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Analysis of the Complete Genome Sequence of Acute Bee Paralysis Virus Shows That It Belongs to the Novel Group of Insect-Infecting RNA Viruses

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The complete genome sequence of acute bee paralysis virus (ABPV) was determined. The 9470 nucleotide, polyadenylated RNA genome encoded two open reading frames (ORF1 and ORF2), which were separated by 184 nucleotides. The deduced amino acid sequence of the 5′ ORF1 (nucleotides 605 to 6325) showed significant similarity to the RNA-dependent RNA polymerase, helicase, and protease domains of viruses from the picornavirus, comovirus, calicivirus, and sequivirus families, as well as to a novel group of insect-infecting RNA viruses. The 3′ ORF2 (nucleotides 6509–9253) was proposed as encoding a capsid polyprotein with three major structural proteins (35, 33, and 24 kDa) and a minor protein (9.4 kDa). This was confirmed by N-terminal sequence analysis of two of these proteins. The overall genome structure of ABPV showed similarities to those of *Drosophila* C virus, *Plautia stali* intestine virus, *Rhopalosiphum padi* virus, and *Himetobi* P virus, which have been classified into a novel group of picorna-like insect-infecting RNA viruses called cricket paralysis-like viruses. It is suggested that ABPV belongs to the cricket paralysis-like viruses. © 2000 Academic Press

INTRODUCTION

Acute bee paralysis virus (ABPV) is a virus of the honeybee (*Apis mellifera*). It was originally discovered as an inapparent infection of honeybees in Britain (Bailey *et al.,* 1963). ABPV has also been found in bumblebees and is the only bee virus known to have a natural alternate host (Bailey and Gibbs, 1964). This virus spreads by way of salivary gland secretions of adult bees and in food stores to which these secretions are added (Ball, 1985). In Europe and North America, ABPV has been shown to kill adult bees and bee larvae in colonies infested with the mite *Varroa jacobsoni.* The mite damages bee tissues and, in so doing, may act as a vector, releasing viral particles into the hemolymph (Scott-Dupree and MacCarthy, 1995). ABPV has a single-stranded RNA genome with a poly(A) tail. The virus particles are isometric in shape and are 30 nm in diameter. ABPV has not been well characterized at the molecular level and has not been definitively assigned to a recognized virus family.

Koonin and Dolja (1993) proposed that positive-stranded RNA viruses be divided into three supergroups based on a comparison of the amino acid sequences of their RNA-dependent RNA polymerases (RdRp). Picorna-like viruses represent a single lineage within RdRp supergroup 1, consisting of members of the families *Picornaviridae, Comoviridae, Sequiviridae,* and *Caliciviridae.* In addition, many insect-infecting viruses have been referred to as picornavirus-like, largely on the basis of biophysical properties and possession of an RNA genome (Moore *et al.,* 1985).

More recently, it has been recognized that a number of insect-infecting RNA viruses have genome structures different from that of picornaviruses and superficially similar to that of caliciviruses. Picornaviruses have monopartite monocistronic genomes with capsid proteins encoded in the 5′ region of the genome and replicase proteins in the 3′ region. Caliciviruses have monopartite bicistronic genomes with replicase proteins encoded in the 5′ region of the genome and capsid proteins in the 3′ region. A calicivirus-like genome organization was first suggested for cricket paralysis virus (CrPV) on the basis of sequence analysis of the 3′ region of the CrPV genome (Koonin and Gorbalenya, 1992). Subsequently, the genomes of *Drosophila* C *virus* (DCV), *Plautia stali* intestine virus (PSIV), *Rhopalosiphum padi* virus (RhPV), and Himetobi P virus (HiPV) were sequenced (Johnson and Christian, 1998; Sasaki *et al.,* 1998; Moon *et al.,* 1998). The genome organization of these viruses was also shown to be superficially similar to that of caliciviruses. All of these viruses have monopartite bicistronic genomes with replicase proteins encoded by a 5′-proximal ORF and capsid proteins encoded by a 3′-proximal ORF.

While caliciviruses and the insect viruses mentioned above share superficially similar genome structures, they differ in that the insect viruses do not produce a subgenomic RNA (Johnson and Christian, 1998; Moon *et al.,* 1998; Sasaki *et al.,* 1998). It has been demonstrated

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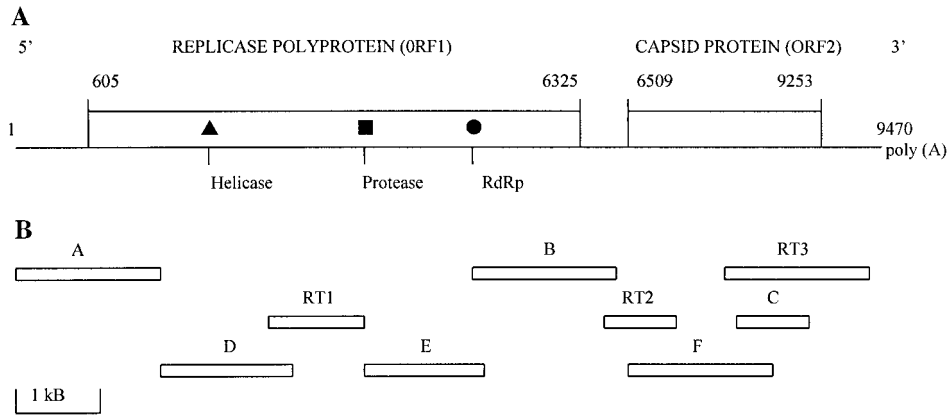

FIG. 1. (A) Schematic representation of the ABPV genome. The ORFs are shown in open boxes. The numbers represent nucleotide positions. Regions of the genome proposed as encoding a helicase (▲), protease (■), and RNA-dependent RNA polymerase (RdRp) (●) are indicated. (B) The sequencing strategy of ABPV. The six cDNA clones (A–F) and the three RT–PCR amplicons (RT1–3) used to determine the nucleotide sequence of ABPV are shown with open boxes. The sizes and relative positions of the clones and PCR products are indicated.

that translation initiation of the capsid protein of PSIV is facilitated by an internal ribosomal entry site (IRES) rather than by the production of a subgenomic RNA (Sasaki and Nakashima, 1999). Equivalent IRES elements appear to be conserved in DCV and RhPV (Sasaki and Nakashima, 1999).

At present, CrPV, DCV, RhPV, PSIV, and HiPV are recognized as a novel group, the "cricket paralysis-like viruses," distinct from the family *Picornaviridae* (van Regenmortel *et al.,* 1999). Here we report the complete nucleotide sequence of the ABPV genome. Analysis of the sequence data showed that ABPV is similar to DCV, PSIV, RhPV, and HiPV. We suggest that ABPV is a member of the cricket paralysis-like viruses.

RESULTS

Nucleotide sequence analysis

The complete genome structure of ABPV is represented in Fig. 1A. The sequence of genomic RNA from ABPV was obtained by compiling sequences from six cDNA clones. Since the clones did not overlap, primers were designed from sequence information and used to amplify the regions between different clones (Fig. 1B). These PCR amplicons were cloned and sequenced. All cDNA clones were completely sequenced in both forward and reverse directions. To confirm the sequence at the 5′ end of the ABPV genome, two independent RACE products were sequenced. The ABPV genome consisted of 9470 nucleotides, excluding the poly(A) tail. A computer analysis of the nucleotide (nt) sequence indicated the presence of two ORFs. ORF1 started at nt 605 and extended to nt 6325 and ORF2 started at nt 6509 and ended at nt 9253. The noncoding regions consisted of a 184-nt intergenic region, separating ORF1 and ORF2, a 605-nt 5′ untranslated region (5′-UTR), and a 217-nt 3′-UTR (excluding the poly(A) tail).

Alignments of amino acid sequences and organization of ORF1

The amino acid sequence of ABPV ORF1 was compared with the amino acid sequences of a calicivirus, a picornavirus, a sequivirus, and a comovirus. It was found to contain three conserved domains. These nonstructural protein domains were the helicase, the protease, and the RNA-dependent RNA polymerase. These three conserved domains are found in viruses from the picorna-like virus superfamily (as reviewed by Koonin and Dolja, 1993). The locations of these domains are indicated in Fig. 1A.

Helicase domain. There are three conserved helicase motifs (A, B, and C) present in the replicase protein of picorna-like viruses (as reviewed by Koonin and Dolja, 1993). The putative helicase domain of ABPV starts at amino acid position 533 and extends to position 654 (Fig. 2). The highly conserved amino acids that are found within motif A (GxxGxGKS) and motif B (QxxxxxDD) were identified in the ABPV sequence, between amino acids 544 and 551 and between 595 and 602, respectively. In ABPV the amino acids that were found within motif C were KKxxxxSxxxxxTSN, which deviates somewhat from the consensus, which is KGxxxxSxxxxxSTN. A computer-assisted pairwise comparison shows that the ABPV helicase domain was similar to that of HAV (32% identity), followed by those of CPMV (28%), FCV (27%), RTSV (26%), and PV (22%).

Protease domain. Only the GxCG domain of 3C-like cysteine protease of the picorna-like viruses is well conserved (as reviewed by Koonin and Dolja, 1993). The putative cysteine protease domain of ABPV was found at amino acids 1163 to 1327 in ORF1. The alignment of the amino acid sequences of the previously studied 3C-like cysteine proteases of positive-stranded RNA viruses with the ABPV sequence suggested that amino acid

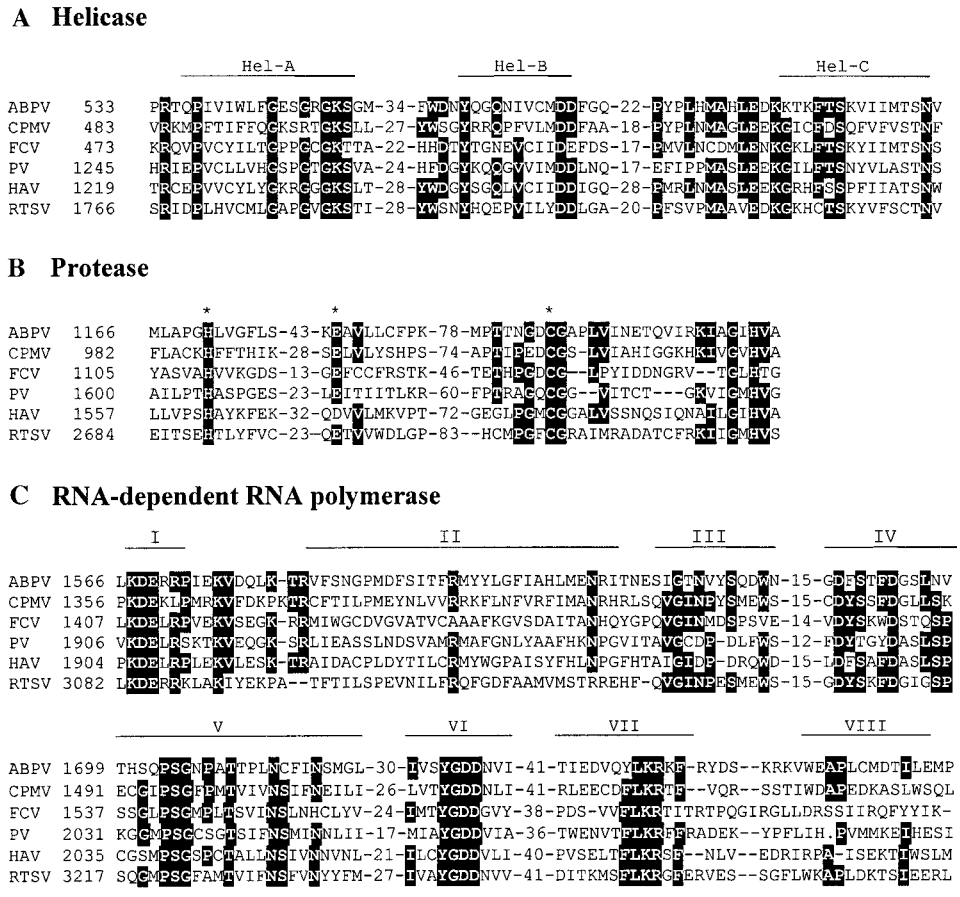

FIG. 2. Multiple amino acid sequence alignment of the conserved regions in replicase polyproteins of picorna-like viruses. Alignments of the conserved regions specific for RNA helicase (A), cysteine protease (B), and RNA-dependent RNA polymerase (C) regions of ABPV with a comovirus (CPMV), a calicivirus (FCV), picornaviruses (PV, HAV), and a sequivirus (RTSV) are shown. The numbers on the left indicate the starting amino acids of the aligned sequences. Residues identical in at least four of the six viruses are shown in reverse. The conserved regions within the helicase regions (Hel-A, Hel-B, Hel-C) and RdRp (I–VIII) regions corresponding to those reviewed by Koonin and Dolja (1993) are indicated. Asterisks indicate amino acids, which are believed to be essential for protease activity.

residues H^{1171}, E^{1221}, and C^{1314} may form the catalytic triad (Fig. 2). However, the amino acids contributing to ABPV protease activity need to be defined experimentally.

RdRp domain. There are eight RdRp amino acid motifs that are conserved in RNA viruses (Koonin and Dolja, 1993). The eight conserved RdRp motifs were found in the C-terminal region of the polyprotein of ORF1 of ABPV. The putative RdRp conserved domain starts at amino acid position 1565 and extends to position 1840 (Fig. 2).

Alignments of amino acid sequences and organization of ORF2

The estimated molecular weights of ABPV proteins correspond to previously estimated sizes, as described by Ball (1985). The estimated molecular weights of the ABPV structural proteins are 35 kDa (VP1), 33 kDa (VP2), 24 kDa (VP3), and 9.4 kDa (VP4). N-terminal sequencing by Edman degradation was successful for the 24-kDa and 33-kDa proteins (Table 1). The N-terminal sequences obtained matched the deduced amino acid sequence of ORF2. A pairwise comparison of the predicted amino acid sequences of ABPV structural proteins was compared with equivalent sequences from the cricket paralysis-like viruses (Table 2).

TABLE 1

Summary of Analysis of the Capsid Proteins

Capsid protein	Molecular mass[a]	N-terminal sequence[b]	Position of N-terminus[c]	Cleavage sites[d]
VP1	35	—	—	—
VP2	33	SKPRNL	407–412	AIF**GW/SK**PRN
VP3	24	INLAN	707–711	M**Q**/INLANK
VP4	9.4	—	—	—

[a] Determined by SDS-PAGE.
[b] Determined by Edman degradation.
[c] Relative to the deduced amino acid sequence of ORF2.
[d] Determined from the N-terminal sequence (the amino acids in bold represent the cleavage sites in ABPV capsid proteins).

TABLE 2

Identity of the Deduced Amino Acid Sequences of ABPV Structural Proteins to the Cricket Paralysis-like Viruses

Virus	Percentage identity
CrPV	31
DCV	33
HiPV	30
RhPV	30
PSIV	30

Alignments of nucleotide sequences of intergenic regions

The intergenic regions of the ABPV, PSIV, DCV, and RhPV genomes were compared by multiple sequence alignment (Fig. 3). Several short nucleotide segments were found to be highly similar. The region in ABPV started at nt position 6257 and extended to position 6519. Sasaki and Nakashima (1999) noted that the intergenic region of the genomes of DCV, PSIV, and RhPV contained several short conserved RNA segments. They demonstrated that this region acted as an IRES, facilitating the cap-independent translation of the 3′-proximal ORF of the PSIV genome.

Phylogenetic analysis

A multiple alignment of the predicted amino acid sequence of the conserved RdRp region of ABPV with selected positive-stranded RNA viruses was conducted. As expected, members of established virus families were grouped with one another while ABPV, DCV, PSIV, HiPV, and RhPV formed a separate group.

DISCUSSION

Recently, the honeybee virus SBV was sequenced (Ghosh *et al.,* 1999). The genome of SBV contained a single, large ORF with the structural proteins encoded at the 5′ end of the genome, which is typical of picornaviruses. Here, it is reported that honeybee virus ABPV has a genome organization that is different from that of SBV and which corresponds to that of the cricket paralysis-like viruses.

ABPV contains two large ORFs encoding a replicase polyprotein and a capsid protein (Fig. 1). The deduced amino acid sequence of ABPV replicase polyprotein contained conserved domains previously identified in picornavirus RNA-dependent RNA polymerase, RNA helicase, and cysteine protease (Fig. 2). The three domains were arranged in the same order as in the genomes of picorna-like viruses (Strauss *et al.,* 1996). The putative capsid protein was encoded at the 3′ terminus, downstream of the replicase polyprotein.

The genome organization of ABPV resembles that of the cricket paralysis-like viruses. It is monopartite and bicistronic and differs from those described for members of established picorna-like virus families. The *Picornaviridae* and *Sequiviridae* have monopartite and monocistronic genomes encoding a single polyprotein with the capsid protein encoded at the 5′ terminus and the nonstructural protein at the 3′ terminus. RTSV is the exception, as it encodes a small ORF2 of unknown function, which may be translated from subgenomic RNA (Shen *et*

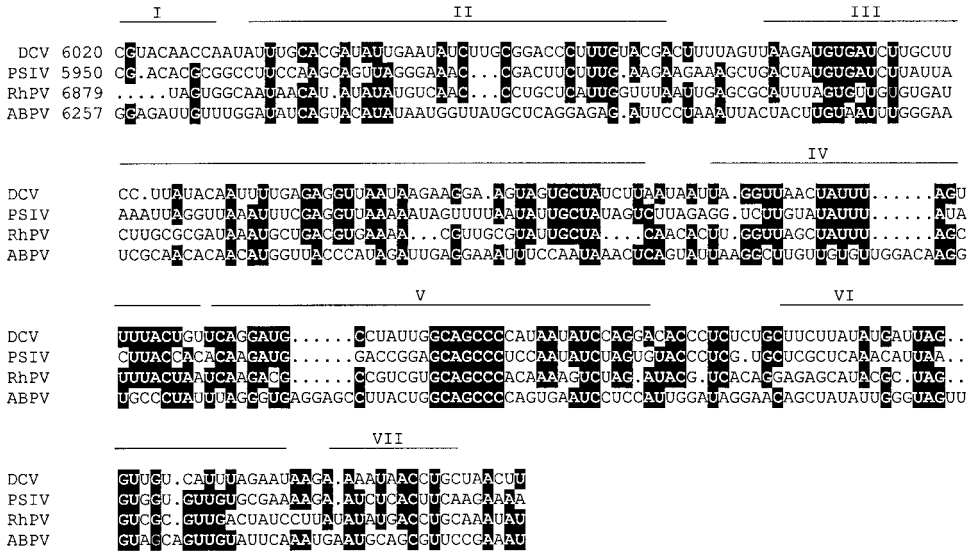

FIG. 3. Multiple nucleotide sequence alignment of the intergenic regions of ABPV, PSIV, RhPV, and DCV and upstream capsid-coding regions. The residues that are identical in at least three of the four viruses are shown in reverse. The numbers on the left show the starting of the nucleotide positions of the aligned sequences. The lines above the alignments correspond to stem-loop structures (I–VII) predicted for the PSIV IRES (Sasaki and Nakashima, 1999).

al., 1993; Thole and Hull, 1996). ABPV resembles caliciviruses in the organization of its genome; in both viruses the capsid proteins are encoded at the 3′ terminus of the genome (Clarke and Lambden, 1997). However, ABPV and caliciviruses differ. Calicivirus particles consist of a single major 58- to 76-kDa protein, and it has been shown that caliciviruses have subgenomic RNA that encodes a capsid protein (Clark and Lambden, 1997).

The unique genome organizations of DCV, RhPV, and PSIV have stimulated an interest in how these viruses produce their capsid proteins effectively without having a subgenomic RNA. Recently, Sasaki and Nakashima (1999) confirmed that the capsid protein gene of PSIV is translated by an IRES *in vitro* by showing that translation of the gene occurs independent of cap. Various site-directed mutants were used to indicate that methionine is not the initiating amino acid in the translation of the capsid protein gene. It was demonstrated that translation is initiated at a CAA codon (Sasaki and Nakashima, 2000). In addition, analysis of the secondary structure of the IRES suggested the presence of a pseudoknot, which plays a primary role in translation initiation of the capsid protein gene (Sasaki and Nakashima, 2000). A multiple nucleotide sequence alignment of the PSIV IRES with DCV and RhPV showed that the nucleotide sequence is similar at this region, which contains short conserved RNA segments that could play an essential role in IRES activity. It was therefore proposed that translation of the capsid proteins of DCV and RhPV is initiated by a similar mechanism as that for PSIV (Sasaki and Nakashima, 1999). A multiple nucleotide sequence alignment of the intergenic regions (separating ORF1 and ORF2) of ABPV, PSIV, DCV, and RhPV was conducted (Fig. 3). The alignment showed that ABPV shared several short conserved segments with these viruses. Despite the high rate of evolution of RNA viruses, this region seems to be conserved in these viruses. It is therefore suggested that this noncoding region functions as an IRES in ABPV, facilitating translation of the capsid protein. The mechanism facilitating translation initiation at the 5′-proximal ORF of cricket paralysis-like viruses has not been experimentally established. The initiation of translation may also be facilitated by IRES.

Four structural proteins were identified for ABPV by SDS–PAGE analysis. N-terminal sequencing for the two ABPV capsid proteins of 33 kDa and 24 kDa was determined by Edman degradation. The capsid proteins were mapped to ORF2, which confirmed that the structural proteins were encoded at the 3′ end of the genome. The N-terminal sequence of the structural proteins of ABPV has been analyzed (Table 1). The region surrounding the N-terminal sequence of the 24-kDa protein is MQ/INLANK, with the scissle bond represented by a forward slash. The presence of a glutamate residue prior to the point of cleavage is consistent with processing by a 3C-like protease at this point. The sequence of the region surrounding the N terminus of the 33-kDa protein is GW/SKPRNL, with the scissile bond represented by a forward slash. This sequence is conserved at equivalent positions on the capsid proteins of other cricket paralysis-like viruses (Tate *et al.,* 1999). The mechanism facilitating cleavage at an equivalent position in CrPV was considered during an analysis of the crystal structure of the CrPV virus particle (Tate *et al.,* 1999). It was proposed that cleavage occurs in a manner analogous to that of the VP0 protein of picornaviruses. Autocatalytic cleavage of VP0 in picornaviruses yields VP4 and VP2 proteins and occurs within the capsid during capsid maturation.

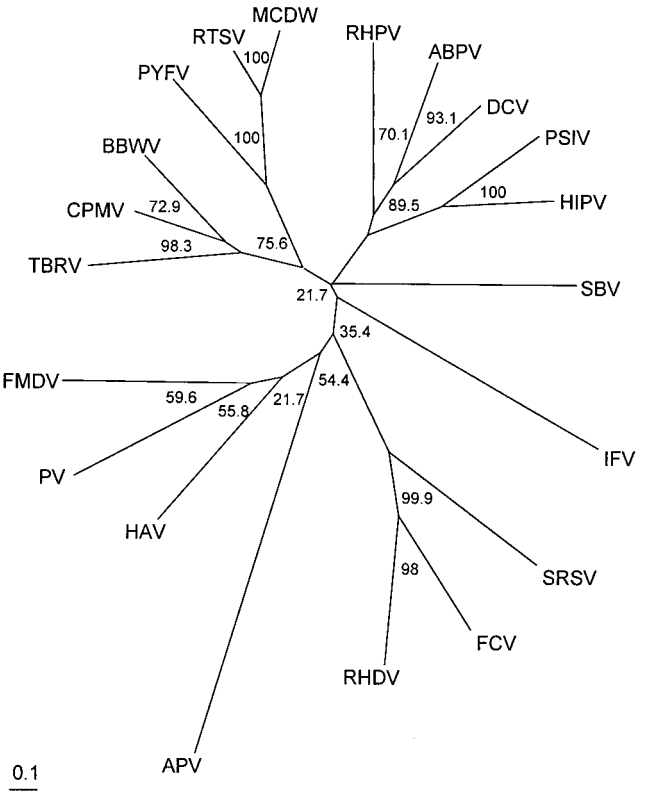

FIG. 4. Phylogenentic analysis of putative RdRp domains of selected positive-stranded RNA viruses. Numbers at each node represent bootstrap values as a percentage of 1000 trials. The scale bar represents 0.1 amino acid substitutions per site. Full virus names and accession numbers for sequences are provided under Materials and Methods.

The RdRp is the only domain that contains universal sequence motifs that are conserved in all positive-stranded RNA viruses with known genome sequences (Koonin and Dolja, 1993). Therefore, it is an ideal sequence to use for phylogenetic analysis. Phylogenetic analysis of the predicted amino acid sequence of the RdRp domain of selected RNA viruses showed that ABPV was most closely related to DCV. In addition, ABPV, DCV, HiPV, PSIV, and RhPV formed a group that branched separately from the other known virus families (Fig. 4). It would be interesting to determine whether this novel genome organization is unique to insect-infecting RNA viruses.

MATERIALS AND METHODS

ABPV source and purification

Acute bee paralysis virus was obtained from IACR-Rothamsted (UK). The virus was propagated by the injection (intrathorasic or intersegmental membrane, situated between the second and third abdominal tergites) of 1 μl of crude virus extract into white-eyed drone pupae (*A. mellifera*). One microliter of 0.01 M phosphate buffer (PB), pH 7.2, was injected into white-eyed drone pupae as a control. After 5–7 days incubation at 30°C, 20 ml 0.01 M PB, 1/10 volume of diethyl ether and carbon tetrachloride and 0.02% diethylthiocarbamate were added to 20 crushed drones. The sample was incubated at 4°C for 16 h. The mixture was centrifuged at 1000 rpm for 10 min and the supernatant was centrifuged at 47,000 rpm for 2 h in a NVT65 Beckman rotor. The pellet was then resuspended in 0.01 M PB, layered on a 10–40% (w/v) sucrose gradient, and centrifuged at 27,000 rpm for 2 h in a SW28 Beckman rotor at 4°C. The band at the 30% region of the gradient was pelleted and layered on a CsCl gradient (1.37 g/ml) and centrifuged at 40,000 rpm for 18 h in a NVT65 Beckman rotor, and the visible band was dialyzed against 0.01 M PB at 4°C for 8 h. The purified virus sample was negatively stained with 2% uranyl acetate and viewed under a transmission electron microscope.

RNA isolation and cDNA synthesis

Viral RNA was isolated by phenol/chloroform extraction after disruption of ABPV particles with 1% SDS at 60°C (Sambrook *et al.,* 1989). cDNA fragments complementary to ABPV RNA were synthesized using AMV reverse transcriptase (Promega) as instructed by the manufacturer. First-strand synthesis was conducted using oligo$(dT)_{15}$ and random hexameric primers. The blunt-ended fragments were ligated into the *Eco*RV site of Bluescript SK(+) (Stratagene). The ligation mixtures were transformed into *Escherichia coli* JM105. To complete the sequence, primers were designed between different clones to synthesize cDNA using the TITAN reverse transcription polymerase chain reaction kit (RT–PCR) (Roche Mannheim). The 5′ end of the genome was amplified using the 5′ RACE (rapid amplification of cDNA ends) system (GIBCO BRL) as described by the manufacturer. The RT–PCR and the 5′ RACE products were cloned into pCR-Script Amp SK(+) cloning vector (Stratagene). All PCR reactions were performed in a Hybaid OMN-E thermocycler in 700 μl PCR tubes.

Nucleotide sequencing

Plasmid DNA was prepared using the Nucleobond isolation kit (Machery–Nagel) as recommended by the manufacturer. The overlapping cDNA clones were digested with exonucleaseIII using the Erase-a-Base kit as recommended by the supplier (Promega). The clones and subclones were sequenced in both directions using an automated dye-terminator sequencer by the dideoxy chain-termination method (Sanger *et al.,* 1977).

Computer analysis of sequence data

Nucleotide sequence data was analyzed and compiled using the University of Wisconsin Genetics Computer Group (GCG) program (Devereux *et al.,* 1984) and overlapping fragments were assembled using a fragment assembly program (PHRAP) (Green, 1997) at the South African National Bioinformatics Institute. ABPV sequences were compared to the GenBank and EMBL databases using BLAST (Altschul *et al.,* 1990). Multiple sequence alignments were conducted using the ClustalW program of Thompson *et al.* (1994). Phylogenetic analysis was conducted using the neighbor-joining method as implemented in the ClustalW program. Confidence levels were estimated using the bootstrap resampling procedure. The GenBank accession number of the ABPV genome sequence is AF150629. The virus genome sequences (with accession numbers) used in this study were Acyrthosiphon pisum virus (APV, AF024514); broad bean wilt virus (BBWV, AAD38152); cowpea mosaic virus (CPMV, X00206 and X00729); cricket paralysis virus (CrPV, P13418); *Drosophila* C virus (DCV, AF014388); feline calcivirus (FCV, M86379); foot and mouth disease (FMDV, P03305); hepatitis A virus (HAV, P06441); Himetobi P virus (HiPV, AB017037); infectious flacherie virus (IFV, AB000906); maize chlorotic dwarf waikavirus (MCDV-TN, AAB58882); parsnip yellow fleck virus (PYFV, Q05057); *P. stali* intestine virus (PSIV, A8006531); poliovirus (PV, V01150); rabbit haemorrhagic disease virus (RHDV, AAB02225); rice tungro spherical virus (RTSV, RTU70989); *R. padi* virus (RhPV, AF022937); sacbrood virus (SBV, AF0924); Southampton calicivirus (SCV, L07418); and tomato black ring virus (TBRV, P18522).

SDS–PAGE and N-terminal sequencing

The molecular masses of the capsid proteins of ABPV were determined. The purified virus was analyzed by electrophoresis on a 12.5% SDS–polyacrylamide gel (Laemmli, 1970). Proteins were blotted onto PVDF membranes and N-terminal sequencing by Edman degradation was conducted using an Applied Biosystems Procise Sequencer.

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REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215,** 403–410.

Bailey, L., and Gibbs, A. J. (1964). Acute infection of bees with paralysis virus. *J. Gen. Virol.* **6,** 395–407.

Bailey, L., Gibbs, A. J., and Woods, R. D. (1963). Two viruses from adult honeybees *Apis mellifera L. Virology* **21,** 390–395.

Ball, B. V. (1985). Acute paralysis virus isolates from honeybee colonies infested with *Varroa jacobsoni. J. Apic. Res.* **24,** 115–119.

Clarke, I. N., and Lambden, P. R. (1997). The molecular biology of caliciviruses. *J. Gen. Virol.* **78,** 291–301.

Devereux, J., Haeberli, P., and Smithies, O. (1984). A comparative set of sequence analysis programs for the Vax. *Nucleic Acids Res.* **12,** 387–395.

Ghosh, R. C., Ball, B. V., Willcocks, M. M., and Carter, M. J. (1999). The nucleotide sequence of sacbrood virus of the honeybee: An insect picorna-like virus. *J. Gen. Virol.* **80,** 1541–1549.

Green, P. (1997). Against a whole genome shotgun. *Genome Res.* **7,** 410–417.

Johnson, K. N., and Christian, P. D. (1998). The novel genome organization of the insect picorna-like virus, *Drosophila* C virus, suggests this virus belongs to a previously undescribed virus family. *J. Gen. Virol.* **79,** 191–203.

Koonin, E. V., and Dolja, V. V. (1993). Evolution and taxonomy of positive-strand RNA viruses: Implications of comparative analysis of amino acid sequences. *Crit. Rev. Biochem. Mol.* **28,** 375–430.

Koonin, E. V., and Gorbalenya, A. E. (1992). An insect picornavirus may have a genome organization similar to that of caliciviruses. *FEBS Lett.* **297,** 81–86.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680–685.

Moon, J. S., Domier, L. L., McCoppin, N. K., D'Arcy, C. J., and Jin, H. (1998). Nucleotide sequence analysis shows that *Rhopalosiphum padi* virus is a member of a novel group of insect-infecting RNA viruses. *Virology* **243,** 54–65.

Moore, N. F., Reavy, B., and King, L. A. (1985). General characteristics, gene organisation and expression of small RNA viruses of insects. *J. Gen. Virol.* **66,** 647–659.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanger, F., Niklen, S., and Coulson, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.

Sasaki, J., and Nakashima, N. (1999). Translation initiation at the CUU codon is mediated by the internal ribosome entry site of an insect picorna-like virus in vitro. *J. Virol.* **73,** 1219–1226.

Sasaki, J., and Nakashima, N. (2000). Methionine-independent initiation of translation in the capsid protein of an insect RNA virus. *Proc. Natl. Acad. Sci. USA* **97,** 1512–1515.

Sasaki, J., Nakashima, N., Saito, H., and Noda, H. (1998). An insect picorna-like virus, *Plautia stali* intestine virus, has genes of the capsid proteins in the 3′ end of the genome. *Virology* **244,** 50–58.

Scott-Dupree, C., and McCarthy, J. (1995). Honey bee viruses. *Bee Cult.* 392–396.

Shen, P., Kaniewska, M., Smith, C., and Beachy, R. N. (1993). Nucleotide sequence and genomic organization of rice tungro spherical virus. *Virology* **193,** 621–630.

Strauss, E. G., Strauss, J. H., and Levine, A. J. (1996). Virus evolution. *In* "Field's Virology," 3rd ed., pp. 153–171. Lippincott–Raven, New York.

Tate, J., Liljas, L., Scotti, P., Christian, P., Lin, T., and Johnson, J. E. (1999). The crystal structure of cricket paralysis virus: The first view of a new virus family. *Nat. Struct. Biol.* **6,** 765–774.

Thole, V., and Hull, R. (1996). Rice tungro spherical virus: Nucleotide sequence of the 3′ genomic half and studies on the two small 3′ open reading frames. *Virus Genes* **13,** 239–246.

Thompson, J. D., Higgens, D. G., and Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22,** 4673–4680.

van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., Carsten, E. B., Estes, M. K., Lemon, S. M., Maniloff, J., Mayo, M. A., McGeoch, D. J., Pringle, C. R., and Wickner, R. B. (1999). "Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses." Academic Press, San Diego.

Acute bee paralysis virus (ABPV) was first described as a cause of inapparent infections of the honeybee (Apis mellifera) (3). The presence of the virus has been reported from several countries worldwide (12, 15, 22, 31, 35). ABPV is considered to be a common infective agent of bees which is frequently detected in apparently healthy colonies.Â The complete nucleotide sequence was determined recently (18). The genome contains two open reading frames (ORFs).Â The importance of viruses in diseases of bees has been investigated in several studies. To date, 18 different honeybee viruses have been described (1, 19). Most of them cause inapparent infections. Viruses belonging to other families have been proposed for use and are under evaluation for safety, specificity, and efficacy on the control of their insect hosts. Viruses has published a Special Issue on this topic (2014).Â Since the discovery that honey bee viruses play a role in colony decline, researchers have made major breakthroughs in understanding viral pathology and infection processes in honey bees. Work on virus transmission patterns and virus vectors, such as the mite Varroa destructor, has prompted intense efforts to manage honey bee health. However, little is known about the occurrence of honey bee viruses in bee predators, such as vespids.Â (This article belongs to the Special Issue Insect Viruses and Pest Management). Analysis of the complete genome sequence of acute bee paralysis virus shows that it belongs to the novel group of insect-infecting RNA viruses. Virology 277: 457-463. Kukielka D, SÃ¡nchez-VizcaÃno JM, 2010. Short commu-nication. First detection of Israeli Acute Paralysis Virus. (IAPV) in Spanish honeybees. Span J Agric Res 8: 308-311.Â First detection of Israeli acute paralysis virus (IAPV) in Poland and phylogenetic analysis of the isolates. J Apicult Sci 55(2): 149-159. Reynaldi FJ, Sguazza GH, Tizzano MA, Fuentealba N, Galosi CM, Pecoraro MR, 2011. First report of Israeli acute paralysis virus in asymptomatic hives of Argentina. Rev Argent Microbiol 43: 84-86. RibiÃ¨re M, Ball B, Aubert MFA, 2008.