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A Densovirus of German Cockroach *Blattella germanica*: Detection, Nucleotide Sequence, and Genome Organization

D. V. Mukha[1] and K. Schal[2]

[1] *Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, 119991 Russia; E-mail: myxa@vigg.ru*
[2] *Department of Entomology, North Carolina State University, Raleigh, NC 27695 United States*

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Abstract—A new *Blattella germanica* densovirus (*Bg*DNV, Parvoviridae: Densovirinae, *Densovirus*) was found. Virus DNA and cockroach tissues infected with *Bg*DNV were examined by electron microscopy. Virus particles about 20 nm in diameter were observed both in the nucleus and in the cytoplasm of infected cells. Virus DNA proved to be a linear molecule sized about 1.2 μm. The complete *Bg*DNV genome was sequenced and analyzed. Five ORF were detected: two coded for structural capsid proteins and were on one DNA strand, and three coded for regulatory proteins and were on the other strand. Potential promoters and polyadenylation signals were identified. Structural analysis was performed for terminal inverted repeats containing extended palindromes. The genome structure of *Bg*DNV was compared with that of other Parvoviridae.

Key words: insect densoviruses, cockroach *Blattella germanica*

INTRODUCTION

Densoviruses (subfamily Densovirinae) belong to the family Parvoviridae, which also includes the subfamily Parvovirinae. These viruses infect arthropods, mostly insects. Densovirus infection has been detected in species of five insect orders (Lepidoptera, Diptera, Orthoptera, Diptyoptera, and Odonate). Densoviruses owe their name to the fact that nuclei of infected cells are hypertrophic and contain dense, dark, Feulgen-positive virion masses [2]. In most cases, densovirus infection causes death of the host [3].

Hexagonal densovirus particles are 19–22 nm in diameter and harbors a single-stranded DNA about 5–6 kb. Some viruses (e.g., *Aedes aegypti* densovirus, *Ae*DNV) contain predominantly a mRNA-complementary DNA strand in their particles, whereas some others (e.g., *Junonia coenia* densovirus, *Jc*DNV) produce particles with different strands in similar amounts [4]. When both plus and minus strands are packed in particles, total DNA isolation at a high ionic strength yields double-stranded DNA (dsDNA).

Terminal inverted repeats (TIR) are at the ends of densovirus DNA and may form secondary structure elements. The nucleotide sequences of the two TIR are identical (*Jc*DNV) [5] or differ (*Ae*DNV) [6]. TIR play an important part in autonomous replication of virus DNA [4]. The densovirus genomes sequenced so far each contain several open reading frames (ORF). ORF occur in one or in both DNA strands depending on the virus type, and code for capsid and regulatory proteins [4].

After gene-engineering modification, densoviruses provide convenient vectors for genetic manipulations in insects. Another line of their employment is the biological control of pests [7–9].

We found a new *Blattella germanica* densovirus (*Bg*DNV), cloned and sequenced its DNA, and compared its genome structure with that of other Parvoviridae.

EXPERIMENTAL

Total DNA was isolated from *B. germanica* individuals of a laboratory colony originating from a natural population (United States). Isolation of total and plasmid DNAs, endonuclease digestion, gel electrophoresis, and fragment elution from a gel were carried out according to published protocols [10, 11].

Electron microscopy. DNA preparations were analyzed by transmission electron microscopy (magnification 30,000). A mixture of virus and plasmid DNAs (2:1) was treated as in [12] and placed on grids with a formvar support film covered with graphite. Preparations were contrasted with uranyl acetate and platinum–palladium vapor.

To obtain ultrathin tissue sections, *B. germanica* individuals were fixed and embedded in a resin. Preparations were contrasted with uranyl acetate and lead citrate as described previously [13].

Virus genome cloning. The gist of the cloning procedure was tailing virus DNA with single-stranded homopolymeric fragments (about 20 nt) and annealing it with pUC19.

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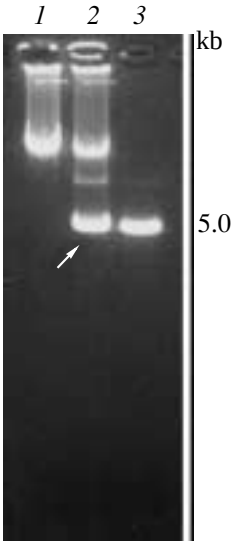

Fig. 1. Electrophoresis in 1% agarose gel of total DNA isolated from (*1*) noninfected and (*2*) infected *B. germanica* individuals and (*3*) of purified virus DNA. Virus DNA is indicated with an arrow.

Virus dsDNA was isolated from agarose gel [10]. The tailing mixture (200 μl) containing 0.3 μg of virus DNA, 100 mM potassium cacodylate (pH 7.2), 2 mM $CoCl_2$, 0.2 mM DTT, 0.1 mM dGTP, and 30 units of terminal deoxynucleotidyl transferase (Gibco-BRL) was incubated at 37°C for 1 h. Plasmid DNA was digested with *Pst*I and resolved in 0.7% agarose gel. The linearized DNA was tailed with poly(dC). The reaction mixture (50 μl) containing 0.1 μg of plasmid DNA, 100 mM potassium cacodylate (pH 7.2), 2 mM $CoCl_2$, 0.2 mM DTT, 0.02 mM dCTP, and 15 units of terminal deoxynucleotidyl transferase was incubated at 37°C for 30 min. Virus and plasmid DNAs were precipitated with ethanol, washed with 70% ethanol, and dissolved in 50 μl of water.

The annealing mixture (10 μl) contained 0.25 μg of virus DNA, 0.05 μg of plasmid DNA, 10 mM Tris-HCl (pH 8.0), 0.1 M NaCl, and 1 mM EDTA. The mixture was incubated at 65°C for 5 min and at 57°C for 1 h. After incubation, 5 μl of the mixture were used to transform competent *Escherichia coli* XL2-Blue MFR' cells (Stratagene).

Sequencing. Plasmids pVir-8 and pPst-Vir containing fragments of the virus genome (see below) were sequenced according to Sanger [14] with a dGTP Big Dye Termination kit (Applied Biosystems, United States) on an ABI PRISM 377 sequencer.

Sequence analysis. Promoters, poly(A) tracts, ORF, and their protein products were predicted and multiple and pairwise comparisons done with the BCM Search Launcher software package [15] (http://searchlauncher.bcm.tmc.edu). The TIR secondary structure was predicted using the FOLD program [16]. A thermal denaturation profile of *Bg*DNV dsDNA was computed according to Poland's algorithm [17, 18] with a program available at http://www.biophys.uni-duesseldorf.de/local/POLAND/poland.html.

RESULTS AND DISCUSSION

Virus Detection and Electron Microscopy

Electrophoresis in 0.7% agarose gel revealed an additional DNA fraction of about 5 kb (Fig. 1, *2*) in some individuals of a laboratory *B. germanica* population maintained for 5 years after capturing in a pigsty (colony P6). Most of these individuals displayed several pathological signs, including flaccidity, poorly coordinated movements, and complete or partial paralysis of the hind legs. Similar signs have been reported for densovirus infection of other insects, cockroach *Periplaneta fuliginosa* and cricket *Acheta domestica* [3, 19–21]. The additional fraction was isolated and purified (Fig. 1, *3*). On electron microscopic evidence, the fraction contained linear DNA about 1.2 μm in length (Fig. 2). The DNA proved to be double-stranded; its restriction map was constructed with *Bgl*II, *Eco*RI, and *Pst*I (Fig. 3a).

Electron microscopy of ultrathin tissue sections of affected individuals showed that cells of the digestive system, fat body, and epidermis were infected with a virus, which dramatically changed the cell ultrastructure. Cells of different tissues were similar in cytopathological features, all having unusually structured chromatin with most of the nucleoplasm occupied by virus particles. Particles about 20 nm in diameter were observed both in the nucleus and in the cytoplasm of infected cells. In the nucleus, virus particles were closely packed in electron-dense virogenic stroma (Fig. 4a). Ultrastructurally, virus particles could be divided into two types, some having an electron-transparent core surrounded by an electron-dense wall and some others representing electron-dense spheres (Fig. 4b). All these features are characteristic of densoviruses [22–24].

Virus DNA Cloning

For cloning, isolated virus dsDNA and pUC19 DNA were 3'-tailed with poly(dG) and poly(dC), respectively; annealed; and then used to transform *E. coli* XL2-Blue MRF'. Clone pVir-8 was selected that contained an insert comparable in size with the full-length *Bg*DNV genome. The orientation of the virus DNA insert relative to the plasmid polylinker was established by restriction enzyme analysis with *Hin*dIII, *Eco*RI, *Pst*I, *Bgl*II, and their combinations (Fig. 3). The cloned fragment proved to lack 150–200 nt of the end close to the plasmid *Hin*dIII site; the other virus DNA end (close to the *Kpn*I site) was intact

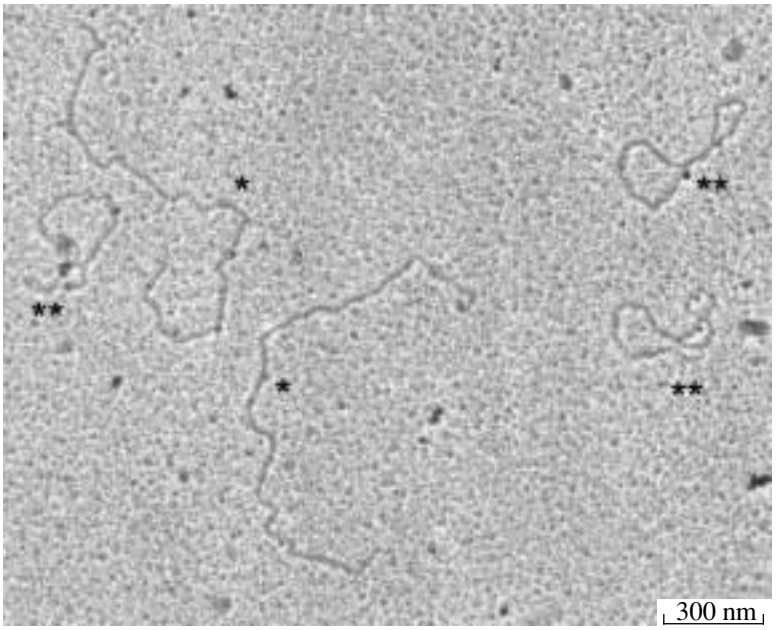

Fig. 2. Electron microscopic image of (*) *Bg*DNV DNA and (**) marker circular DNA of pUC19.

(Fig. 3b). Full-length densovirus genomes are difficult to clone in a circular plasmid vector because of instability of their extended terminal palindromic regions [25]. To clone the lacking fragment of the *Bg*DNV genome, we used the following approach. Virus DNA was isolated from gel and digested with *Pst*I. A fragment containing the region absent from pVir-8 was tailed with poly(dG) and cloned in pUC19 linearized and tailed as above, to produce pPst-Vir. The cloned fragments each containing one end of the virus genome remained stable in the recombinant plasmids maintained in *E. coli* XL2-Blue MRF'.

The resulting plasmids, pVir-8 and pPst-Vir, were used to sequence the *Bg*DNV genome.

Nucleotide Sequence of the *Bg*DNV Genome and Structure of Its Terminal Inverted Repeats

The *Bg*DNV genome is 5335 nt (GenBank accession no. AY189948). Pairwise comparisons with other densovirus sequences available from EMBL and GenBank revealed only a low (48–51%) similarity with the *Bg*DNV genome (data not shown). Yet several motifs proved to be evolutionarily conserved (100% identity) among densoviruses isolated from insects of two distant orders, Lepidoptera and Blattodea (Fig. 5). The motifs are in region 2923–3221 of the *Bg*DNV genome (Fig. 5) and correspond to the 3' end of ORF3 (Fig. 6b). Motifs 1 and 2 may be used to construct universal degenerate primers suitable for seeking new insect densoviruses.

The right and left ends of the *Bg*DNV genome are respectively 216- and 217-nt TIR (Fig. 6a). Analysis with the BLAST program [26] did not reveal any appreciable similarity between these and other virus sequences available from EMBL and GenBank (data not shown). Imperfect palindromes of 192 nt, which are contained in *Bg*DNV TIR, may form secondary structures. Using the FOLD program [16], we identified the most probable secondary structure of *Bg*DNV TIR (Fig. 7). It is known that Parvoviridae TIR may form three types of secondary structures (T-, Y-, or I-shaped hairpins), which are important for replication [4, 27]. In contrast to adenoassociated viruses [28] and *Jc*DNV [5], *Bg*DNV TIR produce I-shaped, but not T- or Y-shaped, hairpins. A similar TIR secondary structure is characteristic of virus B19 [29], *Bombyx*

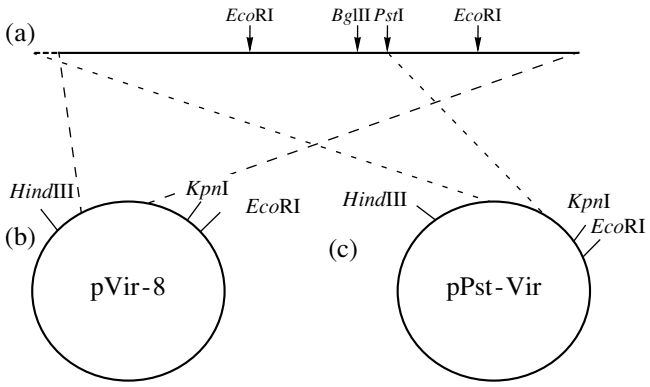

Fig. 3. Virus DNA cloning: (a) restriction map of the virus DNA and plasmids (b) pVir-8, which contained the *Bg*DNV genome lacking 150–200 nt (dashed line in (a)) of TIR close to the *Hin*dIII site, and (c) pPst-Vir, which contained a virus genome fragment with the intact TIR.

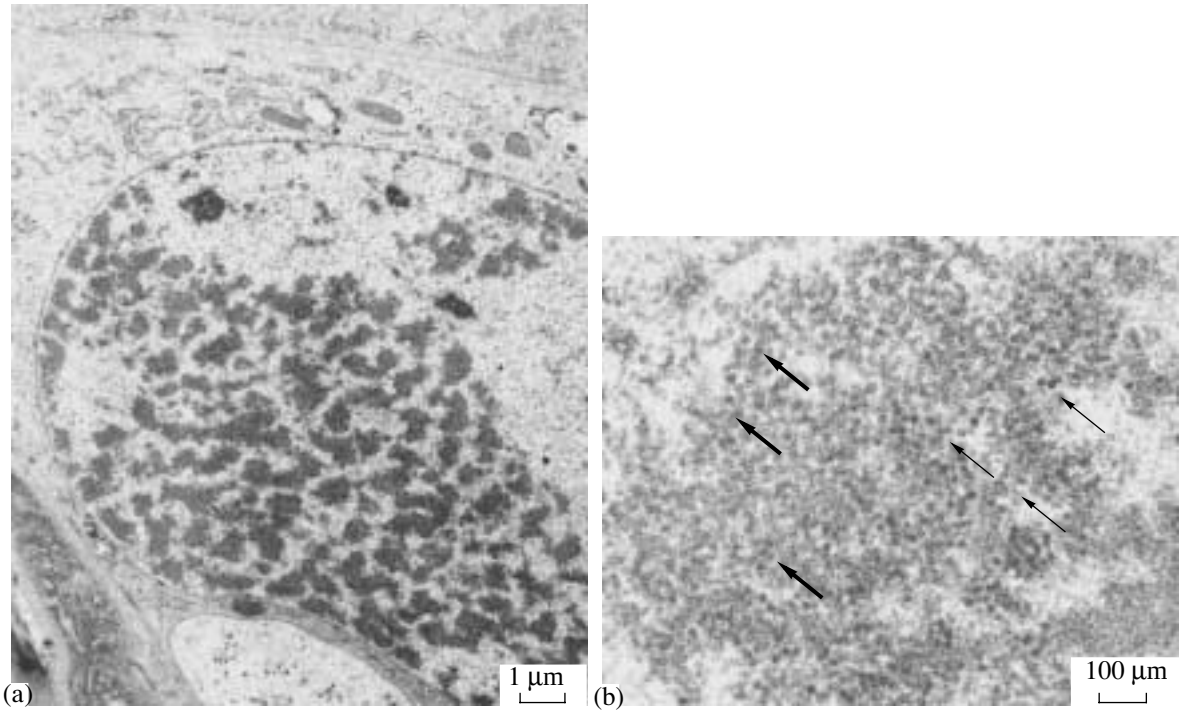

Fig. 4. Electron microscopic image of *Bg*DNV-infected cells of the *B. germanica* digestive tract: (a) virogenic stroma and (b) virus particles having an electron-transparent core and an electron-dense wall (thick arrows) or seen as electron-dense spheres (thin arrows).

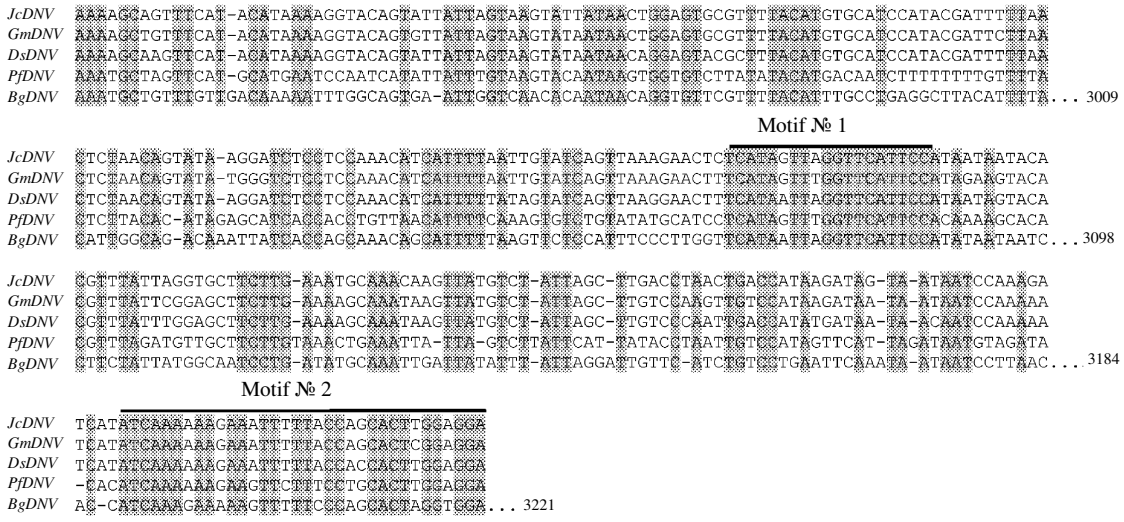

Fig. 5. Comparison of a fragment of *Bg*DNV ORF3 with corresponding sequences of other insect densoviruses. Identical nucleotides are shadowed, and conserved motifs 1 and 2 indicated.

mori densovirus 1 (*Bm*DNV-1) [30], *Galleria mellonella* densovirus (*Gm*DNV, GenBank accession no. L32896), and *P. fuliginosa* densovirus (*Pf*DNV) [31]. The functional importance of the difference in TIR secondary structure among densoviruses is still obscure. Thus the secondary structure of TIR differs between two evolutionarily related densoviruses, *Gm*DNV and *Jc*DNV (genomic DNA similarity about 90%). Possibly, changes resulting in a new TIR conformation take place in early divergence, suggesting saltatory evolution of the taxon [32].

The GC content of *Bg*DNV TIR (59.0%) is higher than that of the entire virus genome (39.6%), which may contribute to the stability of their secondary

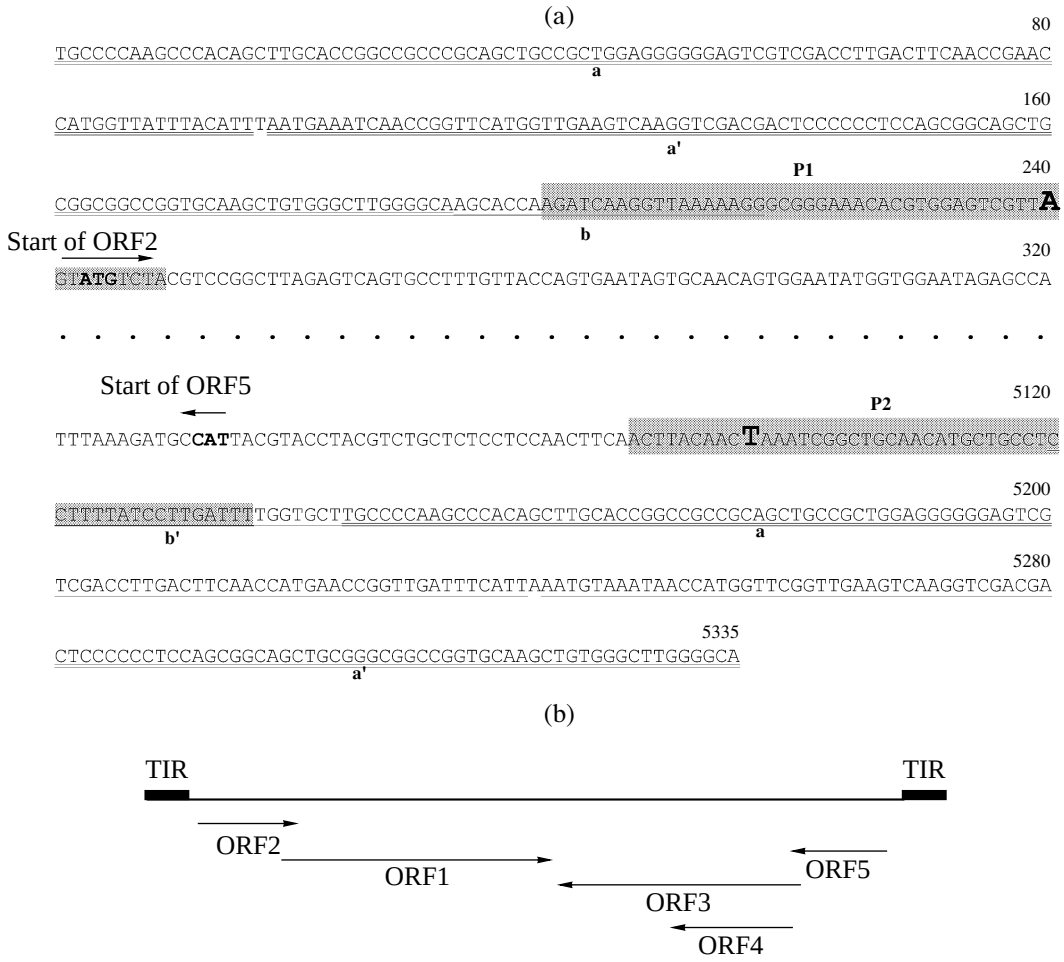

Fig. 6. Nucleotide sequence of TIR (a) and ORF arrangement (b) in the *Bg*DNV genome. Palindromic sequences **a** and **a'** (underlined) and **b** and **b'** (double-underlined) are shown. Translation initiation codons are in bold. Putative promoters (**P1** and **P2**) are shadowed. Transcription initiation start points are shown with large boldface letters. Arrows indicate the direction of transcription.

structure. A thermal denaturation profile was constructed for the first 950 nt (Fig. 6a, on the left) of *Bg*DNV DNA according to Poland's algorithm [17, 18]. The central region of the virus genome proved to have denaturation temperature about 80°C, while two peaks (about 95°C) were observed for TIR (Fig. 8). Hence the palindromes possibly play an important part in replicative dsDNA stabilization [27]. The TIR GC content is usually high in Parvoviridae, being 60% in *Pf*DNV [31], 50% in *Bm*DNV [30], and 46–60% in autonomously replicating parvoviruses [33]. An exception us *Ae*DNV with TIR having only 27% GC [6].

Genome Structure

ORF and amino acid sequences. The *Bg*DNV genome harbors five ORF, two (1 and 2) on one strand and three (3–5) on the other (Fig. 6b). The ORF arrangement is typical for the genus *Densovirus* [4].

ORF1 (922–2808) codes for a protein of 628 amino acid residues (69.7 kDa). ORF2 (243–932) codes for a protein of 229 residues (24.8 kDa). ORF3 (4404–2811) codes for a protein of 530 residues (60.2 kDa). ORF4 (4397–3608) codes for a protein of 262 residues (30.3 kDa). ORF5 (5055–4404) codes for a protein of 216 residues (25.9 kDa). The amino

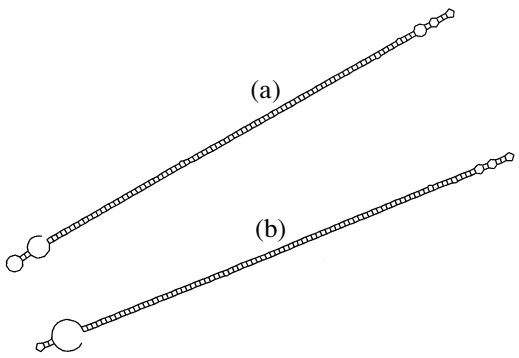

Fig. 7. Secondary structures of (a) 217-nt left and (b) 216-nt right TIR as predicted using the FOLD program [16].

Amino acid sequence comparisons for proteins of *Bg*DNV and other densoviruses

#ORF	Deduced amino acid sequence
ORF1	MPVNYNKPPPYERPNWERMNEGQYNLAVL RRGQYFEPPIAARPPSPANNAIQDLDERLDRLLDNF PIGSPQQSQGGTSNSDQPVAGPSSRPDPVPAQLPVQ PSTIQEPAQTMSAPEEAIVTGKRGAESEPDSASTPTKK NKPSEHSGSALPGTSGNTDGSMGSSTMLDLDASRGI MPISRGIHVEKFEWTFTKKWKFLSFGVADVILPDDII GTTTAPAKRWALTTSLVNIPWEYAFMYMSFAEFNRL REMTGVFATDCDICKIYQYNPRVAFQTADTNSTQAT NQNKFTRIAKGLRNNPHLFGQDSDRDYTFSSDEPMKPL GFETNADTQKFRDRLSKEVTTRTTNTPTVP AISTGKEMGLLRYYCYAKLQVDLSAHHDYQDFF NSMDLIGKQVLSAHHDFKYAPLTTRAHYQDSIYLP GDIPEKKESQPAGVVIPAGSKIVDLQSVRMPSTGFS AVEGANSRKEDAMTLGHLQVGGVDLKTGEALSNSTF TDFDTLYTKFPMEQGGLYNEAGYQGATCGDQEFDL GVRAVPKLGTAVNTINASSWLDCQMYWTVERLRCV STEPFTYPRGNVSDIPLRSQFTAATKTAPMLQTFDR PYFYGKPQRVLNSVEL
ORF2	MSTSGLESVPLLPNSATVEYGGIESPKVHSGYGARS LPVKPTSAGGAGAQYDKIFQSQLGRAASSSGNPLNV FKSRDEYYNSVPWELRRLPFAERDRLIKPYGVKWDK VSKAQYAQHWKLVNPRAGQKRIQQGIVLPFSNNIGP GNTIQDAKTGSDFIDAHYSEAKSDIDIQRADT EAIGQFIQEATHSHNPISQTQGVIGAVGLAGKQLVE KLTGKVQYGKYAS
ORF3	MNYGRLTDFWSRFGVTMGDDDAGRDSVSSSSDMVEAVG GEPSGGPVQGQAEVTSSSVDDQKLQELVDRFVSRLEE KNWKDSGYYISDVYACESSSERANALARRLEQRAESFI GRGFIGIFIHNNHVHTIHACPYTSRTCRCQFKNFPE AKEDIRRLLRKPPAIETFTRRDWENITKYFCTSGRRR ATFFKIFGHLQRLPLELTALSDSTISGQDGGGPDSGG VENCNDPLEFHSGPEVGDIPARPRANRRRKRRDQIV VGGDGGIGIILDLLSKCAVCPLTEIVFTKEYL QDPIVACKRLDSKEVKDAIDTRASVINTWEERED FVA FYNNPNTILIWSARSLNAFDSYYFNYEESFNVVTELL LTFQMGENLVQFCRNLVDTLECNIPKRNCFVVCSPP SAGKNFFFDGVKDYYLNSGQMNNPNKYNQFAYQDCH NRRIIIWNEPNYEPRENMENLKMLFAGDNLSANVKCK PQANVKRTPVIVLTNSLPNFCQQTAFNDRVITYHWT QATFLKDYNKKPRPDACVDVLYSLLQ

Sequence alignment with maximal similarity	Putative function
Junonia coenia DNV (ORF1)[GenBank No. S47266]: 38% identity, 54% similarity *Bg*DNV GTTTAPAKRWALTTSLVNIPWEYAFMYMSFAEFNRLREMTGVFATDCDIKIYQYNPRVAF GT T R +TT L IPW+ +YM+ +EF+ L G +C++K+ R+AF *Jc*DNV GTGTTAVNR–LITTCLAEIPWQKLPLYMNQSEFDLLPP––GSRVVECNVKVIFRTNRIAF *Bg*DNV QTADTNSTQATLNQNKFTRIAKGLRNNPHLFGSDRDYT–FSSDEPMKPLGFETNADQY +T+ T + QATLNQ + A GL N +G DR +T F SD+PM P T+A +Y *Jc*DNV ETSSTATKQATLNQISNLQTAVGL––NKLGWGIDRSFTAFQSDQPMIPTA––TSAPKY	Capsid protein
Periplaneta fuliginosa DNV (ORF2)[GenBank No. AB028936]: 33% identity, 51% similarity *Bg*DNV RIQQGIVLPFSNNIGPGNTIQDAKT––GSDFIAQGHDIHYSEAKSDIDIQRADTEAIGQF +I G+ PF + +GPGN + + + D IA+ HD Y+ AKS ID+ AD +AI F *Pf*DNV KIISGLTYPFHHYLGPGNPLDNNEPVDRDDAIAEEHDKAYANAKSSIDVINADKKAIDHF *Bg*DNV IQEATHSHNPISQTQGVIGAVGLAGKQLVEKLTGKV ++ + N +IG GL K +E+ G + *Pf*DNV SEDFEKNGN––––LHSLIGKTGLQIKTAIEQRFGVI *Junonia coenia* DNV (ORF1) [GenBank No. S47266]: 27% identity, 54% similarity *Bg*DNV QQGIVLPFSNNIGPGNTIQDAKTGS––DFIAQGHDIHYSEAKSDIDIQRADTEAIGQFIQ ++G+ +P +GPGN++ + + D A+ HD Y +AK+ ++ +AD + + + *Jc*DNV RRGLTVPGYKYLGPGNSLNRGQPTNQIDEDAKEHDEAYDKAKTSQEVSQADNTFVNKALD *Bg*DNV EATHSHNPISQTQG–––––VIGAVGLAGKQLVEKLTGKV ++ N + +T G IGA+G+ KQ +EK +G + *Jc*DNV HIVNAIN–LKETPGNAFGAAIGAIGIGTKQAIEKHSGVI	Capsid protein
Periplaneta fuliginosa DNV (ORFα)[GenBank No. AB028936]: 47% identity, 60% similarity *Bg*DNV FCRNLVDTLECNIPKRNCFVVCSPPSAGKNFFFDGVKDYYLNSGQMNNPNKYNQFAYQDC F + LE +PK N V SPPSAGKNFFFD Y +N GQ+ NK N F+ Q+ *Pf*DNV FLNTFYNVLERKLPKCNTICVWSPPSAGKNFFFDVYLHYLMNYGQLGIMNKTNNFSLQEA *Bg*DNV HNRRIIIWNEPNYEPREMENLKMLFAGDNLSANVKCKPQANVKRTPVIVLTNSLPNFCQQ ++R+++WNEPNYE + LKML GD L VK K +V +TP+IVLTN++ F + *Pf*DNV TSKRVLLWNEPNYEDAYTDTLKMLTGGDALCVRVKQKKDCHVYKTPLIVLTNNMIGFMHE *Bg*DNV TAFNDRVITYHWTQATFLKDYNKKPRPDACVDVL AF DRV Y W QA FL +YNKKP P ++L *Pf*DNV LAFVDRVKVYRWKQAPFLAEYNKKPNPLVAFEIL *Junonia coenia* DNV (ORF2) [GenBank No. S47266]: 50% identity, 68% similarity *Bg*DNV LVQFCRNLVDTLECNIPKRNCFVVCSPPSAGKNFFFDGVKDYYLNSGQMNNPNKYNQFAY +V+F NLV+ L+ IPK N F++ SPPSAGKNFFFD + L+ GQ+ N++N FA+ *Jc*DNV IVEFLTNLVNVLDRRIPKLNAFLIISPPSAGKNFFFDMIFGLLLSYGQLGQANRHNLFAF *Bg*DNV QDCHNRRIIIWNEPNYEPREMENLKMLFAGDNLSANVKCKPQANVKRTPVIVLTNSLPNF Q+ N+R+++WNEPNYE + +KM+F GD + VK + A+VKRTPVI+LTN+ F *Jc*DNV QEAPNKRVLLWNEPNYESSLTDTIKMMFGGDPYTVRVKNRMDAHVKRTPVIILTNNTVPF *Bg*DNV CQQTAFNDRVITYHWTQATFLKDYNKKPRP +TAF+DR+I Y W A FLKDY KP P *Jc*DNV MYETAFSDRIIQYKWNAAPFLKDYELKPHP	Nonstructural protein 1 (NS-1), virus replication

Table. (Contd.)

#ORF	Deduced amino acid sequence
ORF4	M A V S P T F G A A L E S L W E M M P D E I A S H P A T W W K L L E E S P L E D R F K D K L K S L L V R W T K N Y K N W L I G S F P A L K K K I G K T V D T I L A M Y M P A N H L N E L M H W L D D W S K E L N L S E E D L S E Y L S T I I T S I Q S T H A P T Q A G R A G A S S R T S L K R K K T L D D C F E S L Q P S K R S H D E T G K I S Q S I F V R Q G D E Q R S L K S L D T Y K D Y L L K L Q L Y P T L Q Y Q A K M E E D R T Q A W R T A M I R L N F T V D Q K S G I F Q R V L E L T D A V K D E I K L L L A E T E E S E E L Q E
ORF5	M A S L K D L C K Q A V L K Y Y R W N W K K T E V L P V T L Q N E L L T D W L K C D E V V L E E F E E L N E R A H Q C D Y E V N V W R R I K Q I M C P Q I Y V G L M E H P D T V P Q F A F D H S H I I T T S I V W Y K E E Y N S E T E S Y E R E R L C S Q C W Y R M A N P R A D D S A D Q W Y M N G W T F G R E Y S H Y C V C S K E D V L D I I Q D K N N W C A I C V T Q S L I D I L T Y D E C V A E T E F H E P G Y R P Y V T R I K G N T L L

Note: Evolutionarily conserved motifs are underlined.

acid sequences and putative functions of the deduced protein products are characterized in the table.

The deduced *Bg*DNV protein sequences were compared with protein sequences of other densoviruses with an adapted version of the BLAST program [34]. The total similarity was very low. However, several relatively short motifs showed a substantial homology, being evolutionarily conserved among various insect densoviruses, including *Bg*DNV, *Pf*DNV, *Jc*DNV, *Gm*DNV, *Ds*DNV, and the *Diatraea saccharalis* densovirus (*Ds*DNV). The results of comparisons are shown in the table.

Promoters. On evidence of computational promoter prediction based on Bucher's algorithm [35, 36], the *Bg*DNV genome contains two putative promoters, P1 and P2, in regions 200–250 and 5136–5086, respectively (Fig. 6a). Probably, P1 controls transcription of ORF1 and ORF2 and P2, that of ORF3–ORF5.

Polyadenylation signals were found in positions 937–942 and 2818–2823 on the DNA strand containing ORF1 and ORF2. The complementary strand contains an adenylation signal in position 2810–2815.

Sequence alignment with maximal similarity	Putative function
Junonia coenia DNV (ORF3) [GenBank No. S47266]: 25% identity, 40% similarity *Bg*DNV LESLWEMMPDEIASHP----ATWWKL--------LEESPLEDRFKDKLKSLLVRWTKNYK +++L+E + +E HP WW++ +E+ + LK W KN+K *Jc*DNV IKTLYESLQEE---HPLVNNVAWWQIHLENVNGHMEDEEQWPALQKNLKKTFNIWQKNWK *Bg*DNV NWLIGSFPALKKKIGKTVDTILAMYMPANHLNELMH–WLDDWSKELNLSEEDLSEYLSTI W+ S L K+ I AM + + +++ W S E + E D S++L *Jc*DNV KWAVNSLDTLLGKVLNLPAHISAMSLSYEIFSSVINVWTSCVSTE–EVDETDCSDFLKKE *Bg*DNV ITSIQSTHAPTQAGRAGASSRTSLKRKKTLDDCFESL----QPSKRSHDETGKISQSIFV ITS ST A T AG S K + D F L S S ++TG +S SI + *Jc*DNV ITSTSSTIALTPIAVAGTSGLV----KSSPSDLFRKLANQSNSSGNSSEQTGTMSSSISL *Bg*DNV RQGDEQRSLKSLDTYKDYLLKLQLYPTLQYQAKMEEDRTQAWRTAMI–RLNFTVDQKS + E + Y + + +Y E + + W A I R+ +V+ KS *Jc*DNV YENGESVQYTLEEKVGKYRVTMNVYD------GPESLKKEKWYQAPIARITMSVNSKS	Nonstructural protein of unknown function
Periplaneta fuliginosa DNV (ORF?)[GenBank No. AB028936]: 50% identity, 62% similarity *Bg*DNV SLKDLCKQAVLKYYRWNWKKTEVLPVTLQNELLTDWLKCD SL D+CK + YR NW + LP T+Q +LL DWL CD *Pf*DNV SLYDMCKLKTRETYRSNWGEVTCLPQTIQKDLLKDWLHCD *Junonia coenia* DNV (ORF4) [GenBank No. S47266]: 23% identity, 43% similarity *Bg*DNV EVNVWRRIKQIMCP–QIYVGLMEHPDTVPQFAFDHSHIITTSIVWYKEEYNSETESYERE E+ W KQ P Q+Y+ +M H + +P++ D + +I V+Y +E + + Y+ + *Jc*DNV ELEHWDWTKQNRLPFQLYLAVM–HLNEIPEW–LDETMLI--ECVYYFKELINHRDPYDTD *Bg*DNV ----------RLCSQCWYRMANPRADDSADQWYMNGWTFGREYSHYCVCSKEDV ++C C+ +P ++ N F V ED+ *Jc*DNV EFNAWNMNGKPFKTMWKICKFCYTNCEDP----DEYRFMYNRTVF--------VEDAEDI *Bg*DNV LDIIQDKNNWCAICVTQSLIDI 186 ++ +QD ++WC IC T L +I *Jc*DNV INRLQDGSSWCQICHTCPLFNI 149	Nonstructural protein of unknown function

The genome structure of *Bg*DNV has several unique features as compared with that of other insect densoviruses, especially as concerns ORF coding for capsid proteins. The two *Bg*DNV ORF coding for structural proteins each contain a polyadenylation signal, whereas synthesis of *Jc*DNV and *Gm*DNV capsid proteins may be initiated from several codons of one ORF [5, 37]. Synthesis of *Pf*DNV capsid proteins may also start from several codons; their mRNAs result from alternative splicing of the primary transcript [31]. As in *Jc*DNV and *Gm*DNV, one promoter, P1, controls transcription of the structural genes in the *Bg*DNV genome. Possibly, two mRNAs are synthesized from this promoter. Transcription starts from a common site and is terminated downstream of the polyadenylation site 937–942 in the case of the ORF2 mRNA or of site 2818–2823 in the case of the other, larger mRNA. Translation of the large mRNA may be initiated at several codons; one of the proteins may also be synthesized from the ORF2 mRNA. To explain such an unusual genome structure, it is possible to assume that the protein encoded by ORF2 is prevalent in the *Bg*DNV capsid.

The genomic arrangement of the nonstructural ORF in *Bg*DNV is similar to that of *Jc*DNV. As in *Jc*DNV, transcription of these ORF is controlled by one promoter, P2 (Fig. 6a) in *Bg*DNV. The polyadeny-

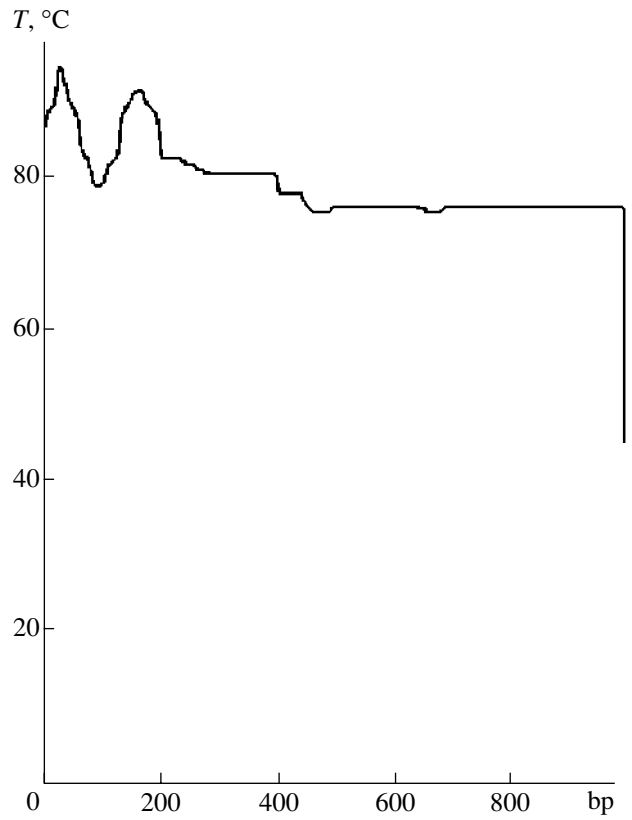

Fig. 8. Thermal denaturation profile of the first 950 bp of the *Bg*DNV dsDNA as constructed according to Poland.

lation signal is at position 2810–1815, exactly at the end of ORF3. Possibly, one mRNA is synthesized from P2, and its translation starts at different sites to yield nonstructural proteins. It should be noted that such a structure is not common for all densoviruses. Thus the *Pf*DNV genes for nonstructural proteins are transcribed from different promoters.

Surprisingly, *Bg*DNV strikingly differs in genome structure and functional organization from *Pf*DNV, which infects the species (cockroach *P. fuliginosa*) evolutionarily closest to *B. germanica*, and is highly similar to *Jc*DNV, a densovirus of Lepidoptera. We think that comparative genome analysis in insect densoviruses may greatly contribute to the understanding of evolution.

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