" Review article "

MOLECULAR DIVERSITY OF INVERTEBRATE PARVOVIRUSES (Densovirinae)

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By

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ABSTRACT

Parvoviruses are defined by having a linear, single-stranded DNA genome. They infect both vertebrates and invertebrates. Those originating from invertebrates are called densoviruses and constitute the Densovirinae subfamily within the Parvoviridae.

The study of different biological, biophysical, biochemical properties is reviewed. In particular, we focused on the great diversity of their genomic organization and emphasized the genomic basis for further classification of the different species of this subfamily.

A better understanding of these viruses could provide the means towards a more judicious use of densoviruses in biological control of insect pests.

Key words: densovirus, densovirinae, parvoviridae, molecular diversity, virus taxonomy.

1. INTRODUCTION

All small non-enveloped icosahedral viruses that contain a linear, single-stranded DNA genome are classified within the family Parvoviridae. The parvoviruses of invertebrates, isolated from several species of Arthropoda, in particular Decapoda (shrimps) and Insecta (mainly lepidopteran insects), form the Densovirinae subfamily (Tijssen and Bergoin, 1995; Bergoin and Tijssen, 2000). In view of the large number of species in these animal orders, it is expected that the real number of densoviruses is very large and that the currently known do not reflect the final distribution and properties of these viruses. Members of this group were originally called Densonucleosis viruses (DNVs) to describe the characteristic histopathologic symptoms, *i.e.*, hypertrophied and densely-stained (Feulgen) nuclei of infected cells in permissive larvae (Amargier *et al.*, 1965). The name was subsequently shortened to Densovirus for all invertebrate parvoviruses. Currently, this subfamily consists of three genera: Densovirus, Brevidensovirus and Iteravirus (Bergoin and Tijssen, 1998).

DNVs are identified by a two-letter abbreviation of the host name, such as *Gm*DNV for the DNV from *Galleria mellonella*, or by a three-letter abbreviation (one-letter abbreviation of the genus and two-letter abbreviation of the species) from insects that would have the same two-letter abbreviation, such as the DNVs from *Lymantaria dispar* (*Ldi*DNV) and *Leucorrhinia dubia* (*Ldu*DNV).

All DNVs are characterized by an autonomous replication and separate packaging of either of the complementary, single-stranded DNA strands. The structure and organization of densovirus genomes are more varied than those of the vertebrate parvoviruses. Although originally densoviruses were classified according to biological, biophysical, biochemical or serological properties, current classifications are based on the organization and expression strategy of the viral genome.

1.1. Biological properties of DNVs
1.1.1. Geographical Distribution

Densoviruses are likely to be widely distributed among all insect species (Table 1). The first DNV was isolated in France in 1964 from

Table (1): Distribution of Densoviruses.

Host	Name	Country of isolation	Year	Ref.
*Insects				
Lepidoptera				
Agraulis vanillae	*Av*DNV	United Kingdom	1980	Kelly *et al.*,
Bombyx mori	*Bm*DNV-1	Japan	1975	Shimizu
Bombyx mori	*Bm*DNV-2	Japan	1983	Seki & Iwashita
Casphalia extranea	*Ce*DNV	Côte d'Ivoire	1981	Fédière *et al.*,
Diatraea saccharalis	*Ds*DNV	Guad eloupe	1977b	Meynadier *et al.*,
Euxoa auxilliaris	*Ea*DNV	United States	1973	Sutter
Galleria mellonella	*Gm*DNV	France	1964	Meynadier *et al.*,
Junonia coenia	*Jc*DNV	United Kingdom	1972	Rivers & Longworth
Lymantria dispar(cell line)	*Ldi*DNV	France	1982	Grignon
Mythimna loreyi	*Ml*DNV	Egypt	1995	Fédière *et al.*,
Pieris rapae	*Pr*DNV	China	1981	Sun *et al.*,
Pseudoplusia includens	*Pi*DNV	United States	1985	Chao *et al.*,
Sibine fusca	*Sf*DNV	Colombia	1977a	Meynadier *et al.*,
Dictyoptera				
Periplaneta fuliginosa	*Pf*DNV	Japan	1979	Suto
Diptera				
Aedes aegypti	*Aae*DNV	Soviet Union	1973	Lebedeva *et al.*,
Aedes albopictus (cell line)	*Aal*DNV	France	1993	Jousset *et al.*,
Aedes pseu doscutellaris (cell line)	*Ap*DNV	Venezuela	1980	Gorziglia *et al.*,
Culex pipiens	*Cp*DNV	France	1998	Baquerizo
Haemagogus equinus (cell line)	*He*DNV	United States	1995	O'Neill *et al.*,
Simulium vittatum	Sv*DNV	United States	1976	Federici
Toxorhynchites amboinensis (cell line)	*Ta*DNV	United States	1995	O'Neill *et al.*,
Hemiptera				
Planococcus citri	*Pc*DNV	USA	2001	MyLo *et al.*,
Odonata				
Leucorrhinia dubia	*Ldu*DNV	Sweden	1979	Charpentier
Orthoptera				
Acheta domesticus	*Ad*DNV	France	1977c	Meynadier *et al.*,
*Crustacea				
Decapoda				
Carcinus mediterraneus	*Cm*DNV	France	1988	Mari&Bonami
Macrobrachium rosenbergii	*Mr*DNV	Malaisia	1990	Anderson *et al.*,
Penaeus merguiensis	*Pme*DNV	Singapour	1985	Lightner&Redman.
Penaeus monodon	*Pmo*DNV	Philippin	1985	Lightner&Redman.
Penaeus orientalis	*Po*DNV	China	1985	Lightner&Redman.
Penaeus semisulcatus	*Pse*DNV	Koweit	1985	Lightner&Redman.
Penaeus stylirostris	*Pst*DNV	Hawü	1989	Lu *et al.*,

larvae of the greater wax moth *Galleria mellonella* (Meynadier *et al.*, 1964). Subsequently, other members of this Densovirinae were isolated from insect orders Lepidoptera, Dictyoptera, Diptera, Odonata, Hemiptera, and Orthoptera, as well as from Crustacea and Decapoda all over the world thus providing evidence of the ubiquity of DNVs in the phylum of Arthropoda.

1.1.2. Host range

As reviewed by Fédière (1996) and Bergoin and Tijssen (1998) investigations on the host range of DNVs indicate a considerable variation. The *Gm*DNV, *Ce*DNV and *Ad*DNV have a host range apparently restricted to their original hosts (Giran, 1966; Jousset *et al.*, 1986; Fédière *et al.*, 1986). In contrast, other DNVs, also isolated from lepidoptera, have a broader host range. The *Jc*DNV can replicate *in Aglais urticae, Bombyx mori, Chrysodeixis chalcites, Lymantria dispar, Mamestra brassicae, Mamestra oleracea, Scotia ipsilon, Spodoptera exigua, Spodoptera littoralis* but not in *G. mellonella* (Rivers & Longworth, 1972; Diallo, 1978). Similary, the *Ml*DNV is infectious for *Chilo agamemnon, G. mellonella, Ostrinia nubilalis, Pectinophora gossypiella, Sesamia cretica, S. littoralis, S. exigua, Agrotis ipsilon, Agrotis segetum, Agrotis spinifera, Autographa gamma, Phthorimaea opercullella, B. mori* and *Helicoverpa armigera* (Fédière *et al.*, 1999; El-Mergawy *et al.*, 2002). According to their sequences and genome organization, the *Ml*DNV is very closely related to *Gm*DNV (95% identity) (Fédière *et al.*, 1998). The close relationship is interesting since their tropism differs greatly, *Gm*DNV being monospecific on its host, whereas *Ml*DNV is polyspecific and infects a large number of lepidoptera pests. The striking differences in tropism related to the short sequence differences offered an ideal system to study these sequence-function relationships and the allotropic determinants (Tijssen *et al.*, 1998). The host range of *Ea*DNV extends to *Pseudaletia unipuncta* and *Heliothis zea* (Sutter, 1973). The host range of the *Pf*DNV was shown to extend to at least four other species of the genus *Periplaneta: P. americana, P. australasiae, P. brunnea* and *P. japonica* (Suto, 1979). The host range of DNVs infecting mosquitoes extends to different species. Larvae of *A.*

albopictus, Aedes cantans, Aedes caspius, Aedes geniculatus, Aedes vexans, Culex pipiens and *Culiseta annulata* are all suceptible to *per os* infection with *Aae*DNV (Lebedeva *et al.*, 1973). The *Aal*DNV isolated from a chronically infected cell line of the C6/36 clone of *A. albopictus* proved to be very pathogenic for *A. aegypti* and *A. metallicus* larvae (Barreau *et al.*, 1996). In the case of *Bm*DNV-1, there is no information concerning its cross-infectivity to other insects except between *B. mori* and the pyralid, *Glyphodes pyloalis*, infecting the mulberry plantations of sericultural farms (Watanabe, 1981). Of practical interest for sericulture was the demonstration that the susceptibility to DNV infections varied from one strain of silkworm to another and that resistant strains could be selected. Among the economically important silkworm strains, severals are suceptible to *Bm*DNV-2. Almost all strains susceptible for *Bm*DNV-1 are resistant to *Bm*DNV-2 and reciprocally, strains resistant to *Bm*DNV-1 are sensitive to *Bm*DNV-2 (Seki, 1984). The mode of inheritance of the resistance to *Bm*DNV infections has been investigated and it was established that for each virus the nonsusceptibility is genetically controlled by a recessive gene that is not sex linked. A practical aspect of this result was to recommend the rearing of silkworm strains homozygous for the nonsusceptible gene, in order to avoid DNV epizootics in sericultural farms (Seki, 1984). Finally, it is worth mentioning that despite their high virulence for their insect hosts, DNVs do not appear to be able to replicate in vertebrates or mamals, including humans. No pathogenic effect was detected following inoculation of *Gm*DNV, *Jc*DNV, *Ce*DNV, and *Ml*DNV to mice or rabbits for production of antisera (Fédière, 1996; Giran, 1966). Similary, no replication of *Aal*DNV could be detected in monkey MA-104 and BGM cells and in human Hela cells (Jousset *et al.*, 1993).

1.1.3. Symptoms

The densoviruses cause fatal diseases to their hosts. The symptoms of *Gm*DNV infections are similar to those of *Av*DNV (*Agraulis vanillae*) and *Ml*DNV infections (Amargier *et al.*, 1965; Fédière, 1996; and Chao *et al.*, 1985). Generally the first symptoms are anorexia and lethargy followed by flaccidity and the inhibition of

moulting and metamorphosis. During the infection, larvae become whitish and progressively paralyzed, followed by a slow melanization (Vago *et al.*, 1966). The cockroach *P. fuliginosa* infected with the *Pf*DNV displays very characteristic symptoms. Prior to the death, the hind legs are paralyzed and their movements uncoordinated. Interestingly, females are particulary affected by this DNV (Suto, 1979). The abdomen is swollen with an hypertrophied fat body, colored milky white in contrast to the brownish-white tissues observed in an uninfected cockroach. More than half of the infected cockroach develop ulcers in the hindgut by a process of accumulation of hemocytes around injured hindgut epithelial cells (Suto *et al.*, 1979). Some other DNVs produce tumor lesions in the intestine of their hosts. Typical tumors were observed in heavily infected slug caterpillar, pests of oil palm, *S. fusca* and *C. extranea* (Meynadier *et al.*, 1977a ; Fédière, 1983). The midgut epithelial cells of diseased larvae undergo intensive proliferation and the progressive thickening and opacity of the gut wall screens off the intestinal content. In the case of *C. extranea*, the larval color changes from green to yellowish brown and the transparent gut becomes opaque (Fédière, 1983). The nymphs of the Swedish dragonfly *L. dubia* infected with *Ldu*DNV become sluggish and flaccid, but there is no other external sign of the disease (Charpentier, 1979). When silkworm larvae are infected *per os* with *Bm*DNV-1, they usually die after seven days showing body flaccidity as a major sign. The alimental canal of the diseased larvae is pale yellow with little internal content (Shimizu, 1975). Mosquito larvae infected with DNV exhibit symptoms of paralysis. Interestingly, despite the lack of cytopathic effect in the mosquito cell culture, the DNVs isolated from cell lines proved to be pathogenic for mosquito larvae by *per os* infection (Jousset *et al.*, 1993; O'Neill *et al.*, 1995; Barreau *et al.*, 1996; Buchatsky *et al.*, 1987). When first instar larvae of *A. aegypti* were infected with *Aal*DNV, the symptoms of the disease appeared at stage IV. Affected larvae lost their mobility and hung near to the water surface. Their bodies were distorted and curved. They lost their pigmentation and exhibited a whitish color. These symptoms appeared one day before death (Barreau *et al.*, 1996).

1.1.4. Histopathology associated with Densoviruses
Most DNVs known so far are polytropic in tissue tropism. In *Av*DNV, *Ds*DNV, *Gm*DNV, *Ml*DNV, *Pi*DNV, *Aae*DNV and *Aal*DNV

infections, almost all larval tissues, *i.e.*, fat body, hypodermis, central nervous system, silk gland, muscular membrane, tracheal cells, malpighian tubules, foregut, hindgut, hemocytes, ovaries and molting gland, are susceptible, with the exception of the midgut epithelium (Kelly *et al.*, 1980; Meynadier *et al.*, 1977b; Chao *et al.*, 1985; Lebedeva, 1973; Jousset *et al.*, 1993; Amargier *et al.*, 1965; Vago *et al.*, 1966; Fédière, 1996; Kurstak *et al.*, 1977). On the other hand, DNVs infecting *B. mori*, *C. extranea* and *S. fusca* multiply predominantly in the columnar cells of midgut epithelium (Meynadier *et al.*, 1977a; Fédière, 1996; Watanabe *et al.*, 1976).

The histopathological aspects of DNVs infections are characteristic. The main lesions occur in the nuclei of infected cells. The nuclei become greatly hypertrophied very rapidly and densely stained (eosinophilic) and Feulgen positive (Amargier *et al.*, 1965). In the *Gm*DNV infections, the first obvious pathological changes occur in cells of the fat body. A voluminous dense homogeneous structure appears in each of the infected nuclei. Later, all cells become progressively involved (Amargier *et al.*, 1965). In the larval tissues of *Aal*DNV-infected *A. aegypti*, no alterations were observed at 2, 3 and 4 days postinfection. Anomalies appeared at day 5 principally in cells of the fat body. Later, the dense nuclei appeared in almost all of the larval tissues (Barreau *et al.*, 1996). Histopathological studies on the midgut epithelium of the silkworm infected with *Bm*DNV-1 showed that the infected nuclei were more than 2.5 times as large as normal nuclei. At the last stage of infection, the degenerated columnar cells were liberated into midgut lumen (Seki & Iwashita, 1983). In the case of *Bm*DNV-2 almost the same features were observed under light microscope (Iwashita & Chun, 1982).

1.1.5. Ultrastructure of infected cells

Ultrastructural studies have led to a comprehensive description of the pathogenesis of DNV infection in larval tissues of *G. mellonella* (Vago *et al.*, 1966; Garzon & Kurstak, 1976; Kawase *et al.*, 1990). The first ultrastructural changes in *Gm*DNV infections are observed both in the cytoplasm and the nucleus. In the cytoplasm, during the first six hours postinfection, polyribosomes disappear and the number of free ribosomes and the formation of microbody-like structures arising from the accumulation of small, spherical particles of 17 to 20

nm inside the vesicles, increased. This step could represent the accumulation and transport of viral proteins to the nucleus. In the nucleus, the heterochromatin becomes very condensed and is localized at the nuclear membrane. The nucleolus undergoes hypertrophy which is accompanied by a segregation of its fibrillar and granular components. The development of the granular portion coincides with the synthesis of double-strand DNA of the replicative form. Simultaneously, a virogenic stroma appears in close vicinity to the nucleolus. As the infection progresses, the granular portion of the nucleolus regresses in favour of the fibrillar portion. After one or two days, the virions are assembled inside the virogenic stroma which invades the whole nucleus and leads to a nuclear hypertrophy. By day four or five, mature virions replace progressively the virogenic stroma and paracrystalline viral concentration takes place, pushing the chromatin and the nucleolus to the nuclear periphery. At the end of infection, the nuclei are so hypertrophied that the nuclear envelope is disrupted, allowing the virions to accumulate in the cytoplasm and viral inclusions, often arranged in paracrystalline arrays that can be then observed. Similar ultrastructural changes of nuclei infected with other DNVs have been observed (Meynadier *et al.*, 1977c; Sutter, 1973; Suto, 1979; Fédière, 1983; Barreau *et al.*, 1996). In several DNV-infected insects, the formation of cytoplasmic paracrystalline virion arrays occurs prior to or without destruction of the nuclear membrane (Chao *et al.*, 1985; Charpentier, 1979; Diallo, 1978). Althoutgh both DNV-1 and DNV-2 multiply in the nucleus of columnar cell, difference in the ultrastructural studies of infected cells is obvious when the sections are observed in the electron microscope. On the contrary of *Gm*DNV and *Bm*DNV-1, the virogenic stroma of cell infected with *Bm*DNV-2 is less electron-dense than the surrounding nuclear matrix and occupies most of the nucleus. Discrete sites where virions replicate in linear array appear early in infection. These increase in size with each round of multiplication until they eventually fuse (Watanabe & Kurihara, 1988).

1.2. Biophysical and biochemical properties

As reviewed by Kurstak *et al.*, (1977) and Tijssen and Bergoin (1995), DNVs are small (20-25) nm in diameter and the nonenveloped, icosahedral particle contains a linear molecule of

single- stranded DNA with a size between 4 and 6 Kb. The capsid consists of 2 to 4 structural polypeptides, generally between 40 and 100 kda.

1.3. Molecular characterization of the genome

It is possible to separate the molecular analysis of virus genomes into two types of approaches: (1) Physical analysis of structure and nucleotide sequence (essentially performed *in vitro*). (2) Genetic analysis of the structure-function relationships of the intact virus genome and its individual genetic elements (usually involving analysis of the virus phenotype *in vivo*).
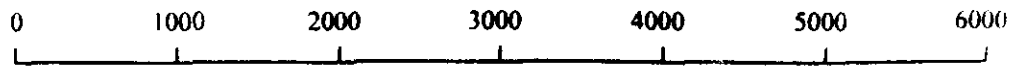
As Densoviruses constitute an increasingly diversified subfamily based on the structure and the organization of their genome, a new taxonomic structure was proposed (Bergoin and Tijssen, 1998). This subfamily has been subdivided into three genera which can be clustered into five types, three belonging to genus Densovirus and two belonging to genus Iteravirus and Brevidensovirus (Table 2). As shown in Table (2), the classification was based on:

-The size of the genome.

-The presence or absence of the inverted terminal repeats.

-The size of the extremities.

-Genomic organization, ambisense or monosense.

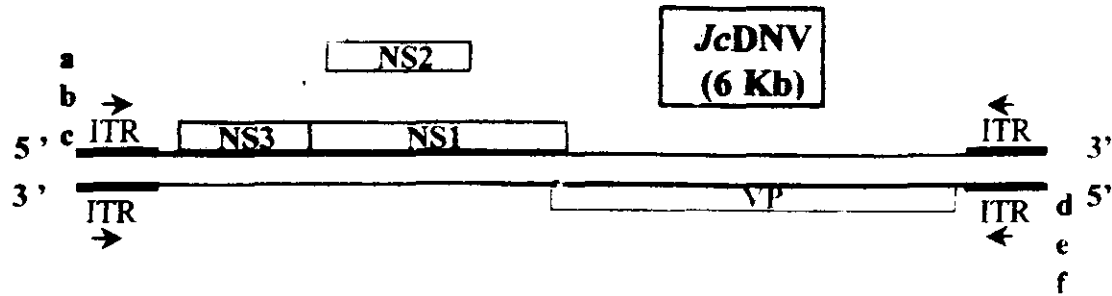-Models of replication.

-Transcription and translation.

The genome of DNVs contains two sets of ORFs (open reading frames) coding for NS (nonstructural proteins) and VP (structural proteins), respectively. The great variation in the organization and size of these ORFs indicates also a great diversity in their mode of expression. All the members of the genus Densovirus (Fig. 1) share the property of having the NS and VP genes equally distributed in the 5' halves of the two complementary strands. As exemplified by *Gm*DNV, those infecting *Lepidoptera* (subgroup A) thus far exhibit an identical organization of their genome. Three ORFs coding for three NS proteins are present on one strand, the left-most ORF (near the 5' extremity) being in frame with, and separated from the largest NS ORF (NS1) by only a TAA stop codon, codes for NS3. The sequence of the third ORF (NS2) is overlapping that of the largest
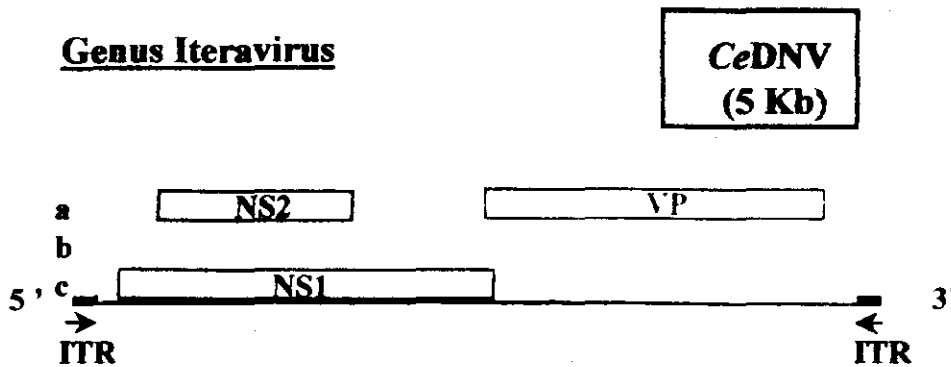
Table (2): Classification of Densoviruses.

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Genus Densovirus	- Large genome: 5.5-6.0 kb - Large ITRs, both with "flip"/"flop" orientations - Ambisense genomic organization.
Subgroup A: **(Lepidoptera)**	- Genome size: 6 kb - ITRs over 500 nt - Four structural proteins (47, 53, 58 and 89 kDa) -Representatives: GmDNV: *Galleria mellonella*, JcDNV: *Junonia coenia*, DsDNV: *Diatrea saccharalis*, PiDNV: *Pseudoplusia includens*, MlDNV: *Mythimna loreyi*.
Subgroup B: (Orthoptera)	- Genome size: 5.5 kb - ITRs shorter than in subgroup A - Four structural proteins (41.5, 55, 88 and 120 kDa for AdDNV, 48, 52, 61 and 76 kDa for PfDNV) - Distinctive properties: splicing within VP mRNA -Representatives: AdDNV: *Acheta domesticus*, PfDNV: *Periplaneta fuliginosa*.
Subgroup C: (Diptera)	- Genome size: 6kb - ITRs over 300 nt - Four structural proteins (12, 57, 64 and 90k Da) - Distinctive properties: probable splicing in NS mRNA Representative:CpDNV:*Culex pipinus*.
Genus Iteravirus	- Genome size: 5 kb - ITRs of about 250 nt, both with "flip"/"flop" orientations - Monosense genomic organization -Structural proteins: two doublets (49-54 and 74-82 kDa) -Representatives: BmDNV: *Bombyx mori*, CeDNV: *Casphalia extranea*.
Genus Brevidensovirus	- Genome size: 4 kb - No ITRs, but terminal hairpins - Monosense genomic organization -Structural proteins: one doublet (38-40 kDa) and sometimes an additional protein at 53 kDa (*Aal*DNV) -Representatives: AaeDNV: *Aedes aegypti*, AalDNV: *Aedes albopictus*.
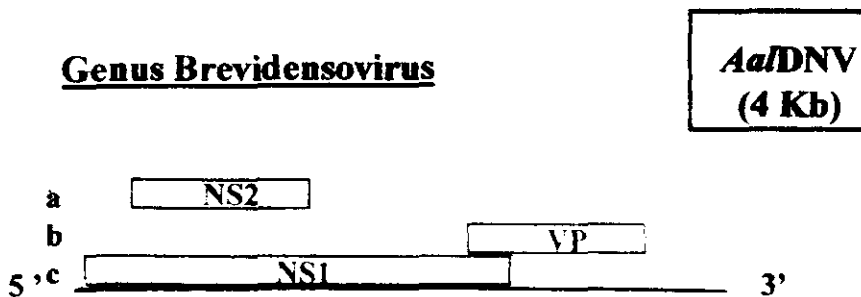
Fig. (1) : Overall organization of the genomes of insect parvoviruses (*Densovirinae*).

ORF and starts 4 nucleotides downstream of the initiation codon of NS1. A single large ORF codes for the four structural polypeptides on the complementary strand. In the subgroup B, containing *Ad*DNV, *Pc*DNV and *Pf*DNV, the genomes differ from that of subgroup A by the presence of two overlapping ORFs on one strand, each coding very likely for a single NS protein, and by the VP gene coding for the four structural polypeptides on the complemetary strand which is split into two reading frames. The *Cp*DNV is so far the only representative of subgroup C. On one strand of its genome two overlapping genes code probably for two NS proteins. The coding sequence of each gene is split into two ORFs, so that four ORFs distributed in the three reading frames presumably participate to yield these NS proteins (Bergoin and Tijssen, 2000).

DNVs belonging to genera Iteravirus and Brevidensovirus (Fig. 1) share with the vertebrate Parvovirus a monosense organization of their genome, *i.e.*, the sequences coding for NS and VP proteins are located in the left and the right halves of the same strand, respectively. In both Iteravirus and Brevidensovirus genera, two overlapping ORFs encode for two proteins with the typical motifs of the NS proteins whereas the structural proteins originate from the right ORF, but their size, location and sequence differ considerably.

A shared characteristic of all Parvoviruses is the presence of self-priming hairpins at the ends of the genome. This enables a host cell DNA polymerase to convert the single strand into a double strand and, subsequently, a cellular ligase to create covalently linking of the newly synthesized strand to the hairpin at the other end of the genome. This double stranded DNA can then be transcribed by host cell RNA polymerase and be translated into NS protein required for replication (Bergoin and Tijssen, 2000).

CONCLUSION

The molecular analysis of Densovirus genomes permits an unequivocal identification of individual genera, species and strains, and is also predictive of the properties of previously unknown or novel virus.

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(مقالة مرجعية)

التنوع الجزيئي لفيروسات البارفوفيروس (Parvovirus) التي تصيب
اللافقاريات (تحت عائلة فيروسات الدينسو Densovirinae)

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ملخـــــص

تعتبر فيروسات الدينسو Densovirus (DNV) مـــن الفيروسـات الصغيرة ذات العشرون وجها، والتي تم عزلها من أنواع عديدة من الحشـــرات، وبصفة أساسية من حشرات حرشفية الأجنحة. وهذه الفيروسات مصنفة داخـــل عائلة فيروسات البارفوفوريدي (Parvoviridae). ولقـــد أكتسـبت فيروسـات الدينسو أهمية في المكافحة البيولوجية لأن زيادة المعرفـــة بـــها يسـاعد علـــي الاستخدام الأمثل لها في مكافحة الآفات الحشرية. لقـــد تـم سـرد الخصــائص المختلفة لهذه الفيروسات، مع التركيز علي التباين الكبير في التنظيـــم الجينومـــي genomic organization لها. و تؤكد دراســة الخصـــائص المختلفـة لـــهذه المجموعة من الفيروسات مثل الخصائص البيولوجية، الخصــائص البيوفيزيقيـــة والبيوكيمائية والخصائص الجزيئية للجينوم أن التحليل الجزيئي لجينوم الفـــيروس يعتبر هو الأسلوب الأكثر سرعة ودقة لفصل وتمييز الفيروسات المختلفة التابعـــة لنفس المجموعة.