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GENOME ANNOUNCEMENT

Organization of the Ambisense Genome of the *Helicoverpa armigera* Densovirus

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A natural densovirus (DNV) of a serious phytophagous pest, *Helicoverpa armigera*, was isolated. The genome of HaDNV contained 6,039 nucleotides (nt) and included inverted terminal repeats (ITRs) of 545 nt with terminal Y-shaped hairpins of 126 nt. Its DNA sequence and ambisense organization with four typical open reading frames (ORFs) demonstrated that it belonged to the genus *Densovirus* in the subfamily *Densovirinae* of the family *Parvoviridae*.

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Noctuidae), is one of the most serious polyphagous pest species, causing huge losses predominantly in the Old World. Economic damage is greatest in cotton and vegetables. In grain legumes, which are staple foods for people in many countries, up to 80% of the crop can be destroyed. The cotton bollworm also attacks a great number of cereal, vegetable, and garden crops.

The cotton bollworm has become increasingly resistant to pesticides (3), necessitating biological or integrated pest management. Densoviruses (DNVs) have shown great potential, e.g., complete control of *Casphalia extranea* in the oil palm industry in Ivory Coast (1). Since the late 1980s, we have been developing a program to obtain natural DNV isolates from pests in agricultural fields in Egypt. This program led previously to the isolation of the MlDNV (a pathogen of the maize worm) (2). Here we report the isolation, cloning, and sequence analysis of a densovirus, HaDNV, from the cotton bollworm. Infected larvae stopped feeding within a few days and died within a week. The virus remained infectious for months in cadavers in the field.

HaDNV DNA, extracted from the virus under conditions of high ionic strength to anneal the single-stranded DNA (ssDNA), has a size of around 6 kb. This DNA was blunt-ended by a mixture of Klenow fragment and T4 DNA polymerase and cloned into the EcoRV site of the pBluescript vector. The restriction profiles of the viral DNA indicated a close relationship to both MlDNV and *Galleria mellonella* densovirus (GmDNV). Digesting viral DNA with BamHI gave rise to a 5.5-kb fragment and a 0.3-kb fragment. This small DNA fragment was probably a doublet, resulting from the existence of symmetrical BamHI sites within the viral putative inverted terminal repeats (ITRs), similar to fragments from some other densoviruses (2, 4, 5). This observation helped to clone the viral DNA by methods that were successfully employed for GmDNV and MlDNV (2, 5). Two complete clones, pHa2 and pHa8, were sequenced, both in both directions, using Sanger's method and the primer-walking method as described before (5). The sequences of the two clones were identical.

The HaDNV genome contained inverted terminal repeats (ITRs), typical of members of the *Densovirus* genus, with a length of 545 nucleotides (nt). The terminal Y-shaped hairpins of 126 nt are completely conserved between HaDNV and MlDNV. The overall sequence is 90% identical to the corresponding sequence of MlDNV and 86% identical to that of GmDNV.

The large open reading frame (ORF) 1 (nt 657 to 1352) on the plus strand had a coding capacity for NS1 of 547 amino acids (aa). ORF2 corresponded to NS2 (nt 1359 to 2996) with 275 aa, and ORF3 (nt 1366 to 2193) corresponded to NS3 with 231 aa. On the complementary minus strand, a large ORF (also on the 5′ half at nt 3028 to 5463) with a potential coding region of 811 aa corresponded well to those of the VP structural proteins of related densoviruses. The distribution of the putative coding sequences of HaDNV on the two viral strands thus implied an ambisense organization, and HaDNV contained the typical NS-1 helicase superfamily III and VP phospholipase A2 (6) motifs observed in parvoviruses.

Nucleotide sequence accession number. The GenBank accession number of HaDNV is JQ894784.

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