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Morphological and genomic characterization of the polydnavirus associated with the parasitoid wasp *Glyptapanteles indiensis* (Hymenoptera: Braconidae)

Y. P. Chen and D. E. Gundersen-Rindal

USDA-ARS Insect Biocontrol Laboratory, Beltsville, MD 20705, USA

Correspondence
Dawn Gundersen-Rindal
gundersd@ba.ars.usda.gov

Glyptapanteles indiensis polydnavirus (GiPDV) is essential for successful parasitization of the larval stage of the lepidopteran *Lymantria dispar* (gypsy moth) by the endoparasitic wasp *Glyptapanteles indiensis*. This virus has not been characterized previously. Ultrastructural studies of GiPDV showed that virions had a rod-like or rectangular form and each contained as many as ten nucleocapsids enclosed by a single unit membrane envelope. Field inversion gel electrophoresis (FIGE) analysis of the virus genomic DNA revealed that GiPDV had a segmented genome composed of 13 dsDNA segments, ranging in size from approximately 11 kb to more than 30 kb. Four genomic segments were present in higher molar concentration than the others. Further characterization of the GiPDV genome yielded several cDNA clones which derived from GiPDV-specific mRNAs, and Northern blot analysis confirmed expression of isolated cDNA clones in the parasitized host. Each was present on more than one GiPDV genomic DNA segment, suggesting the existence of related sequences among DNA segments. It has been proposed previously that in polydnavirus systems, genome segmentation, hypermolar ratio segments and segment nesting may function to increase the copy number of essential genes and to increase the levels of gene expression in the absence of virus replication. The present data support this notion and suggest that GiPDV morphology and genomic organization may be intrinsically linked to the function and evolutionary strategies of the virus.

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INTRODUCTION

Polydnaviruses (PDVs) are complex insect viruses characterized by segmented double-stranded circular DNA genomes that vary in size and molar ratio (Stoltz *et al.*, 1984). Viral DNA is integrated into the wasp genome as provirus and transmitted vertically through the germ-line (Stoltz *et al.*, 1984, 1986; Fleming & Summers, 1986, 1991; Fleming, 1992; Fleming & Krell, 1993; Stoltz, 1993). PDVs replicate only in the nucleus of calyx epithelial cells of the wasp ovaries during the pupal stage, and are stored in the lumen of the oviduct (Norton & Vinson, 1983; Theilmann & Summers, 1986). During oviposition, PDVs are injected with eggs into the host, and enter different host tissues. Within the host tissues, PDVs do not replicate, but host-specific viral genes are expressed. PDV gene products function to disrupt the host immune system as well as host development, which appear to be essential for successful parasitoid development (Stoltz, 1993; Lawrence & Lanzrein, 1993, 1994; Lavine & Beckage, 1995; Strand & Pech, 1995).

The PDVs are recognized as two genera: the ichnoviruses found exclusively in parasitoid wasps of the Ichneumonidae, and the Bracoviruses found only in parasitoid wasps of the Braconidae. *Ichnovirus* and *Bracovirus* differ from each other in morphology, host range and molecular characteristics (Stoltz & Whitfield, 1992). Ichnoviruses characteristically contain one lenticular nucleocapsid per virion surrounded by two unit membranes, whereas bracoviruses characteristically contain one or more rod-shaped nucleocapsids per virion surrounded by a single unit membrane envelope. It is thought that Ichnoviruses are released into the ovary lumen by budding from calyx cells, while bracoviruses, in contrast, are believed to be released through lysis of the calyx cells.

The PDV genome structure is complex and poorly understood. Only a few PDV genomes have been described in detail. Estimates of PDV genome sizes have been complicated by the characteristic presence of unequal molar ratios of genomic segments, comigrating DNA segments of equal size, and direct terminal repeats (Fleming, 1992; Fleming & Krell, 1993; Stoltz, 1993). Comparisons of various PDV genomes showed that the DNA segment number and molar ratio and the total genome size appeared to be specific to

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the PDVs of each individual PDV-containing wasp species (Krell, 1991; Stoltz, 1993; Fleming & Krell, 1993). Descriptions of ichnovirus genomes have been largely based on studies of PDVs isolated from the parasitoid wasps *Campoletis sonorensis* (Krell *et al.*, 1982), *Hyposoter exiguae* (Krell & Stoltz, 1980) and *Diadegma terebrans* (Krell, 1987). Of these ichnoviruses, the *C. sonorensis* polydnavirus (CsPDV) genome has been by far the most systematically characterized, and appears to contain 28 DNA segments ranging in size from 5·5 to 21 kbp. Genome analyses of bracoviruses have been less complete than those described for Ichnoviruses. The available information on bracovirus genomes comes mainly from descriptions of the PDVs associated with the parasitoids *Cardiochiles nigriceps* (Varricchio *et al.*, 1999), *Chelonus inanitus* (Albrecht *et al.*, 1994; Johner & Lanzrein, 2002; Wyder *et al.*, 2002) and *Microplitis demolitor* (Strand *et al.*, 1992). In general, the genome segments of bracoviruses tend to be fewer in number but larger in size than those of the ichnoviruses, and exhibit a relaxed open circular topology (Albrecht *et al.*, 1994; Webb, 1998). For example, characterization of the bracovirus of *C. inanitus* showed that its genome consisted of 10 different segments ranging in size from 7 to 31 kbp, and that individual DNA segments appeared to be singly encapsidated (Albrecht *et al.*, 1994).

The braconid endoparasitic wasp *Glyptapanteles indiensis* parasitizes the larval stage of the gypsy moth, *Lymantria dispar*. Previous studies on *G. indiensis* polydnavirus (GiPDV) in our laboratory demonstrated that GiPDV could be integrated not only as a provirus within the parasitoid wasp genome, but also *in vitro* within the chromosomal DNA of cells derived from the natural host *L. dispar* (Gundersen-Rindal & Dougherty, 2000). More recently, a GiPDV putative protein tyrosine phosphatase gene believed to be associated with gene regulation during immune response was shown to be differentially expressed in various tissues of the parasitized host (Chen *et al.*, 2003). Although information on the *in vitro* and *in vivo* properties of GiPDV has been gathered, fundamental knowledge pertaining to GiPDV virion morphology and genomic organization remains undescribed. To fill this gap, the morphological features, genomic organization and molecular features of GiPDV were investigated and are reported here. The isolation of cDNA clones representing GiPDV sequences from mRNA isolated from parasitized host and confirmation of the expression of cDNA clones in the parasitized host are described. Further, mapping of these cDNA fragments to the GiPDV genome is shown.

METHODS

Insect rearing. The parasitoid wasp was reared from larvae of the host insect, *L. dispar*. Adult wasps were fed with 30 % honey water. Both *G. indiensis* and its host were maintained at 26 °C, 50 % relative humidity and a 16 h light : 8 h dark photoperiod according to the methodology established by Bell *et al.* (1981). To obtain parasitized larvae, individual *L. dispar* larvae were exposed to a single *G. indiensis* female within a 35 × 10 mm Petri dish to ensure 100 % parasitization. After a single oviposition was observed, each parasitized larva was removed from the Petri dish and reared in a plastic cup containing high wheat-germ diet for 24 h prior to RNA extraction.

Virus purification and viral DNA isolation. Virus was purified from the calyx fluid of female wasps. Female wasps were anaesthetized in 75 % ethanol for a few seconds and then rinsed in sterile H_2O. Each pair of ovaries was dissected in a 15 µl drop of cold PBS and punctured individually with forceps, causing the calyx fluid to diffuse into the PBS drop. The calyx fluid was filtered through a 0·45 µm filter to remove eggs and cellular debris by the methods of Beckage *et al.* (1994). The resulting viral fluid was transferred into a 1·5 ml microcentrifuge tube and immediately processed for viral DNA isolation and electron microscopy of viral ultrastructure.

Electron microscopy of viral particles. For negative staining, GiPDV virus particles in filtered calyx fluid were absorbed onto a 400 mesh Formvar-coated nickel grid for 2 s, quickly washed in distilled water, stained with 4 % uranyl acetate for 1 min, and then viewed in an H-7000 Hitachi electron microscope at 75 kV. For thin sections, embedded ovaries dissected from female *G. indiensis* were fixed in 3 % glutaraldehyde/0·05 M sodium cacodylate buffer (pH 7·0) for 2 h at room temperature and placed in a 4 °C refrigerator overnight. After washing in sodium cacodylate buffer six times within 1 h, ovaries were post-fixed in 2 % buffered osmium tetroxide for 2 h, dehydrated in graded ethanol solutions and infiltrated with Spurr's low-viscosity embedding resin. Ovaries were sectioned on a Riechert/AO Ultracut microtome with a Diatome diamond knife. Silver sections of the ovaries were mounted on 200-mesh Ni grids, stained with 4 % uranyl acetate and 3 % lead citrate, and observed in an H-7000 Hitachi electron microscope at 75 kV.

Viral DNA isolation and field inversion gel electrophoresis (FIGE). Filter-purified viral fluids were incubated in an equal volume of extraction buffer (4 % sarcosyl, 1 % SDS, 50 mM EDTA, 10 mM Tris, 0·2 M NaCl, 20 µg RNase ml^{-1}, 50 µg proteinase K ml^{-1}) at 50 °C for 2 h, followed by two extractions with ultrapure buffer-saturated phenol (Invitrogen). DNA was precipitated in 2 vols of cold ethanol and centrifuged at 15 000 r.p.m. for 30 min. The viral DNA pellet was resuspended in TE buffer and stored at 4 °C. The purified viral DNA was analysed by FIGE, an electrophoretic method used to resolve DNAs in the 10–150 kb range. Undigested viral DNA (2 µg) and a molecular size standard (DIG-labelled linear λ DNA digested with *Hin*dIII) were loaded onto a 0·8 % (w/v) pulse field certified agarose gel (Bio-Rad) in 0·5 × TBE buffer (50 mM Tris/borate, 1 mM EDTA, pH 8·3). Gel electrophoresis was performed at 4 °C for 2 × 24 h using a 0·1–0·4 s switch time ramp with 180 V forward voltage and 120 V reverse voltage for each 24 h run. The gels were stained with ethidium bromide for 15 min, destained with sterile H_2O for 30 min, and visualized using a UV transilluminator.

cDNA library construction and screening. Total RNA was isolated from parasitized *L. dispar* larvae 24 h post-parasitization. Insects were frozen in liquid nitrogen and ground to a fine powder. RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA samples were resuspended in nuclease-free water in the presence of ribonuclease inhibitor (Invitrogen). The RNA quality was verified by formaldehyde gel electrophoresis, and mRNA was isolated from total RNA using the Messagemaker Reagent Assembly kit (Life Technologies) following the manufacturer's instructions. The concentration of mRNA was measured using a spectrophotometer. cDNA libraries were prepared using the Superscript Choice System for cDNA Synthesis (Invitrogen) following the manufacturer's instructions. mRNA was converted into size-fractionated, *Eco*RI-adapted cDNA. The cDNA was ligated into the *Eco*RI-digested λZIPLOX expression vector (Life Technologies). The ligated vector-cDNAs were packaged in Gigapack III Gold

Packaging Extract (Stratagene). The packaged cDNA-containing phage were introduced into Y1090 (ZL) *E. coli* that had been grown overnight in LB medium with 10 mM $MgSO_4$ and 0·2 % maltose. The transformants were plated on LB-agar and incubated overnight at 37 °C. The cDNA libraries were titrated and amplified, and approximately 5×10^4 p.f.u. of library were plated onto ten large LB plates. The plaques were transferred to nitrocellulose membrane (Roche) and the phage DNAs were denatured, neutralized and UV cross-linked to membrane according to standard procedures. The GiPDV genomic DNA used as a probe was radiolabelled to high specific activity with the Random Primers DNA Labelling System (Invitrogen) following the manufacturer's instructions. Hybridization screening of cDNA libraries using the ^{32}P-labelled GiPDV probe was carried out in the hybridization solution (6× SSPE, 0·5 % SDS, 5× Denhardt's solution, 100 µg denatured fragment salmon sperm DNA ml^{-1}) at 65 °C for 16 h. Membranes were washed once in lower stringency solution (1× SSPE, 0·1 % SDS) for 15 min at room temperature and twice in medium stringency solution (0·5× SSPE, 0·1 % SDS) at 48 °C for 15 min. After air-drying, membranes were exposed to Hyperfilm (Amersham Pharmacia) at −80 °C overnight. Positive plaques were identified and subjected to a second round of screening. Two positive clones confirmed by secondary screening were picked with a Pasteur pipette and resuspended in 500 µl of SM buffer (50 mM Tris/HCl, pH 7·9, 100 mM NaCl, 10 mM $MgSO_4$, 0·01 % gelatin) and stored at 4 °C with 20 µl of chloroform. Conversion of recombinant phage into plasmid (*in vivo* excision) was done by infecting *E. coli* DH10B(ZIP), according to the manufacturer's instructions (Life Technologies). Plasmid DNA containing the cDNA insert was purified for each using a Plasmid Miniprep kit (Bio-Rad).

DNA sequence analysis and nucleotide sequence accession numbers. Sequencing reaction of isolated cDNA clones was carried out with the ABI PRISM Big Dye Terminator Cycle Sequence kit (Applied Biosystems) using T7 and SP6 promoter-specific primers, followed by primer walking. The nucleotide sequences of the cDNA clones were determined using an ABI PRISM 310 Genetic Analyser (Applied Biosystems). Sequence fragments were edited and assembled into contiguous regions using the SeqManII sequence assembling software of the DNASTAR package. Sequence homology searches were done using BLAST (Altschul *et al.*, 1990) and searched against the GenBank database. Sequences have been deposited in GenBank with accession numbers AF414845 and AF414846, respectively.

Northern blot analysis. Expression of isolated cDNA clones in the parasitized host was confirmed by Northern blot analysis. The RNA samples isolated from parasitized *L. dispar* larvae 24 h post-parasitization and nonparasitized *L. dispar* larvae were resolved on denaturing formaldehyde/1 % agarose gel and blotted onto nylon membrane. DIG-labelled RNA probes complementary to two isolated cDNA clones were synthesized using the DIG RNA labelling kit (Roche). The membrane was prehybridized in prehybridization solution (50 % formamide, 5 % blocking reagent, 5× SSC, 0·1 % sarcosyl, 0·1 % SDS) at 52 °C for 2 h, followed by hybridization with DIG-labelled RNA probes overnight. After hybridization, the membrane was washed twice in low stringency wash solution (2× SSC, 0·1 % SDS) at room temperature for 5 min and washed twice in high stringency wash solution (0·1× SSC, 0·1 % SDS) at 52 °C for 15 min. The hybridization signals were detected with anti-digoxigenin–AP Fab fragments (Roche) and visualized with chemiluminescence substrate CDP-Star, ready-to-use (Roche). The same membrane was probed with each DIG-labelled RNA probe (1·1 and 1·8) and a DIG-labelled β-actin cDNA probe, individually, under identical conditions. For repeated hybridization to the same Northern blot, the nylon membranes were washed in stripping solution (50 % formamide, 50 mM Tris/HCl, pH 7·5, 5 % SDS) for 2× 45 min at 60 °C to remove the probe.

Genome mapping of cDNAs. Southern hybridization was performed to map the two isolated cDNA fragments to a specific region(s) of the GiPDV genome. FIGE-separated viral genomic DNA segments were transferred from the gel to nylon membrane (Hybond-N+; Amersham Pharmacia) and UV cross-linked for analysis. cDNA clones were DIG-labelled using the DIG DNA labelling kit (Roche) according to the manufacturer's instructions. Membranes with separated GiPDV genomic segments were hybridized with a DIG-labelled cDNA probe in DIG Easy Hybridization solution (Roche) for 16 h at 48 °C. After hybridization, the membranes were washed twice in low stringency wash solution (2× SSC, 0·5 % SDS) at room temperature for 5 min and washed twice in high stringency wash solution (0·1× SSC, 0·5 % SDS) at 48 °C for 15 min. After the washes, anti-DIG antibody was used to bind to the DIG-labelled probe and the chemiluminescent substrate, CSPD, was used to visualize antibody binding. The banding pattern and signal were detected on X-ray film (Amersham) after a 30 min exposure. The same membrane was probed with two different cDNA probes individually. For repeated hybridization of the same blot, the nylon membranes were washed in stripping solution (0·2 M NaOH, 0·1 % SDS) for 30 min at 37 °C to remove the probe.

RESULTS

Electron micrographs of GiPDV

Thin section analysis of the calyx region in the lumen of the *G. indiensis* oviduct revealed the presence of large quantities of viral particles (Fig. 1a). Variable densities of viral particles were seen among different calyx cells (Fig. 1b). Each viral particle contained, at most, ten nucleocapsids enveloped by a single unit membrane, with an average of six nucleocapsids per virion (Fig. 1c). The size and shape of virions were variable, with a mean length of $273 \pm 34{\cdot}24$ nm, ranging from 175 to 350 nm, and a mean width of $162 \pm 19{\cdot}32$ nm, ranging from 140 to 190 nm ($n = 80$) (Fig. 2a).

A negative stain of GiPDV particles present in calyx fluid showed the nucleocapsids were rod-like or rectangular in shape. The electron-dense cores were probably DNA genome with their associated proteins (Fig. 1d). The sizes of nucleocapsids were slightly variable, with a mean length of $58 \pm 5{\cdot}21$ nm, ranging from 46 to 66 nm, and a mean width of $38 \pm 6{\cdot}37$ nm, ranging from 34 to 52 nm ($n = 60$) (Fig. 2b).

Electrophoretic profiles of GiPDV genomic DNA

GiPDV FIGE hybridization patterns showed that GiPDV comprised multiple DNA segments, similar to other previously described polydnaviruses, and 13 visible bands were recognized ranging in size from approximately 11 kb to more than 30 kb (Fig. 3). The various DNA segments were present in non-equimolar ratios, as indicated by differing band intensities. Four segments (D, F, K and M) were present in higher molar concentration than the other segments (Fig. 3). Assuming that each DNA segment was unique, and that there were no co-migrating DNA segments, GiPDV genomic size was estimated to be 250 kb.

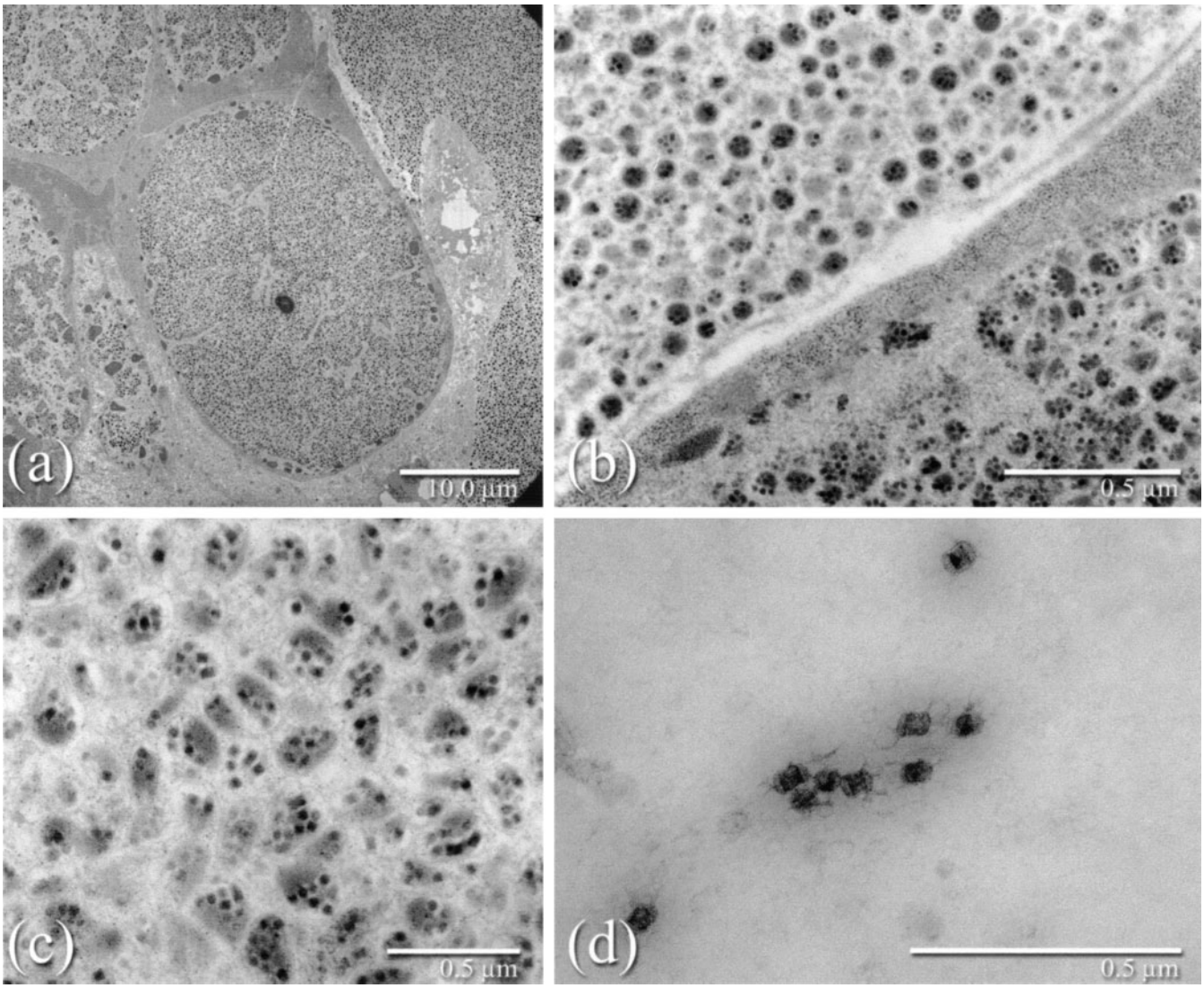

Fig. 1. Electron micrographs of *G. indiensis* polydnavirus. (a) Thin section showing calyx region in the lumen of the *G. indiensis* oviduct. Large numbers of viral particles were seen in calyx epithelial cells. (b) Thin section showing separate desmosomes joining two calyx cells. Different densities of viral particles between the calyx cells may show different levels or stages of replication occurring in the two cells. (c) Thin section showing virus particles within the calyx fluid. Each virion consists of 5 to 10 nucleocapsids enclosed by a single membrane envelope. Bar marker represents 0·5 μm. (d) Negative staining of liberated GiPDV nucleocapsids (35 nm wide × 46 nm long). Electron micrograph: Charlie Murphy and Christopher Pooley.

Screening of parasitized host cDNA libraries and isolation of expressed GiPDV-specific cDNA clones

To further characterize the complexity of the GiPDV genome, cDNA was synthesized and cloned from mRNA isolated from parasitized *L. dispar* larvae 24 h after *G. indiensis* parasitization. After screening the library with GiPDV, two positive cDNA clones, which derived from GiPDV-specific mRNA expressed in parasitized hosts, were identified, isolated and sequenced. The first cDNA clone, cDNA 1·1, contained a 1·1 kb insert, whereas the second cDNA clone, cDNA 1·8, contained a 1·8 kb insert. The sequences of cDNA 1·1 and cDNA 1·8 are presented in Fig. 4 and Fig. 5. Clone 1·1 contained a single large open reading frame (ORF) encoding 325 amino acids. Clone 1·8 contained one large ORF comprising 494 amino acids. BLAST searches of these sequences did not yield any significant homology to nucleotide or amino acid sequences in GenBank.

Northern blot analysis of cDNA clones

The expression of cDNA 1·1 and cDNA 1·8 in parasitized host was confirmed by Northern hybridization. Using

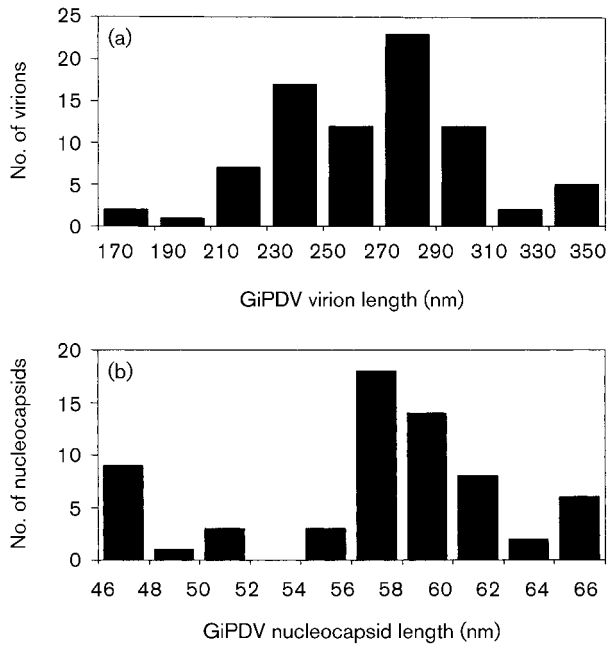

Fig. 2. (a) Distribution of GiPDV virion length (nm) ($n=80$). (b) Distribution of GiPDV nucleocapsid length ($n=60$).

DIG-labelled RNA complementary to cDNA 1·1 as a hybridization probe, one transcript of 1·1 kb was identified. Using DIG-labelled RNA complementary to cDNA 1·8 as a hybridization probe, one major transcript of 1·8 kb and two minor transcripts of 1·7 kb and 2·6 kb were identified. No hybridization signal was detected with RNA extracted from non-parasitized gypsy moth host (Fig. 6).

Mapping of isolated cDNA clones to the GiPDV genome

In order to determine which GiPDV genomic DNA segments encoded these two expressed GiPDV-specific mRNAs, cDNA 1·1 and cDNA1·8 were DIG-labelled and used as probes to hybridize individually to an identical Southern blot containing size-fractionated genomic DNA. Mapping studies indicated that both cDNAs hybridized to more than one genomic DNA segment and different cDNAs were present on the same genome segment. cDNA 1·1 probe hybridized to four viral DNA segments (C, D, E, K) and cDNA 1·8 probe hybridized to two viral DNA segments (J, K), as shown in Fig. 7.

DISCUSSION

Characteristics of GiPDV morphology

Polydnaviruses have been found to be mutualistically associated with some hymenopteran endoparasites in the families Braconidae and Ichneumonidae and have been characterized in approximately 40 species of wasps (Fleming, 1992). The morphology of the braconid polydnavirus GiPDV was

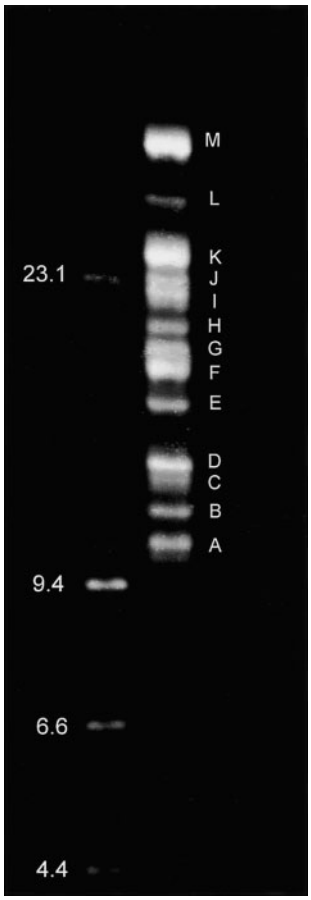

Fig. 3. FIGE profile of GiPDV genomic DNA. Undigested GiPDV genomic DNA and DIG-labelled linear λ DNA markers digested with *Hin*dIII were loaded into a 0·8 % pulse field certified agarose gel. FIGE was performed at 4 °C. Major DNA segments are indicated by upper-case letters. Fragment sizes (kb) are labelled.

observed by transmission electron microscopy and the morphological characteristics were similar to those of other described bracoviruses. The calyx fluid of *G. indiensis* females contained large quantities of GiPDV viral particles. The GiPDV virions were ovoid in form and contained electron-dense nucleocapsids enclosed by a single unit membrane envelope. Compared with previously characterized bracoviruses, virions of GiPDV seemed to contain more numerous nucleocapsids; as many as six to ten nucleocapsids in a single virion were frequently observed. Variation in the number of nucleocapsids that appeared in the virions may have been due to the different angles at which the virions were observed under electron microscopy. Compared with those of other bracoviruses, GiPDV nucleocapsids were fairly large, with a mean length of $58\pm5{\cdot}21$ nm, ranging from 46 to 66 nm, and a mean width of $38\pm6{\cdot}37$ nm, ranging from 34 to 52 nm. The largest nucleocapsids reported to date were found in the PDV associated with *Chelonus texanus*, with each PDV having a

Clone 1.1:

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1    ccg gcc gcg tcg act gta gct ggc atg ttg tgc cgc acc ata ttg acg ttg ctc atc gct
                                     M   L   C   R   T   I   L   T   L   L   I   A

61   ttc gtt gga att tca aga att gaa gcg agg gaa ata gga gaa cga aca gca caa ctg aat
      F   V   G   I   S   R   I   E   A   R   E   I   G   E   R   T   A   Q   L   N

121  aaa aca ccc gta gtt gga gtc aat acc gcg atc gaa gcc tta acg gta cag aat gac aac
      K   T   P   V   V   G   V   N   T   A   I   E   A   L   T   V   Q   N   D   N

181  act ttc aac act ggc cca aat gat cag agc ata tat gga tcg gtc gta aag gtg gat aat
      T   F   N   T   G   P   N   D   Q   S   I   Y   G   S   V   V   K   V   D   N

241  aac ata tat gaa tca ccc aat agc cgt caa cca acc agt aca caa act cgc acc gtt gtt
      N   I   Y   E   S   P   N   S   R   Q   P   T   S   T   Q   T   R   T   V   V

301  gcg cca ggt ggt cct gga cac gat ggg gta ttc tac gca aag tca gaa gaa cgc gat caa
      A   P   G   G   P   G   H   D   G   V   F   Y   A   K   S   E   E   R   D   Q

361  caa gga gaa ccg aaa aac aat gtg agt gca aca act tat atg aag ggc aat act ttt aac
      Q   G   E   P   K   N   N   V   S   A   T   T   Y   M   K   G   N   T   F   N

421  ctc gcg cct gat agc aaa aac gtg ttt gga tcg tac ata gaa caa aat aat aat gta tat
      L   A   P   D   S   K   N   V   F   G   S   Y   I   E   Q   N   N   N   V   Y

481  aga tta aga ggc ggt aat aac caa acg caa cca aat gaa gaa cat aaa tcc tct aat agg
      R   L   R   G   G   N   N   Q   T   Q   P   N   E   E   H   K   S   S   N   R

541  ggt aac atc gga cta aca gct gaa act ata cta act aac agc gtt ttt caa acg agt gga
      G   N   I   G   L   T   A   E   T   I   L   T   N   S   V   F   Q   T   S   G

601  aat atg gaa atc aat gca ggg tca tct ata act cga aac ggt aat gtt tat acc tcc aac
      N   M   E   I   N   A   G   S   S   I   T   R   N   G   N   V   Y   T   S   N

661  aag aat tca caa act gga gaa aag aca atc aat ggt acg gag aga caa gat aat tta tcc
      K   N   S   Q   T   G   E   K   T   I   N   G   T   E   R   Q   D   N   L   S

721  gat atc atg act gta ctt cta aag gac ata caa aca cga gaa aag ata tac aaa gac gga
      D   I   M   T   V   L   L   K   D   I   Q   T   R   E   K   I   Y   K   D   G

781  aga atc aat tat act tcg gga acc aat cga ata tcc aac aag acc caa ttg aaa gta aac
      R   I   N   Y   T   S   G   T   N   R   I   S   N   K   T   Q   L   K   V   N

841  caa att cat cat tta ttt ttt atg att ctt acc cga att cag aaa cgg aaa aga atg cag
      Q   I   H   H   L   F   F   M   I   L   T   R   I   Q   K   R   K   R   M   Q

901  aag aca cca agg aga aag aag cat ccg ccg aga agc acg acg gac cta acg gac aac cga
      K   T   P   R   R   K   K   H   P   P   R   S   T   T   D   L   T   D   N   R

961  gtt tgt cac cta atg tat att act tat tta cat att ttt tga taa tat tga taa aat tat
      V   C   H   L   M   Y   I   T   Y   L   H   I   F   *

1021 ctg ttt taa act aaa act ctt aga aat cta tta tag ttt att aaa taa agc agc ttt ata

1081 gta taa aaa aaa aaa aaa aaa agt cga cg
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Fig. 4. Nucleotide sequence and deduced translated amino acids of GiPDV cDNA 1·1. The putative translation initiation site is in bold and indicated by an arrow. Stop codon is bold and indicated by an asterisk.

diameter of 35 to 40 nm and a length of 50 to 100 nm (Stoltz & Vinson, 1979). The smallest nucleocapsids were seen in the PDV associated with *Cardiochiles nigriceps*, with a diameter of 25 to 50 nm and a length of 10 nm (Stoltz & Vinson, 1979). As a general rule, the number and size of nucleocapsids were species-specific among the bracoviruses and were positively related to the size of DNA segments enclosed. It was reported that nucleocapsids of bracovirus contained only one DNA segment and DNA molecules were singly encapsidated, as indicated by the release of

cDNA 1.8:

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1     gcg gcc ggt cga cct atg cta atc taa ttc att tat aag acc acg agg gaa acc gtg gga
61    tgg ttt cat gat cta cac ata gac ttt ttc aga aaa aat ctt ctt acc cca agg cag tag

121   agt ttg aga atc gaa aca atg cgt ggt gct gcg gtc tgt gtt tta ttg tgt atc ggc acc
                          M   R   G   A   A   V   C   V   L   L   C   I   G   T

181   gtc gtc tca aat agc aga gcg act gag aat gga gaa tat gga gat ggc atg acc gtc aaa
      V   V   S   N   S   R   A   T   E   N   G   E   Y   G   D   G   M   T   V   K

241   gaa gaa gtc gga aaa aga gta att gtc ctc cag ggt agc aag aac gac gtg act att gca
      E   E   V   G   K   R   V   I   V   L   Q   G   S   K   N   D   V   T   I   A

301   gag caa act aat gag gat ggt tcg aga tcc atg atc tat gag aaa gag aca agg gga aaa
      E   Q   T   N   E   D   G   S   R   S   M   I   Y   E   K   E   T   R   G   K

361   tta gga gat acc cga acg act gag agt aga aaa gat gaa ggt ggc atg acc gtc aaa gac
      L   G   D   T   R   T   T   E   S   R   K   D   E   G   G   M   T   V   K   D

421   gaa ccc gga aaa aga cta att atc acc cgg ggt ggc aag tac aag tat gaa gag tca att
      E   P   G   K   R   L   I   I   T   R   G   G   K   Y   K   Y   E   E   S   I

481   aca gac aaa aat tat gat gat gga ttg aga tcc acg atc ttt aag aga gag aca agg cga
      T   D   K   N   Y   D   D   G   L   R   S   T   I   F   K   R   E   T   R   R

541   aaa tta gaa aat aaa aca aac ctg atc gtc gtg acg gaa gaa gtt ttt aag aac gag att
      K   L   E   N   K   T   N   L   I   V   V   T   E   E   V   F   K   N   E   I

601   tat cct aag gga ttc aaa gtt att cga acg gta gtc aca gga agt gtc gac gat aaa gat
      Y   P   K   G   F   K   V   I   R   T   V   V   T   G   S   V   D   D   K   D

661   cct cga aaa aat gct ctt tta tcc gac cag tcg gaa act tat ccg gat ttc gaa tca tgg
      P   R   K   N   A   L   L   S   D   Q   S   E   T   Y   P   D   F   E   S   W

721   aga cca atg ttt cct cga cca atc gtc ctg tca aaa aaa act aac aat cag caa gaa aac
      R   P   M   F   P   R   P   I   V   L   S   K   K   T   N   N   Q   Q   E   N

781   gtg cct caa ccg aca ggt gaa ata tta gca act cct cct ccc cag cat gac cct gaa tcg
      V   P   Q   P   T   G   E   I   L   A   T   P   P   P   Q   H   D   P   E   S

841   gga cgt aac cac cag ggt tct tct ata gtc aat gaa cgc cct cct ttt gga aca cat aat
      G   R   N   H   Q   G   S   S   I   V   N   E   R   P   P   F   G   T   H   N

901   ggc gag aaa gta tca gcc cag aca gtt ggt gga agg aca gga ggt tat cca cct cag tat
      G   E   K   V   S   A   Q   T   V   G   G   R   T   G   G   Y   P   P   Q   Y

961   gga cct gga tta gga cat aac ccg cag gat cct tct gaa gtc aat gaa cgc cct cct ctc
      G   P   G   L   G   H   N   P   Q   D   P   S   E   V   N   E   R   P   P   L

1021  gca gca cat aat ggc gag aaa gta tca gcc cag aca gtt ggt gga atg gca aga ggt tat
      A   A   H   N   G   E   K   V   S   A   Q   T   V   G   G   M   A   R   G   Y

1081  ccg tct cag tat gga cct gga tct gga tat aac ccg cag gat cct tct ata gtc aat caa
      P   S   Q   Y   G   P   G   S   G   Y   N   P   Q   D   P   S   I   V   N   Q

1141  tat cct act gtg gca gct cag aat gga gga aaa gta tca gcc cag aca ttt aat gga atg
      Y   P   T   V   A   A   Q   N   G   G   K   V   S   A   Q   T   F   N   G   M

1201  gca gga gct tat ccg tct cag tat gaa cca aga tca gga cat aac ttt cag gat cct gct
      A   G   A   Y   P   S   Q   Y   E   P   R   S   G   H   N   F   Q   D   P   A

1261  ata gtc aac gag cgc cct cct ttc gca gca gat tat agc aag aga gta tca ccc cag aca
      I   V   N   E   R   P   P   F   A   A   D   Y   S   K   R   V   S   P   Q   T

1321  gtt gat gga agg aca gga gct tat ccg act cag tat gga cct gga tca gga aat tac ttt
      V   D   G   R   T   G   A   Y   P   T   Q   Y   G   P   G   S   G   N   Y   F

1381  cag gag ccc tct gta gtc aac gag cac cct cct tcc aca gct cat aat agc gag aga cta
      Q   E   P   S   V   V   N   E   H   P   P   S   T   A   H   N   S   E   R   L

1441  tca gcc caa aca gtt ggt aaa agg aca gga ggt tat cca cct cag tat gga cct gga tta
      S   A   Q   T   V   G   K   R   T   G   G   Y   P   P   Q   Y   G   P   G   L

1501  gga cat aac ccg cag gat cct tct gta gtc aat gaa cga cct cct tcc aca gct gat tac
      G   H   N   P   Q   D   P   S   V   V   N   E   R   P   P   S   T   A   D   Y

1561  ggt caa gta tac gtc ggg cca gtt ttt aga cag tcg cca cct ttt tct ggg acc ctg aat
      G   Q   V   Y   V   G   P   V   F   R   Q   S   P   P   F   S   G   T   L   N

1621  tag att att tgt gga aga atc gtt ttg tct taa taa tcg ccc gac aat aat aaa taa ttg
      *
1681  gcc gtt tta cta tga atg aac aac cta gcg aac aaa ata cta tgt tcc gtg tta gtt tta
1741  gga gat ttt ttt ttt tta atc gtc tga ttg ctt gta ata aag tta tga tca cct aaa gtc
1801  gac gcg g
```

Fig. 5. Nucleotide sequence and deduced translated amino acids of GiPDV cDNA 1·8. The putative translation initiation site is in bold and indicated by an arrow. Stop codon is bold and indicated by an asterisk.

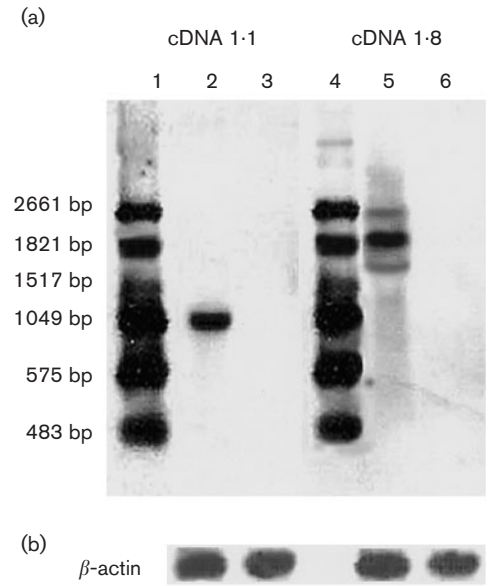

Fig. 6. Northern blot analysis of cDNA 1·1 and cDNA 1·8. (a) Total RNA was extracted from parasitized and non-parasitized *L. dispar*. The RNA samples were resolved on a denaturing formaldehyde 1 % agarose gel, blotted to nylon membrane and individually hybridized to the DIG-labelled RNA probes complementary to cDNA 1·1 and cDNA 1·8. The mobility and sizes (bp) of DIG-labelled RNA molecular mass marker I are indicated. (b) Labelled insect β-actin was hybridized to the same membranes to show that uniform quantities of RNA were loaded. Lanes 1 and 4, DIG-labelled RNA molecular mass marker. Lanes 2 and 5, RNA extracted from parasitized host. Lanes 3 and 6, RNA extracted from non-parasitized host.

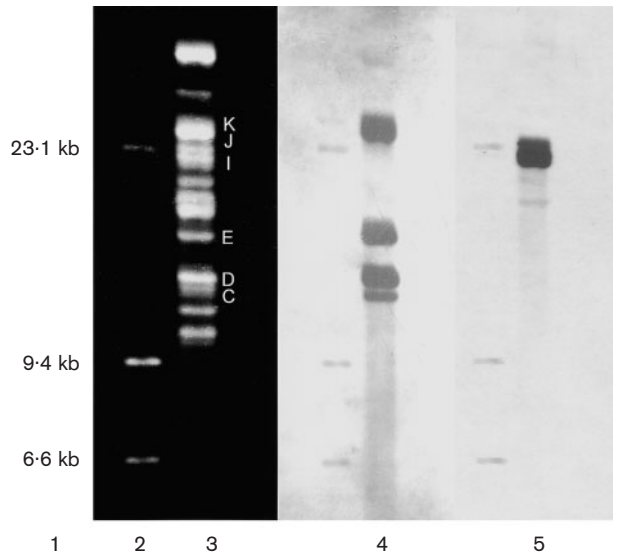

Fig. 7. Localization of cDNAs 1·1 and 1·8 within the GiPDV genome. DIG-labelled λ DNA/*Hin*dIII (lane 2; fragment kb sizes labelled in lane 1) and GiPDV genomic DNA (lane 3) were separated by FIGE and blotted to nylon membrane. The Southern-blotted GiPDV genomic DNA was probed individually with cDNA 1·1 (lane 4) and cDNA 1·8 (lane 5) to map the two isolated cDNA fragments to specific regions of the GiPDV genome. cDNA 1·1 hybridized to four genomic DNA segments (C, D, E and K) and cDNA 1·8 hybridized to two genomic DNA segments (J and K).

individual closed circular DNA segments from the nucleocapsids after osmotic shock (Albrecht *et al.*, 1994).

Characteristics of the GiPDV genome

Bracoviruses appear to comprise fewer but larger DNA molecules than ichnoviruses, and exhibit a relaxed open circular topology (Albrecht *et al.*, 1994; Webb, 1998). Electrophoretic profiles of genomic DNA showed that the GiPDV genome contained 13 segments ranging in size from 11 kb to more than 30 kb. The aggregated GiPDV genome size was approximately 250 kb. The visible GiPDV segments may represent distinctive genomic molecules or the same genomic molecules in different conformations. Since supercoiled, relaxed circular and linear forms of viral DNA were very difficult to differentiate, the potential increase in sequence complexity associated with co-migration of DNA segments of the same size or the same DNA segment present in more than one conformation was not included in the size estimate for the GiPDV genome in this study. The ongoing GiPDV sequencing project in our laboratory will provide a better and more precise characterization of genome size and organization in the future. Compared with the most systematically characterized ichnovirus, *C. sonorensis* polydnavirus (CsPDV), containing 28 DNA segments ranging in size from 5·5 to 21 kbp, GiPDV comprised fewer but larger DNA segments. Consistent with previous findings for other ichno- and bracoviruses, GiPDV consisted of multiple DNA segments with variable sizes and molar ratios (Theilmann & Summers, 1987, 1988; Summers & Dib-Hajj, 1995; Cui & Webb, 1997; Strand *et al.*, 1997; Webb & Cui, 1998, Varricchio *et al.*, 1999). Four segments (D, F, K and M) were present in higher molar concentration than other segments, as indicated by a higher band intensity. The higher molar concentration of DNA segments indicated the presence of multiple DNA segments of similar size with either homologous or polymorphic sequences.

Identification of cDNA clones encoding GiPDV-specific mRNA and expression of cDNA clones in the parasitized host

Construction and screening of a cDNA library allowed identification of cDNA clones deriving from GiPDV-specific mRNA expressed in parasitized host 24 h post-parasitization. Both cDNA clones contained a single ORF. Analysis of amino acid sequences indicated that isolated transcripts have no significant homology to other ichnovirus or bracovirus gene families (Webb & Cui, 1998). Northern blot analysis confirmed the expression of cDNA

1·1 and cDNA 1·8 in the parasitized host, with cDNA 1·1 expressed as a single 1·1 kb transcript and cDNA 1·8 expressed as one major 1·8 kb transcript, with minor 1·7 and 2·6 kb transcripts also detected in the parasitized host. Whether the minor transcripts resulted from alternative splicing of the same precursor transcript or were derived from independent genes is unknown.

Southern hybridization showed that cDNA 1·1 resided on four separate viral DNA segments, while cDNA 1·8 apparently resided on two viral DNA segments, indicating multiple gene loci within GiPDV. The multiple gene loci suggested the probability of homologous sequences or related genes among different GiPDV DNA segments, as is characteristic of other described polydnaviruses (Theilmann & Summers, 1988; Cui & Webb, 1997, 1998; Strand *et al.*, 1997; Volkoff *et al.*, 1999). These are the first internal sequence homologies noted for GiPDV. The features of genome segmentation, hypermolar segment ratios and sequence homology have been suggested to be involved in increasing the copy number of essential genes and the levels of gene expression in the absence of virus replication (Xu & Stoltz, 1993; Cui & Webb, 1997). Homologous viral genes residing on different DNA segments could be transcribed simultaneously or separately to exert additive or complementary functions in host regulation. Webb & Cui (1998) reported that abundantly expressed genes are often associated with nested DNA segments, while genes that are expressed at a lower level are not generally present on nested DNA segments. The multiple loci among GiPDV genome segments suggested that cDNA 1·1- and 1·8-encoded GiPDV-specific genes represent related genes of importance in the parasitized host, members of gene families, and are probably not associated with nested segments.

The morphological features and genomic organization of the bracovirus of GiPDV are unique, although characteristic of braconid PDVs in general. Isolation of cDNAs encoding GiPDV-specific mRNA expressed in the parasitized host, as well as mapping of these cDNAs to multiple GiPDV genomic DNA segments, supported the notion that GiPDV morphology and genomic organization are intrinsically linked to the function and evolutionary strategies of the virus. This study enhances knowledge about this virus and provides a basis for future functional characterization of GiPDV.

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