INSECT VIRUSES DIVERSITY, BIOLOGY, AND USE AS BIOINSECTICIDES

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Summary

Viruses are often considered non-living things because they lack some of the basic characteristics of life. However, viruses share important features with the living things, such as their chemical complexity, the need to reproduce, and the ability to evolve. Still, viruses are totally dependent on living things as they are obligate pathogens. Viruses are known for their ability to cause disease and death in practically all known living things, from microorganisms to plants and animals, including humans. However, viruses are beneficial to humans in some instances. One example of this is the relationship that viruses have with insect. Insects are also attacked by a great diversity of viruses, and frequently their infection may cause the death of the infected individuals. Many insect

species are called pests because they cause damage to humans, most frequently as competitors for the food that humans grow. Many of these pests are also susceptible to viral infections and hence, these viruses can be used as biological control agents. Although there is a great diversity of insect viruses, only a few are frequently observed in insect populations, such as the ascovirus, iridovirus, polydnavirus, baculovirus, cypovirus, entomopoxvirus; and among these, only few show potential to be used as control agents, mainly the baculoviruses. This chapter focuses its attention towards the description of the main groups of insect viruses, their characteristics, life cycle, genetics, use as bioinsecticides, production, application, examples on their use as control agents, and finishing with a review on their genetic manipulation and its potential to improve their virulence towards the insect pests, as well as their potential to express heterologous proteins with importance in the pharmaceutical industry, when used as expression vectors.

1. Introduction

The word "virus" comes from Latin, meaning "poison", and is normally associated with disease and death. First definitions of viruses were simple and refer to disease-causing agents, showing ability to self-replicate within living cells and having such a small size enough to pass through ultra thin filters (normally porcelain filters), where no bacteria can pass through. More modern definitions refer to them as the most elementary biosystems that show macromolecular complexity and are able to self-replicate and evolve, but lack irritability (i.e. the ability to react to environmental factors), self-movement, and their own source of metabolic energy, in contrast with the basic features that characterize all living things. The basic virus particle comprises a nucleic acid (DNA or RNA), which directs the virus replication and is encapsulated within a protein case known as "capsid", which plays an important role in the host cell infection process. Once a viral particle gains entry into a permissive (susceptible) cell, its nucleic acid takes charge of the cell's metabolic system and profusely replicates into new virus particles, until the cell is normally depleted of all its content and dies. This is the reason why a virus is considered an obligate parasite, as it not only uses the cell's own material but also most of its own metabolic machinery. That is, viruses cannot replicate "*in vitro*" (i.e. on artificial media, where only organic material sources are provided). Although all viruses show the same basic structure and the same need for host cells to replicate, a great variety of viruses can be found in nature. Each group of viruses shows some intrinsic features such as morphology, genome, infectivity, host range, etc., which are distinctive of each group.

The basic replication cycle of a virus starts when it gains entry into a permissive host cell, using a variety of infective mechanisms: endocytosis, phagocytosis, pinocytosis, membrane fusion, etc. Subsequently, the viral genome (DNA or RNA) is dissociated from the capsid and released into the host cell in a great variety of mechanisms. Once in the cell (either in the nucleus or in the cytoplasm) the viral genome replicates itself, frequently using the host cell's enzymatic machinery, creating a great number of identical copies. Also, structural, functional, and auxiliary viral genes are transcribed and translated into proteins, which will be used to assemble, part by part, new viral particles. The new copies of the viral genome are packed within the new capsids and the new viral progeny is released by a variety of procedures, which are distinctive of each

viral group. Some viruses are released by inverted pinocytosis, which engulfs the viral particle within a membrane vesicle, creating what is known as "enveloped viruses".

Enveloping is a distinctive feature of some groups of viruses, just as some other attributes such as capsid morphology and size, the type of nucleic acid, mode of entry, replication mechanism, host range, etc. These and other characteristics are taxonomically important and are used by virologists to classify viruses into families, genera and species. Nevertheless, virus classification is not an easy task, as their origin is unclear and their evolution is complex. To date it is agreed that different groups of viruses had different and independent origins, all of them highly related to their hosts. This is in contrast to the living things, which all had a single evolutionary origin. Viral groups kept appearing independently along the evolutionary development of the living things, and as derivatives from the host genomes. That is the reason why there is such an extensive diversity of viruses. Recently, the 8th report of the International Committee on Taxonomy of Viruses (ICTV) approved three orders, 73 families, nine subfamilies, 287 genera, and 1950 species of viruses, although the final list includes a total of 5450 viruses. It is important to note that this list also includes viroids, virusoids (satellite viruses) and prions.

Earlier to the development of the electronic microscope, the nature of viruses was unknown. The idea of naked genes with the ability to move from one cell to another was suggested. Later on, viral particles were able to be purified, once ultracentrifugation procedures were developed. By the 1940s it was certain that viruses contain nucleic acids and performed functions separately, although dependent, of the host metabolism. This host dependency and their high specificity indicate that hosts evolved before their own viruses, and suggest that virus precursors were nucleic acid fragments which developed the ability of self-replication, similar to extrachromosomal replicons known as plasmids. In fact, viral genomes made of either DNA or RNA can be considered self-replicating molecules, with a single origin of replication in their sequences, just as plasmids. However, this is all they have in common, as viral genomes are coated with capsid proteins and have the ability to invade cells and "capture" their metabolism. It has been suggested that primitive viruses may be similar to the actual viroids (short, non-coding, self-replicating, non-encapsulated, infective fragments of RNA) derived from chromosomal or transcriptional RNA from host cells. More complex virus groups may have evolved from DNA plasmids which acquired genes coding for capsid proteins and evolved to self-replicating, infective particles. Complex virus groups may contain large and highly developed genomes as well as intricate structure as a consequence of a long and interrelated co-evolution with their hosts.

Virology is a complex discipline, and its development has revolutionized our knowledge due to the multiple effects that the great diversity of viruses have on animals, plants, and microorganisms. Virology has developed the basis for valuable knowledge in medicine, genetics, and biology. Viruses have been a key factor in the understanding of many genetic, pathological and molecular bases of life, such as genetic expression and regulation, cell growth and development, medical treatment of diseases, etc. Virology has been very important in the development of Medicine as a science, as viruses have caused devastating diseases throughout the human history. Viruses, such as those causing influenza, polio, hepatitis, rabies, yellow fever, encephalitis, dengue, herpes,

and more recently AIDS and Ebola, among many others, have had a significant social, economic, and even political impact in the development of civilization.

Viral infections are not restricted to humans, as many viruses infect other animals, plants, and microorganisms, some with devastating effects. However, their destructive consequences can also be used to benefit mankind. Several viruses specifically attack many pest animals and plants, undesirable in ecosystems and agro-ecosystems of the world. Also, viruses are the basis for the development of viral infection treatments, as most vaccines are based on the use of attenuated viral particles which, once within the body, stimulate the immunological system to form antibodies against the same virus. Additionally, some viruses from the family *Baculoviridae* are extensively used to overproduce proteins important in scientific research, although its use in the medical industry is starting to show its potential. This chapter deals with one of the beneficial uses of viruses because, as any other living organisms, insects are subject to virus infections. Many of these infection cause lethal diseases to susceptible individuals and, therefore, viruses can be important biotic factors that keep insect populations densities under natural control. Pests, by definition, are animal populations harmful to humans, whose densities are out of natural control. The deliberate introduction of some biotic factors into those populations is called "Biological Control" of pests, and viruses have demonstrated to be important biological control agents, in many cases.

2. Entomopathogenic Viruses

Insects constitute the most divers group of living things on the world. They can be found practically in every terrestrial and fresh-water habitat. Some have been associated with humans throughout their evolution and play an important role since the beginning of the agriculture, by becoming natural competitors for the food humans grow. We call these competitors "pests" and try to develop technique to counteract this competition. Although many measures have been used for this purpose, an efficient technique was developed by the middle of the 20th century, which made obsolete and impractical any other kind of control: the use of chemical insecticides. Consequences of the practically exclusive use of this technique soon were apparent: pollution, toxicity to humans and domestic animals, development of resistant pest populations, damage to beneficial insects and to wilderness, development of new pests, etc. A modern, more moderate, environmentally oriented society has made possible to reduce the use of chemical insecticides by about 1.5% annually. Also, new control alternatives have been developed, especially those environment-friendly, such as biological control. Three main groups of organisms constitute the backbone of biological control: parasitoids, predators, and pathogens. The first two groups are mostly represented by other type of insects, while the third one is constituted by infectious microorganisms causing lethal or deleterious effects on susceptible individuals. These can be bacteria, viruses, fungi, protozoa, and nematodes, and are frequently used as "bioinsecticides" (living insecticides), which are sprayed on pest populations. Nowadays, the market for bioinsecticides is about 2.5% of the total insecticide market and it is estimated that will rise to 4.2% by 2010. Although bacterial bioinsecticides represent the greatest majority of them, viruses constitute an important component of this type of agents, especially the baculoviruses. These constitute the most diverse group of entomopathogenic viruses which have been found practically exclusively on insect populations, mainly within the

orders Lepidoptera, Hymenoptera, and Coleoptera, although some few have been found in crustaceans and spiders. Nonetheless, the diversity of entomopathogenic viruses is extensive (Table 1).

Diseases caused by entomopathogenic viruses are known since the 16th century. A disease called *jaundice o grasserie*, now identified as a nucleopolyhedrosis, observed in silkworm (*Bombyx mori* L.; Lepidoptera: Bombycidae) rearing facilities was described since 1524 by Vida, and later on another viral disease was described in the honeybee (*Apis mellifera* L.; Hymenoptera: Apidae). By 1856, two Italian scientists, Maestri and Cornalia, described the occlusion bodies (OBs, see below) of the silkworm nucleopolyhedrosis, and by 1926, Paillot described the granuloviruses (GVs) for the first time. Likewise, by 1934, Ishimori described a new type of polyhedrosis in the silkworm, whose OBs were formed in the cytoplasm of the infected cells, rather than in the nuclei, now known as cypoviruses (see below). The introduction of electron microscopy greatly advanced the knowledge of entomopathogenic viruses, allowing Bergold to observe the rod-shaped capsids within the OBs of baculoviruses. Later on, since the 1950s to the 1970s, Steinhaus and his collaborators tested baculoviruses as biological control agents in the field by applying a nucleopolyhedrovirus (NPV) to control the alfalfa caterpillar (*Colias eurytheme* Boisduval; Lepidoptera: Pieridae). It is worth noticing that the first commercial bioinsecticide based on a virus was developed in 1975 by the company Sandoz under the name Elcar, focused to control the *Heliothis*/*Helicoverpa* complex (Lepidoptera: Noctuidae). During the 1970s and 1980s important advances were made on the genetics of entomopathogenic viruses, especially on baculoviruses. Plaque isolation was developed of non-occluded virions and first recombinant baculoviruses were developed. To date, studies on the genetics of entomopathogenic viruses are focused on the study of complete genomes as a consequence of the development of genomics. So far, more than 29 complete sequenced genomes have been obtained.

FAMILY	**NUCLEIC ACID**	**NUCLEOCAPSID SIMETRY**	**OCCLUSION BODY**
Baculoviridae	dsDNA	Baciliform	+
Reoviridae	dsRNA	Isometric	+
Poxviridae	dsDNA	Ovoid	+
Iridoviridae	dsDNA	Icosahedral	-
Parvoviridae	ssDNA	Isometric	-
Picornaviridae	ssRNA	Spherical	-
Ascoviridae	dsDNA	Allantoid	-
Polydnaviridae	dsDNA	Ovoid	-
Rhabdoviridae	ssRNA	Baciliform	-
Nodaviridae	ssRNA	Icosahedral	-
Rhabdoviridae	ssRNA	Baciliform	-
NON-CLASSIFIED RNA VIRUSESs			
Divided genome	ssRNA	Isometric	-
β *Nodaurelia*	ssRNA	Isometric	-
Kelply group	ssRNA	Isometric	-
5-virus group	ssRNA	Isometric	-
Minivirus	ssRNA	Isometric	-
Ovoid virases	ssRNA	Ovoid	-

Drosophila X Virus	dsRNA	Isometric	-

ds= double-stranded, ss= single-stranded.

Table 1. Groups of entomopathogenic viruses

3. Taxonomic Classification

Entomopathogenic virus classification, just as any other type of viruses, follows the indications of the International Committee on Taxonomy of Viruses (ICTV). Therefore, they follow the same criteria to classify the diversity of viral groups that attack insects, such as: type of genetic material (i.e. singe- or double-stranded DNA, singe- or double-stranded RNA, positive or negative strand), virion morphology and size (i.e. icosahedral, rod-shaped, etc.), presence of an envelope surrounding the virion, presence of an occlusion body engulfing the virions, host and host range, among many others. However, the ultimate criterion is the sequencing of the genetic material which determines not only the discrimination between viral species, but also establishes the evolutionary relationship among viruses within the same group. Insect viruses are named in acronyms, according to their host and the viral group to which it belongs to. For example, the *Autographa californica* multiple nucleopolyhedrovirus is named AcMNPV. Therefore, all nucleopolyhedroviruses are named NPV, just as the granuloviruses are named GV, the entomopoxviruses are EPV, the iridoviruses are IV, and the cytoplasmic polyhedrosis viruses (cypoviruses) are CPV. As observed in Table 1, insect viruses are highly diverse; however, only few groups are frequently found in insect populations and show potential to be used as biological control agents, highlighting the group of baculoviruses. The main features of the most important insect viruses are shown next.

3.1. Ascovirus

Ascoviruses are members of the family *Ascoviridae*, which include a few species isolated only from insects, specifically from the order Lepidoptera. Enveloped virions of ascoviruses are bacilliform, ovoid, or allantoid in shape and are occluded within vesicle-like OBs. Their genomes contain circular dsDNA of 100 to 180 Kb. So far, eight species have been identified from larvae of the same number of noctuid (Lepidoptera: Noctuidae) species.

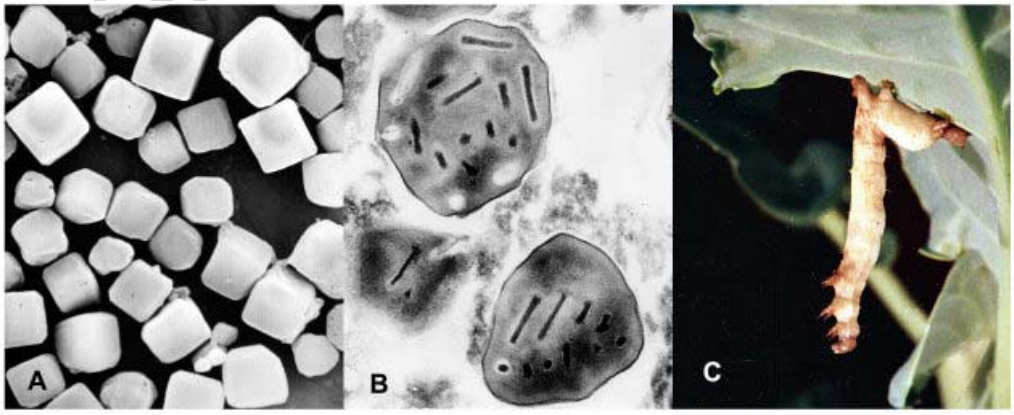

Figure 1. A) Scanning electron microscopy of baculovirus polyhedra, B) Transmission electron microscopy of baculovirus polyhedra, C) Cabbage looper larva infected with baculovirus.

3.2. Iridovirus

Members of the family *Iridoviridae* show non-enveloped, non-occluded, icosahedral viral particles . The most distinctive feature of this family is a particular iridescence of the infected tissues, whose color varies according to the species. Iridovirus genomes are large, fluctuating from 140 to 303 Kb of linear dsDNA. Although some iridoviruses infect frogs and fishes, those infecting insects belong to two genera: *Iridovirus*, whose viral particles fluctuate between 120 to 130 nm in size and the type species was isolated from the Asiatic rice borer *Chilo suppressalis*; and *Chloriridovirus*, with a larger viral particle (180 nm) and the type species was isolated from mosquito larvae of *Aedes taeniorhinchus*. The ICTV only recognizes 20 species of iridoviruses, in spite of reports describing infections in 28 species of insects within the order Diptera, 12 from Lepidoptera, and four from Coleoptera.

3.3. Polydnavirus

Polydnaviridae is a family of insect viruses characterized by infecting exclusively to endoparasitic Hymenoptera. Members of this family show non-occluded, ovoid virions, containing multipartite dsDNA, totaling 75 to 200 Kb when all the fragments are added. ICTV recognizes two genera within this family: *Ichneovirus*, whose type species infects the ichneumonid wasp *Campoletis sonorensis*; and *Bracovirus*, whose type species infects the braconid wasp *Cotesia melanocella*.

3.4. Baculovirus

Members of the family *Baculoviridae* contain circular dsDNA with a size fluctuating between 80 and 130 Kb, within enveloped, rod-shaped viral particles. These are occluded either within polyhedral or granular OBs (Figure 1). Although there have been more than 600 isolates reported from a variety of insect species, the ICTV only recognizes 30 species within two genera: *Nucleopolyhedrovirus* or NPVs; and *Granulovirus* or GVs. NPVs also are divided into single nucleopolyhedroviruses or SNPVs, with only one virion per envelope; and multiple nucleopolyhedroviruses or MNPVs, with several virions per envelope. The SNPV type species (BmSNPV) infects silkworms (*B. mori*), and the MNPV type species (AcMNPV) infects larvae of *Autographa californica* (Lepidoptera: Noctuidae). NPV virions replicate only in the nuclei of susceptible cells and their OBs fluctuate between one and 15 μm in size, clearly visible under light microscopy. On the other hand, GV type species infects codling moth larvae (*Cydia pomonella*; Lepidoptera: Tortricidae). GV virions are always single within the envelope. OBs are very small (0.2 to 0.5 m) as compared with NPV polyhedra, but this is because there is only one virion per OB. GV virions replicate in the cytoplasm of susceptible cells. Additionally, there is a group not recognized by ICTV as belonging to the family *Baculoviridae*, but due to many similarities with baculoviruses and its use as a biological control agent, we include it in this review. This is the nudivirus or non-occluded baculovirus (NOB) specific of the

coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). The rod-shaped virions are very similar to those of baculoviruses, except that they are not occluded within an OB.

3.5. Cypovirus

Members of the family *Reoviridae* are highly diverse in their host range, as they include many vertebrates and invertebrates. The well-known rotavirus is included in this family. Those specific to insects are called cytoplasmic polyhedrosis viruses or cypoviruses or just CPVs. Icosahedral virions show 12 lateral projections and are occluded within large isometrical polyhedra of up to 10 µm in size. Virions replicate in the cytoplasm of susceptible cells and contain 10 to 12 segments of dsRNA of about 12 to 32 Kb in total. Size and number of fragments depends on the species. ICTV assemble all the insect-specific reoviruses within the genus *Cypovirus*, and recognizes 70 species, all hosted by lepidopteran species. The *Bombyx mori* cytoplasmic polyhedrosis virus or BmCPV is the type species of the genus. However, there are more than 200 reports of isolates from lepidopteran species, as well as 20 more from dipterans.

3.6. Entomopoxvirus

Members of the family *Poxviridae* also show a wide host range, with vertebrate and invertebrate hosts. The well-known chicken-pox and small-pox viruses belong to this family. Those specific to insects form a compact group called entomopoxviruses (subfamily *Entomopoxvirinae*). They show allantoid- to brick-shaped virions, occluded within ovoid OBs called spheroids. Virions are some of the largest in the virus world, measuring up to 400 nm in length and 250 nm in width, and contain dsDNA ranging in size from 270 to 320 Kb. Virions replicate in the cytoplasm of susceptible cells. Entomopoxviruses have been isolated from 27 species of orthopterans, lepidopterans, dipterans, and coleopterans. The subfamily *Entomopoxvirinae* includes three genera called simply as *Entomopoxvirus A*, *Entomopoxvirus B*, and *Entomopoxvirus C*. The first one infects only coleopteran species and the type species infects *Melolontha melolontha*; the second one infects lepidotperan and coleopteran species and the type species infects *Amsacta moorei*; and the third one infects only dipteran species and the type species infects *Chironomus luridus*. A fourth group (D) has been proposed to the ICTV which attacks hymenopterans.

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Biographical Sketches

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