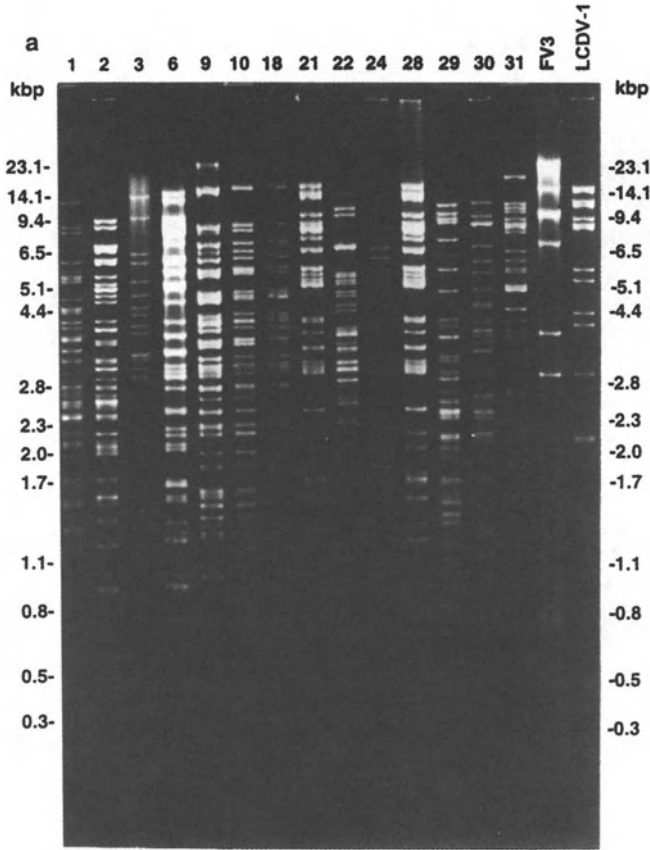


FIGURE 2. (a) *Hind*III restriction endonuclease profiles of DNA from IVs identified by type number and two vertebrate isolates, FV3 and LCDV-1, showing clear similarities among IV9, IV10, and IV18 and between IV21 and IV28. (b) Autoradiograph following hybridization of an MCP gene probe from IV22 to a Southern blot of the *Hind*III gel under conditions of high stringency. Hybridization of the probe was only detected to one large group of the IVs tested. (c) Autoradiograph following hybridization of the IV22 gene probe under conditions of low stringency, showing detectable hybridization to comigrating fragments of IV6, IV21, and IV28 and weak hybridization to IV31. No hybridization was detected between the probe and IV3 or either of the vertebrate viruses tested.

group of related isolates was also apparent; these are IV6, IV21, and IV28, with extremely high hybridization between IV21 and IV28 (also strains of a single virus). IV31 appeared to be only distantly related to the other IVs by hybridization and IV3 even more so. The vertebrate viruses, FV3 and LCDV-1, consistently failed to hybridize to any of the invertebrate isolates or to one another.

From these results a clear pattern emerged involving three discrete complexes of interrelated isolates. This genetic analysis was in close agreement with previous serological findings with the exception of the placement of IV24 and IV21/IV28. Consequently, the largest complex representing a total of

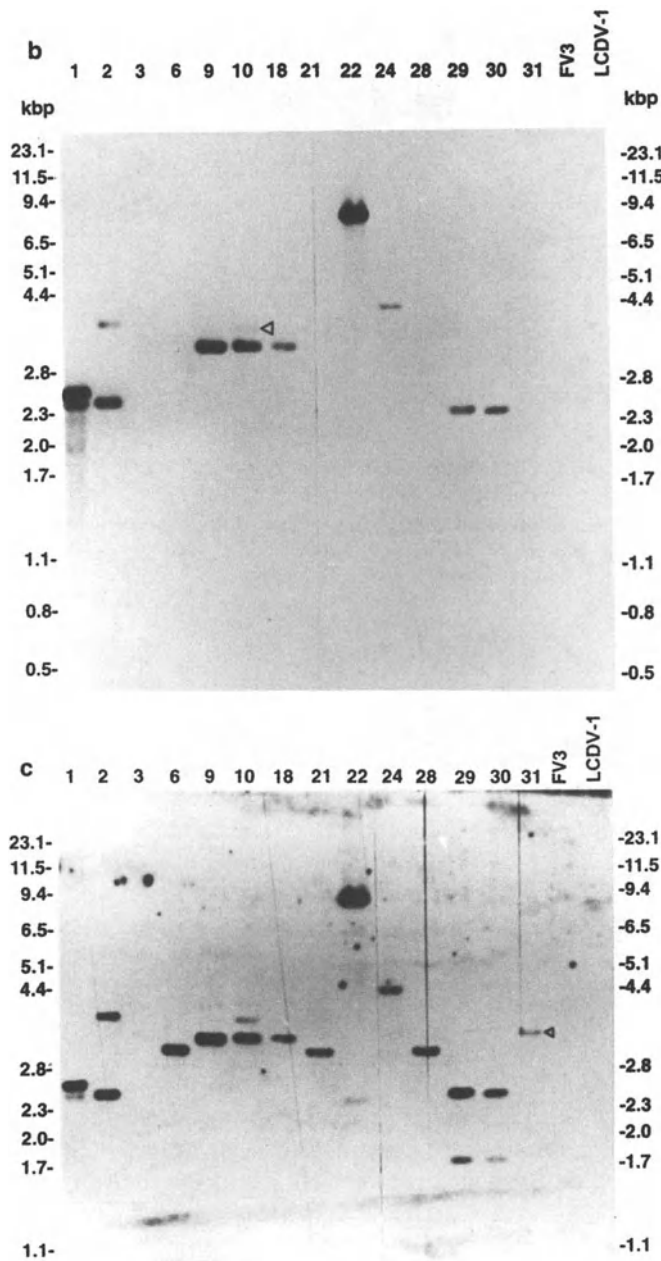


FIGURE 2. (Continued).

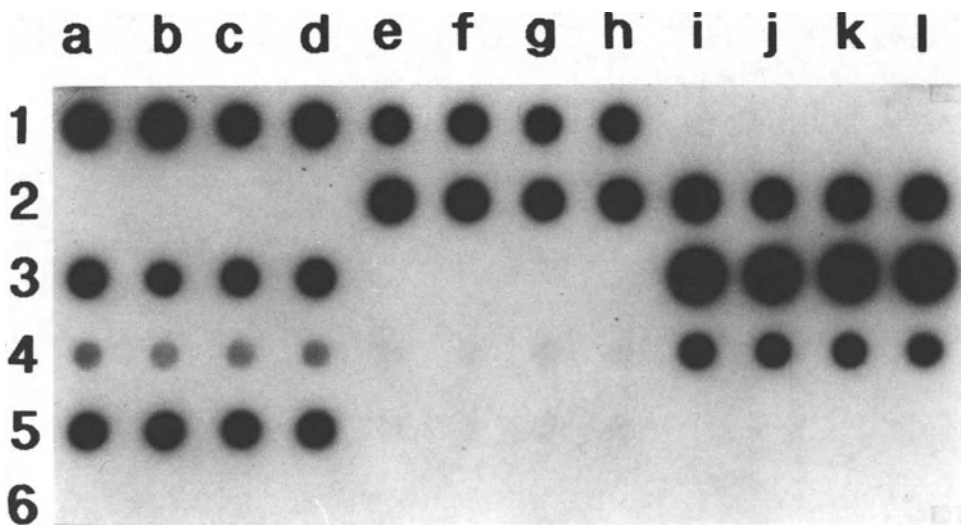


FIGURE 3. Autoradiograph of DNA-DNA dot-blot hybridization of ^{32}P -labeled genomic DNA of IV22 to four replicate dots of IV1 1a-1d; IV2 1e-1h; IV3 1i-1l; IV6 2a-2d; IV9 2e-2h; IV10 2i-2l; IV18 3a-3d; IV21 3e-3h; IV22 (homologous) 3i-3l; IV24 4a-4d; IV28 4e-4h; IV29 4i-4l; IV30 5a-5d; IV31 5e-5h; FV3 5i-5l; LCDV-1 6a-6d; Salmon sperm DNA 6e-6h. Hybridization was only detected toward members of the Polyiridovirus complex.

nine viruses (13 isolates) was named the Polyiridovirus complex. The second smaller group of isolates comprising IV6 and IV21/IV28 were assigned to a separate complex, the Oligoiridovirus complex. Finally, IV31 from terrestrial isopods was assigned as the sole member of a third complex: the Crustaceo-iridovirus complex (Fig. 4). Subsequent characterization of *Anticarsia gemmatalis* IV indicated it was an additional member of the Polyiridovirus complex (Williams, 1994; Kinard *et al.*, 1995). Serological and some genetic data suggest that IV16 and IV23 merit inclusion as probable members of the Polyiridovirus complex (Kalmakoff, *et al.*, 1972; Longworth *et al.*, 1979; McMillan *et al.*, 1990). IV32 was previously demonstrated to be the same virus as IV31 by restriction endonuclease profile (Cole and Morris, 1980).

The results indicated that no change was necessary to the status of the genus *Chloriridovirus* (type species IV3) or with respect to the vertebrate viruses, FV3 and LCDV-1, as the type species of two distinct vertebrate genera. Previous DNA studies supported the concept that the vertebrate viruses were not closely related to their invertebrate counterparts (Bellett and Fenner, 1968; Darai *et al.*, 1983).

C. DNA Sequence Analysis

Stohwasser *et al.* (1993) used PCR primers targeted at highly conserved sequences of the MCP gene to amplify a fragment (506 bp) of the gene of IV6.

Genus: <i>Iridovirus</i>			Genus: <i>Chloriridovirus</i>
Polyiridovirus Complex	Oligoiridovirus Complex	Crustaceoiridovirus Complex	
IV1	IV6	IV31/IV32	IV3
IV2	IV21/IV28		
IV9/IV10/IV18			
IV16			
IV22			
IV23			
IV24			
IV29			
IV30			
<i>Anticarsia gemmatilis</i> IV			

FIGURE 4. Hybridization complexes within the *Iridovirus* genus reported by Williams and Cory (1994).

This product was labeled and used to probe a genomic library. Fragments showing hybridization to the probe were sequenced to find the ORF for the MCP gene, and thereby obtain a putative amino acid sequence for the capsid protein. Amino acid sequence alignments reveal that the MCP of IV1 and IV22 is identical in all but a short section of the C-terminus. The amino acid sequence identity between IV1 or IV22 and IV6 is 64.7% (Tajbakhsh *et al.*, 1990a; Cameron, 1990; Stohwasser *et al.*, 1993).

Kinard *et al.* (1995) used PCR to amplify a 500-bp fragment of the MCP gene from IV21 and *Anticarsia gemmatilis* IV (AgIV). Direct sequencing of the PCR product followed by alignment of the deduced amino acid sequences revealed 100% sequence identity between IV21 and IV6. Amino acid sequence identities were 97.3% between AgIV and IV1 and 58.0% between AgIV and IV21 or IV6.

Apparently, the MCP of IV16 from *Costelytra zealandica* is also quite similar to those of IV1 and IV22, although details have not yet been published (Davison *et al.*, 1992). All sequence comparisons performed to date agree with the pattern of interrelationships described previously (Williams and Cory, 1994; Williams, 1994) in that AgIV and IV16 are related to IV1 and IV22 (Polyiridovirus complex) and IV21 and IV6 are closely interrelated but are distinct from other IVs (Oligoiridovirus complex).

The MCP of FV3 showed 47–49% amino acid sequence identity to IV1, IV6, and IV22. Similarly, these IVs showed a 49–53% amino acid sequence identity to the fish iridovirus LCDV-1 (Mao *et al.*, 1996; Schnitzler and Darai, 1993). Such comparisons support the concept that the vertebrate isolates are distinct from the invertebrate IVs and should be assigned to separate genera within the family.

D. Biochemical

Williams and Thompson (1995) reasoned that the lipid composition of IVs may be a useful indicator in comparative studies. Eight IVs grown in the same host, *G. mellonella*, were purified and analyzed for their fatty acid composition by high-pressure liquid chromatography (HPLC). Viruses fell into two main groups: one comprising IV1, IV2, IV9, and IV31; the other comprising IV21, IV22, IV30, and *Anticarsia gemmatilis* IV. Analysis of fatty acid composition was not consistent with genetic or serological findings and seems to be of little use for comparative studies.

VII. ECOLOGY

Ecological studies of IVs are sparse, probably because the incidence of patent disease is typically very low. Even fundamental aspects of the virus–host relationship, such as the route of infection, remain poorly understood. Hopefully, this may change following new information on patterns of IV infection in certain host populations and the gradual adoption of molecular techniques to study invertebrate pathogens.

Ecological studies on all entomopathogenic viruses are faced with two problems: detection and identification of the pathogen. However, the majority of studies on IVs use only iridescence as the criteria for diagnosing infection; inapparent infections go undetected. Some have used, or proposed to use, serological or nucleic acid hybridization techniques to detect inapparent infections, with generally favorable results (Carter, 1973c, 1974; Kelly *et al.*, 1978; Ward and Kalmakoff, 1991; Tesh and Andreadis, 1992), while insect bioassay and PCR have also been employed successfully (Williams, 1993, 1995).

A. Transmission

1. Route of Infection

The route of infection is unknown or uncertain for most IV–host systems. Moreover, the often low incidence of patent disease following ingestion of inoculum has led to speculation that IVs depend on other mechanisms for their transmission. Cannibalism or predation has been highlighted as a probable route by which massive doses of IVs could be ingested, and indeed it appears to be the principal mechanism of transmission in populations of mosquitoes, isopods, tipulids, and mole crickets (Linley and Nielsen, 1968b; Federici, 1984; Carter, 1973b; Fowler, 1989). Carter (1973a) detected the presence of IV1 in the feces of patently infected *Tipula* larvae, but at a concentration insufficient to cause patent infection in conspecific larvae following a *per os* challenge. Similarly, infective IV6 was detected in the meconium of adult *Bombyx mori* that had been infected by injection as larvae (Ohba, 1975). *Per os* inoculation of

Helicoverpa zea and *Anticarsia gemmatilis* larvae or mole cricket nymphs, however, resulted in numerous patent infections (Sikorowski and Tyson, 1984; Sieburth and Carner, 1987; Boucias *et al.*, 1987).

The fact that IVs are highly infective when injected may mean that parasites or parasitoids could potentially vector the virus between hosts. Hess and Poinar (1985) mentioned two unpublished reports in which IV31/IV32-infected isopods appeared more prone to infection by nematodes. Particles also may gain entry to the host via cuticular abrasions or when the host integument is vulnerable, for example, at molting.

Electron microscope studies have observed that IV3 particles are degraded in mosquito larvae shortly after entering the midgut; intimate contact between virus and midgut cells was prevented by the peritrophic membrane. This led to speculation that the virus relies on physical breaks in the peritrophic membrane or that viral DNA released from degraded virions is itself infective (Stoltz and Summers, 1971; Hall and Anthony, 1971). Virus particles have been observed in a midgut epithelial cell following consumption of IV3 by mosquito larvae. The cell was located at the very anterior of the midgut where the peritrophic membrane is produced and is relatively thin (Hembree and Anthony, 1980).

Undeen and Fukuda (1994) reasoned that damage to the peritrophic membrane should enhance the probability of infection in mosquitoes. To test this, *Ae. taeniorhynchus* larvae were exposed to IV3 in the presence or absence of silicon carbide fibers. The incidence of the resulting patent disease rose from 4.8% with virus alone to 17.5% in the presence of fibers. Moreover, when larvae were shaken in the presence of fibers to provoke external damage to the cuticle, no discernible effect on the probability of transmission was detected, suggesting that the gut rather than the external integument is indeed the principal route of entry of the virus.

2. Transmission

Alternating horizontal and vertical transmission has been proposed as the mechanism by which IV3 persists in *Ae. taeniorhynchus* populations. Larvae develop patent disease and usually die in the fourth instar just prior to pupation. Infected cadavers often remain suspended from the water surface and are prone to cannibalism. This results in horizontal transmission to early and late instars alike, although their respective fates are stage-dependent: early instar larvae go on to develop patent infections and die in the fourth instar, whereas when larvae are infected as late instars, pupation and adult eclosion proceed as normal (Linley and Nielsen, 1968a,b). Female mosquitoes infected in this manner appear capable of transmitting the virus to between 19 and 46% of their progeny (Woodard and Chapman, 1968). Progeny larvae develop normally until the third or fourth instar, when the symptoms of disease become manifest and the larvae die, leading to a further cycle of horizontal transmission to early and late instar conspecifics.

The pattern of vertical transmission has been observed to be highly aggregated; patently infected progeny appeared in the broods of just 8% of the fe-

males that had been infected as late instar larvae. However, 100% of the larvae from these broods showed patent infections (Linley and Nielsen, 1968a), although others have indicated that vertical transmission of IV3 may not be so efficient (Fukuda and Clark, 1975). Clearly inapparent infections are common in this system, although the incidence of vertical transmission resulting in covert infection of progeny larvae is not known.

No evidence for intrinsic (genetic) resistance to IV3 was found when the progeny of individual *Ae. taeniorhynchus* females were challenged with IV3 and compared, in terms of variation in susceptibility, to an experimental population (Undeen and Fukuda, 1994). There also was no evidence that serial passage of IV3 in 30 generations of cultured mosquitoes increased the virulence of the pathogen (Woodard and Chapman, 1968).

One report notes that the transmission of IV3 may be sensitive to the presence of other host pathogens. The incidence of infection of *Ae. taeniorhynchus* by IV3 was between two and three times higher when a mosquito picornavirus was present in the inoculum. Moreover, the titer of each virus recovered from infected larvae was higher when the other virus was also present. The picornavirus was vertically transmitted and persisted as a low-level asymptomatic infection in the insect culture. It appears that the picornavirus enhanced both the transmission and replication of the iridescent virus (Wagner *et al.*, 1974).

In *Porcellio scaber* (Isopoda) populations infected with IV31/IV32, increasing intraspecific competition by doubling the host density resulted in an insignificant increase in the incidence of disease (from approximately 7.5 to 11%). However, interspecific competition arising from the introduction of an equal density of *Porcellio laevis* caused a significant increase in percentage infection (to approximately 22%). *P. scaber* mortality was highest in treatments where *P. laevis* was present and the availability of food was restricted. This may have provoked high levels of interspecific aggression (wounding) and predation-cannibalism, resulting in enhanced virus transmission. Vertical (transovarial) transmission in this system is not suspected to occur, as virtually all infections are patent and lethal and infected gravid females abort their offspring. Nematodes were absent or extremely rare in this study (Grosholz, 1992).

B. Persistence

1. Outside the Host

The ability of IVs to persist outside of a host is not well understood. It has been stated that IVs show marked stability in water and that virus particle structure may be responsible for this, particularly the internal lipid membrane (Kelly, 1985). However, there are few data that support this assertion. The ability of IV2 to produce patent infections in *G. mellonella* fell by 50% after 32 days at 4°C (Day and Gilbert, 1967).

IVs may decay rapidly in the environment. Using patent infections as the indicator, the infectivity of IV3 to mosquito larvae fell markedly after 2 days in

fresh or brackish water at 27°C and was generally undetectable after 5 days, although some infectivity was detected after 20 days in one trial. When inoculated onto damp soil, the virus was almost completely inactivated within 24 hr (Linley and Nielsen, 1968b). Virus in the cadavers of isopods remained infective for up to 5 days at ambient laboratory temperatures (Grosholz, 1993). IV6 incorporated into a bait gradually lost infectivity when placed outside over 14 days and was inactivated more rapidly when sprayed on cotton plants (McLaughlin *et al.*, 1972).

2. In Host Populations: How Virulent Are IVs?

The persistence of pathogens in host populations is intimately related to the degree of virulence and the strategies of transmission adopted (Ewald, 1995; Lipsitch *et al.*, 1995). It has recently become apparent that IVs can differ in their degree of virulence. ELISA tests on isopods indicated that >99% of infections were patent and lethal (Grosholz, 1992). Likewise, *Helicoverpa zea* larvae inoculated *per os* with IV30 only developed patent infections; larval homogenates bioassayed in *G. mellonella* gave no evidence of sublethal infection (T. Williams, unpublished data). Certain IVs have been reported to cause epizootics of disease, including IV30 in *H. zea*, the IV from *Anticarsia gemmatalis*, and an IV from *Scapteriscus borellii*. In northwest Russia, 130 ponds were studied over a 2-year period. Patently infected mosquito and dixid larvae were found in just one, but the incidence of infection was 100% (Fedorova, 1986).

In contrast, inapparent and nonlethal infections have been detected in a population of the blackfly *Simulium variegatum*. Homogenates of blackfly larvae caused patent disease when injected into *G. mellonella*. This bioassay technique was used to reveal the presence of abundant covert infections in the springtime population in the River Ystwyth, Wales. The incidence of covert infection at three sites varied between 17 and 37% in March and fell to 0–20% in April. In May, a small number of patently infected larvae were found. Monitoring of this population continued for the summer months, but the incidence of covert and patent infection remained at virtually undetectable levels. In September, covert infections reappeared at levels of up to 20%. Laboratory rearing of larvae confirmed that the covert infections detected by bioassay were indeed inapparent and nonlethal, and not simply an early stage of what would later develop into overt lethal disease (Williams, 1995).

Covert infection was confirmed using a nested PCR technique with two sets of primers targeted at G/C-rich sequences of the major capsid protein gene. Treatment with *Xho*I was used to verify the product identity by demonstrating the presence of a restriction site reported by Cameron (1990) one third the way along this amplicon (Williams, 1993).

The covert infections of blackflies did not appear to be latent in the sense generally used for insect viruses (e.g., Hughes *et al.*, 1993), because the infection could be experimentally transmitted to another insect by injection. Probably the infection persists as particles within certain host cells but at a low density.

The blackfly–IV story is also intriguing from the standpoint of virus identity. Restriction endonuclease and Southern blot techniques were used to ana-

lyze the genetic relationships among the various isolates detected in blackfly larvae. Three types of virus could be distinguished genetically, two of which occurred in both patently and covertly infected larvae and a third that was observed only as a covert infection. Such was the magnitude of the differences in the restriction profiles and the degree of hybridization to an MCP gene probe that the three types were tentatively suggested to be three distinct IV species. The isolates detected in the spring were clearly strains of IV22, the virus reported from blackfly larvae in the same river two decades earlier (Batson *et al.*, 1976). The isolates detected in the autumn were all strains of a different virus that had been isolated from a patently infected larva in September of the previous year. The third virus appeared to be unlike any other isolate.

The fact that strains of the same virus were found producing covert and patent disease indicates that these viruses may show different grades of virulence in different individuals. Moreover, in all cases a marked degree of genetic heterogeneity was observed among the various isolates analyzed; identical isolates were never recovered from two different host larvae (Williams and Cory, 1993).

Recently, Marina *et al.* (1998) reported the replication of IV6 in *Aedes aegypti* larvae in the absence of patent disease. Exposure to virus inoculum resulted in extended juvenile development times; adult female fecundity was also reduced and showed increased variability. Males appeared capable of passing virus to uninfected females during the mating process, in contrast to previous observations based on patent infection (Hembree, 1979). After death, female mosquitoes were bioassayed and classified as "covertly infected" or as "uninfected survivors" of an inoculum challenge. Covertly infected females were smaller and had shorter lifespans than control or virus-challenged females. A conservative estimate for the reduction in the net reproductive rate (R_0) of such insects was calculated at slightly more than 20% relative to controls.

IV infections of isopods were endemic at population densities of over 2000 individuals/m², but were absent in low-density populations nearby (Grosholz, 1992). Host density and spatial heterogeneity in host populations were seasonally influenced. During the dry months of late summer in California, isopod populations were highly aggregated; the within-patch density was high and the distance between patches was large. The incidence of disease during this period was 1–2%. Within-patch density fell and the spatial structure of the host populations broke down somewhat during the wetter months of winter and spring. These conditions appeared more suitable for virus transmission and the prevalence of disease rose to 13%. The probability of infection was not dependent on the sex or size of hosts, but species was significant. The incidence of infection was highest in *Porcellio laevis* (5.7% overall) followed by *P. dilatatus* (4.6%), *P. scaber* (2.7%), and *Armadillidium vulgare* (1.6%). Experiments in which the spacing between patches was manipulated showed that the probability of isopod dispersal was negatively correlated with interpatch distance. The prevalence of disease was highest when interpatch distance was minimal. These observations indicate that IV disease was more frequent when the isopods could disperse freely among available patches but fell when the population

structure became highly aggregated and isopods tended to stay within their patch (Grosholz, 1993).

The incidence of infection in *Tipula* populations also appeared to be correlated with host density and spatial heterogeneity. Damp soil attracted host densities twice as high as dry soils. An epizootic of IV infection developed over the course of a 2-year study until the incidence of infection reached 90% and the host population crashed. The number of patently infected tipulid larvae was ten times higher in moist compared to dry habitats (Ricou, 1975).

C. Host Range

1. Laboratory Studies

The range of host species in which IVs may replicate depends very much on the route of infection. Most of the IVs tested have shown remarkably broad host ranges when the inoculum is administered by intrahemocoelomic injection compared to a reduced host range following a *per os* challenge.

IV6 has been most studied in this respect and has been shown to cause patent infections in numerous species from the major insect orders, including species of agricultural and medical importance (Fukuda, 1971; Ohba, 1975) and a number of other arthropods, including terrestrial isopods (woodlice) and a centipede (Ohba and Aizawa, 1979). Experiments wherein the inoculum is injected tell us little, apart from the fact that once inside a host, many IVs can replicate and cause lethal infections. Moreover, such studies can be misleading when emphasis is placed on biocontrol of pest species that have been infected in this manner (Mitsuhashi, 1967; Jensen *et al.*, 1972; McLaughlin *et al.*, 1972), leading to the interpretation that IVs have a potential for control in situations where they do not.

Occasionally, accidental transmission of IVs during laboratory experiments has been observed between overtly different hosts. For example, Federici (1984) reported the transmission of IV31/IV32 from isopods to *Acrolophus* sp. (Lepidoptera) breeding in the detritus of isopod-rearing jars. Fowler (1989) observed devastating epizootics of IV disease in termite colonies that had inadvertently fed on filter paper contaminated by an IV from the mole cricket *Scapteriscus borellii*.

Most IVs can be grown in the standard laboratory host, *Galleria mellonella* (Lep.: Pyralidae). However, IV16 from the scarabid *Costelytra zealandica* is highly specific in its host requirements. For this virus, one additional natural host species is suspected to exist, but attempts to infect other insects or to infect insect cell lines have all failed (N. McMillan, personal communication). Likewise, of the ten mosquito species challenged by immersion in a suspension of IV3 from *Ae. taeniorhynchus*, only *Ae. sollicitans*, *Ae. vexans*, and *Ae. nigromaculis* developed patent infections, usually at very low frequencies (Woodard and Chapman, 1968; Fukuda and Chapman, 1973). IV3 and the bee virus, IV24, also fail to replicate in *G. mellonella*.

A few studies have attempted to determine host range given asymptomatic infection. Ward and Kalmakoff (1991) reported that of 11 insect species challenged by injection of IV9 from *Wiseana* spp. (Lepidoptera), dot-blot DNA hybridization could be used to detect viral replication in seven species prior to or in the absence of iridescence of host tissues. Of these seven, virus was reisolated from just five, suggesting that DNA hybridization is a sensitive indicator of virus replication.

Serological evidence was presented for asymptomatic infection of mosquitoes, sandflies, and a triatomid bug following injection of IV22 from *Simulium variegatum*. Indirect fluorescent antibody technique demonstrated the presence of virus antigen in head and abdominal squashes. The quantity of antigen was proportional to the interval between inoculation and testing. Electron microscopy of one mosquito species showed particles scattered irregularly throughout the cytoplasm of host cells, not in the crystalline arrays required to induce iridescence of host tissues (Tesh and Andreadis, 1992). It is clear that accurate appraisal of IV host range requires diagnosis of infection on grounds other than patent infection alone.

2. Host Range in Nature

Certain IVs appear to exploit multiple hosts in their natural habitat. Soil-dwelling insects in New Zealand appear to share a common IV pathogen in the example presented earlier of IV9/IV10/IV18 from two lepidopteran and one coleopteran species. Similarly, relationships have been inferred for IV16 from the scarabids *Costelytra zealandica* and *Odontria striata*, a mosquito IV infecting *Ae. antipodeus* and *Ae. subalbirostris*, and probably for IV22 from *Simulium variegatum* and a tipulid in the United Kingdom. These host ranges are based on the tentative identification of strains of a single virus in different hosts (N. McMillan personal communication; Elliott *et al.*, 1977; Anderson, 1983; Williams and Cory, 1994).

It has also been suggested that at least eight North American woodlice (Isopoda) species, representing six different genera, and the nematode *Thaumamermis cosgrovei* are host to IV31/IV32 (Cole and Morris, 1980; Poinar *et al.*, 1980; Schultz *et al.*, 1982; Grosholz, 1993). Alternative hosts have also been suspected as providing a source of infection in Japanese beetle, *Popillia japonica*, populations. About 1 in 10,000 beetle larvae were patently infected, an incidence at which, the authors argued, the disease could not persist unless it originated from another soil arthropod (Lacey and Adams, 1994).

VIII. USE OF IRIDESCENT VIRUSES IN BIOCONTROL

Virtually every biocontrol-orientated research article or review of IVs arrives at the conclusion that they have little real potential as biocontrol agents. Field trials have confirmed laboratory and field observations of the low infectivity and slow speed of kill of IVs.

For control of *Tipula* spp., four treatments of IV1 were applied to soil plots in northern England: virus formulated as a spray or a bran bait and 50 infected *Tipula* larvae either living or dead. The resulting incidence of infection was highest in the sprayed plots (4.4%) and lowest in the baited plots (2.1%), with release of infected larvae giving intermediate values (Carter, 1978).

Trials with IV3 for control of *Ae. taeniorhynchus* were performed using pans to isolate experimental larvae from the natural population. Each pan received between 1 and 100 patently infected mosquito larvae either living or macerated. Experimental larvae were challenged as fourth instars and collected after pupation and allowed to emerge and mate. The incidence of patent infection in the progeny of these larvae was related to the concentration of the inoculum, but never exceeded 10% (Linley and Nielsen, 1968b).

The results of plastic tub trials of IV9 for control of *Wiseana* spp. were so disappointing that subsequent field trials were not performed. Artificial infestation of the tubs with *Wiseana* eggs followed by the application of virus sprays to grass tufts at three concentrations (up to 10^9 particles/m²) resulted in no patent infected larvae. The possibility of inapparent infections was investigated by electron microscopy and serological testing, with no positive results (Moore *et al.*, 1974).

Moderately high levels of infection have been achieved in boll weevils, *Anthonomus grandis*, following the application of IV6 as a bait spray to cotton plants in field cages. When leaves were collected and bioassayed by feeding to laboratory weevils, 47.5% became patently infected from the leaves collected after 1 day, falling to 11.8% infected from the leaves collected after 5 days. After 19 days, a collection of weevils from the field showed no patent infections, but a blue pellet was obtained following trituration and centrifugation, suggesting a sublethal infection in apparently all the test insects. In a repeat experiment, the incidence of infection was about half that observed in the initial trial. The bait formulation appears to have provided considerable protection against inactivation of the virus by desiccation and UV irradiation (McLaughlin *et al.*, 1972).

Perhaps the only real hope for the successful use of IVs in insect biocontrol lies in the development of formulations that augment the infectivity of the virus (e.g., by interacting with other pathogens), through the detection of marked sublethal effects arising from covert infections, or via the discovery of factors that trigger covert infections to become patent and lethal. If covert infections by IVs are common, then these viruses are likely to be more infective than is currently believed. Given such a scenario, it may be possible to insert a host-killing gene into the virus genome to produce an IV bioinsecticide, particularly for the control of insect vectors with aquatic stages and possibly soil pests. However, the true host range, persistence, and transmission mechanisms of IVs need to be clearly defined before the feasibility of using modified IVs could be determined.

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