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GENOME ANNOUNCEMENT

Papilio polyxenes Densovirus Has an Iteravirus-Like Genome Organization

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The genome of *Papilio polyxenes* densovirus was cloned and sequenced and contained 5,053 nucleotides (nt), including inverted terminal repeats (ITRs) of 271 nt with terminal hairpins of 175 nt. Its DNA sequence and monosense organization with 3 open reading frames (ORFs) are typical of the genus *Iteravirus* in the subfamily *Densovirinae* of the *Parvoviridae*.

The larvae of the black swallowtail (*Papilio polyxenes*; family *Papilionidae*), a butterfly found throughout eastern North America, feed gregariously on host plants of the carrot family (*Umbelliferae*), such as dill, parsley, and fennel. These plants produce stimulatory compounds for chemoreception of this insect but also furanocoumarins that are toxic and serve as a defense mechanism against various insect predators. *Papilio polyxenes*, which adapted to furanocoumarin-containing host plants, therefore provides an excellent laboratory model to study insect-plant coevolution (1, 10). During recent years, significant mortality was observed in laboratory animals and electron microscopy examination revealed isometric particles of about 20 nm in diameter (F. Pringle, unpublished observations), characteristic of densoviruses. A significant proportion of the larvae obtained from the field were also infected.

Papilio polyxenes densovirus (PpDNV) was partially purified from 0.5 g larvae as described previously for *Galleria mellonella* DNV (GmDNV) (5) and *Sibine fusca* Stoll DNV (SfDNV) (11). PpDNV DNA, extracted under conditions of high ionic strength to anneal the single-stranded DNA (ssDNA), had a size of around 5 kb. A sequence-independent, single-primer amplification (SISPA) method (6) was used as previously described (11). The amplicons were cloned into the pGEM-T vector by the TA cloning method (3) and sequenced by Sanger's method as described previously (8). A unique SacI restriction site detected near the middle of a 4.7-kb sequence was used to clone the blunt-ended PpDNV DNA, obtained from virus, restricted with SacI in the EcoRV and SacI sites of the PCR2.1 vector. Clones with a 1.6-kb insert and clones with a 3.4-kb insert were obtained, and four inserts of each set were sequenced in both directions using Sanger's method and the primer-walking method as described previously (8). Insert sequences were identical in each set except for the flip-flop sequences.

The overall sequence had a high identity with iteraviruses (identity of about 78% to *Casphalia extranea* DNV [CeDNV], about 75% to *Bombyx mori* DNV [BmDNV-1], about 74% to SfDNV, and about 67% to *Dendrolimus punctatus* DNV [DpDNV]). The PpDNV genome contained typical inverted terminal repeats (ITRs) of the four members of the *Iteravirus* genus (BmDNV-1, CeDNV, SfDNV, and DpDNV), albeit a bit longer (271 versus 230 nucleotides [nt]) (7). The terminal J-shaped hairpins of 175 nt were about 80% conserved with BmDNV-1 (4), CeDNV (2), SfDNV (11), and DpDNV (9). In the hairpins, nt 67 to 109 and nt 4945 to 4987 occurred in two orientations, "flip" and its reverse-complement orientation "flop," that were close to 100% identical to the flip-flop of the other iteraviruses.

Similar to other iteraviruses, the PpDNV monosense genome contained three intronless genes with essentially identical positions and sizes. The largest, open reading frame 1 (ORF1) (nt 349 to 2631), had a coding capacity of 760 amino acids (aa) and the typical nucleoside triphosphatase (NTPase) motif for NS1 (2). ORF2 (nt 2686 to 4707) with the phospholipase A2 motif typical for parvovirus VP (12) had a coding capacity of 673 aa. ORF3 corresponded to NS2 with a 455-aa coding capacity and typically overlapped the N terminus of NS1 (nt 482 to 1849). As a comparison, NS1 is 753 to 775 aa, NS2 is 451 to 453 aa, and VP is 668 to 678 aa for the other iteraviruses.

Nucleotide sequence accession number. The GenBank accession number for PpDNV is JX110122.

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