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Genome sequence and organization of a nucleopolyhedrovirus that infects the tea looper caterpillar, *Ectropis obliqua*

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Abstract

The complete nucleotide sequence of *Ectropis obliqua* nucleopolyhedrovirus (EcobNPV), which infects the tea looper caterpillar, was determined and analyzed. The double stranded circular genome is composed of 131,204 bp and is 37.6% G+C rich. The analysis predicted 126 putative, minimally overlapping open reading frames (ORFs) with 150 or more nucleotides that together compose 89.8% of the genome. The remaining 10.2% constitute non-coding and three homologous regions. Comparison with previously sequenced baculoviruses indicated that three ORFs were unique to EcobNPV, while the remaining 123 ORFs shared identity with other baculovirus genes. In addition to two *bro* homologues, three other repeat ORFs, including *dbp*, *p26*, and *odv-e66*, were identified. Phylogenetic analysis indicated that each member of the paired ORFs was acquired independently. Gene parity plot analysis and percent identity of gene homologues suggested that EcobNPV is a Group II NPV, although its genomic organization was highly distinct.

Keywords: *Ectropis obliqua*; Nucleopolyhedrovirus; Complete genome; Gene parity plot; Phylogeny

Introduction

The Baculoviridae represent a large and diverse family of rod-shaped viruses with circular, double stranded DNA genomes ranging in size from the 81.7 kb genome of *Neodiprion lecontei* nucleopolyhedrovirus (NeleNPV) to the 178.7 kb genome of *Xestia c-nigrum* GV (XcGV). The baculovirus family includes two genera, the nucleopolyhedroviruses (NPV) and granuloviruses (GV). Lepidoptera NPVs are subdivided into Groups I and II based on their molecular phylogenies (Zanotto et al., 1993). Updated classification has proposed to expand the baculovirus family into four genera: the alphabaculoviruses (Lepidopteran-specific NPV), betabaculoviruses (lepidopteran-specific GV), gammabaculoviruses (hymenopteran-specific NPV), and deltabaculoviruses (dipteran-specific baculovirus) (Jehle et al., 2006). To date, 34 completely sequenced baculovirus genomes have been determined, including 22 alphabaculoviruses, eight betabaculoviruses, three gammabaculoviruses, and one deltabaculovirus based on this proposed scheme.

The tea looper caterpillar, *Ectropis obliqua* (Lepideptera: Geometride), infests tea plants in East Asia and can cause considerable damage (Chen and Huang, 2001). *E. obliqua* nucleopolyhedrovirus (EcobNPV) is a singly embedded NPV (Ma et al., 2006) that is pathogenic to this caterpillar. As a result, EcobNPV has become an important biological insecticide (Hu et al., 1994) that is now commercially available in China.

Restriction maps of EcobNPV have been assembled and 15.5 kb of the *polyhedrin* region is characterized (Ma et al., 2006). In this study, the complete genome of EcobNPV has been sequenced and analyzed in order to better understand how this virus evolved, as well as its molecular mechanisms of infection and replication.

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Results and discussion

Nucleotide sequence analysis of the EcobNPV genome

The EcobNPV genome consists of 131,204 bp (GenBank accession no.DQ837165), which is similar to the 127.7 kb size predicted by restriction enzyme analysis (Ma et al., 2006), and within the 81.7 and 178.7 kb range for baculovirus genomes. The EcobNPV genome is highly AT-rich, having only 37.6% G+Cs, similar to *Chrysodeixis chalcites* NPV (ChchNPV, 39.1%), *Helicoverpa armigera* NPV (HearNPV, 39.1%), *Helicoverpa zea* SNPV (HzSNPV, 39.1%), *Rachiplusia ou* MNPV (RaouMNPV, 39.1%), and *Adoxophyes honmai* NPV (AdhoNPV, 35.6%), but lower than most other sequenced NPVs.

According to the adopted convention (Vlak and Smith, 1982; Hayakawa et al., 1999; Ijkel et al., 1999), the adenine residue at the translation initiation codon of the *polyhedrin* gene represented the zero point on the EcobNPV physical map, and was designated ORF 1 (Fig. 1 and Table 1). 126 putative ORFs and three homologous regions (hrs) were detected in the EcobNPV genome using computer-assisted analysis to select ORFs starting from the methionine-initiated codon (ATG) and including at least 50 amino acids (aa) having minimal overlap with other ORFs. All 126 ORFs are shown in Table 1 by location, orientation, size, and potential baculovirus homologues. The number of ORFs was similar to other fully sequenced baculoviruses, which range from 89 (NeleNPV) to 181 (XcGV), and was most like AdhoNPV (125). EcobNPV

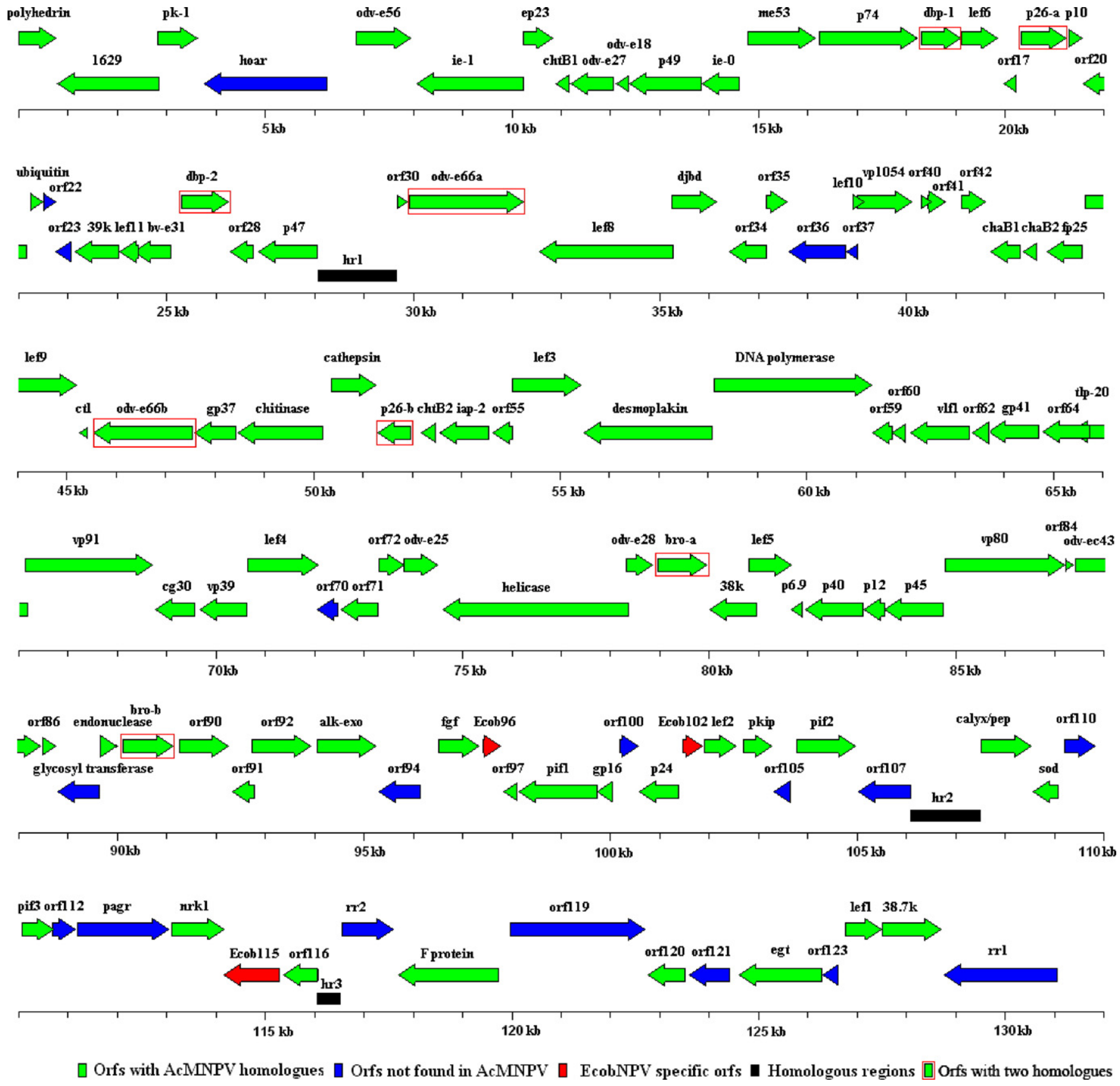

Fig. 1. Linear map of the 126 predicted ORFs for the complete EcobNPV genome.