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Genomic and host range studies of *Maruca vitrata* nucleopolyhedrovirus

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The complete genome of the *Maruca vitrata* nucleopolyhedrovirus (MaviNPV) isolated from the legume pod borer, *Maruca vitrata* (Lepidoptera: Pyralidae), was sequenced. It was found to be 111 953 bp in length, with an overall 39 % G+C content, and contained 126 open reading frames (ORFs) encoding predicted proteins of over 50 aa. The gene content and gene order of MaviNPV have the highest similarity to those of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and their shared homologous genes are 100 % collinear. In fact, MaviNPV seems to be a mini-AcMNPV that is native to Taiwan and possesses a smaller genome with fewer auxiliary genes than the AcMNPV type species. Except for one ORF (Mv74), all of the MaviNPV ORFs have homologues in the AcMNPV genome. MaviNPV is the first lepidopteran-specific baculovirus to lack homologues of *vfgf* and *odv-e66*. In addition, MaviNPV lacks the baculovirus repeat ORF (*bro*) gene that corresponds to AcMNPV ORF2. Five homologous regions (*hrs*) were located within the MaviNPV genome, and these contained a total of 44 imperfect palindromes. Phylogenetic analysis of the whole genome revealed that MaviNPV was separated from the common ancestor of AcMNPV and *Bombyx mori* nucleopolyhedrovirus before these two viral species diverged from each other. Moreover, replication of MaviNPV in several cell lines and an *egfp*–MaviNPV infection assay revealed that IPLB-LD-652Y cells are only partially permissive to MaviNPV, which supports our conclusion that MaviNPV is a distinct species of the group I lepidopteran NPVs.

INTRODUCTION

The legume pod borer, *Maruca vitrata* (Lepidoptera: Pyralidae), is a pantropical pest of legume crops, particularly cowpea (Jackai, 1995), pigeonpea (Shanower *et al.*, 1999) and beans (Abate & Ampofo, 1996). In the 1990s, infestation rates reached 50–80 % for cowpea in West Africa (Afun *et al.*, 1991; Dreyer *et al.*, 1994). Most nucleopolyhedroviruses (NPVs) studied so far are pathogenic for insect species of the orders Lepidoptera, Diptera and Hymenoptera (Theilmann *et al.*, 2005). A new baculovirus isolated from *M. vitrata* (*M. vitrata* nucleopolyhedrovirus, MaviNPV) has great potential to allow us to investigate the biological control of this pest (Lee *et al.*, 2007). The family *Baculoviridae* is subdivided into two genera, *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV). Based on the *polyhedrin* (*polh*) gene, NPVs are further subdivided into group I and group II (Bulach *et al.*, 1999; Herniou *et al.*, 2001, 2003). Recent evidence shown from the comparison of 29 baculovirus genomes indicates that baculovirus phylogeny is aligned with the classification of the hosts more closely than with morphological traits (Afonso *et al.*, 2001; Garcia-Maruniak *et al.*, 2004; Lauzon *et al.*, 2004; Jehle *et al.*, 2006). An updated classification of the family *Baculoviridae*, which includes four genera – *Alphabaculovirus* (lepidopteran-specific NPV), *Betabaculovirus* (lepidopteran-specific GV), *Gammabaculovirus* (hymenopteran-specific NPV) and *Deltabaculovirus* (dipteran-specific NPV) – was thus proposed (Lauzon *et al.*, 2004; Jehle *et al.*, 2006). Using whole genome sequence data to compare the genetic makeup of different baculoviruses may help to determine which genes are essential for these viruses and help in understanding host range,

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The GenBank/EMBL/DDBJ accession number for the complete genome sequence of MaviNPV reported in this paper is EF125867.

virulence and morphology. Genes found in all baculoviruses are more likely to be essential genes, while auxiliary genes found in only some baculoviruses may give viruses a selective advantage in nature (O'Reilly, 1997).

The type species of the genus *Nucleopolyhedrovirus* is *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). This virus can infect more than 33 species of Lepidoptera (Groener, 1986) and a wide variety of lepidopteran cell lines (Hink, 1970; Hink & Hall, 1989; Goodman & McIntosh, 1994; McIntosh *et al.*, 2005). In contrast, most NPVs generally have a more restricted host range, for example, *Bombyx mori* nucleopolyhedrovirus (BmNPV) replicates strongly in Bm5 cells or larvae, but only weakly in Sf9 cells (Martin & Croizier, 1997); *Lymantria xylina* multiple nucleopolyhedrovirus (LyxyMNPV) replicates only in *Lymantria dispar* and *Lymantria xylina* cells (Wu & Wang, 2005, 2006). Limits on the host range of baculoviruses appear to be controlled and influenced by the cell's factors (Miller & Lu, 1997).

The apparent size of the MaviNPV genome (111.95 kbp in this study) is smaller than that of AcMNPV (133.9 kbp) (Ayres *et al.*, 1994) and BmNPV (128.4 kbp) (Gomi *et al.*, 1999). Based on the sequences of 29 baculoviral core genes in phylogenetic analyses in this study, MaviNPV belongs to group I NPVs and is closely related to AcMNPV and BmNPV.

In this report, in addition to describing the whole genomic sequence and gene structure, and performing a phylogenetic analysis, we also investigated the replicative host range of MaviNPV in lepidopteran cell lines using wild-type and *egfp* recombinant MaviNPV.

METHODS

Insect cell lines, virus and viral DNA. *Lymantria dispar* cell line [IPLB-LD-652Y; Goodwin *et al.*, 1978 (LD)], *Perina nuda* cell line [NTU-PN-HH; Wang *et al.*, 1996 (PN)], *Lymantria xylina* cell line [NTU-LY4; Wu & Wang, 2006 (LY)] and *M. vitrata* cell line [NTU-MV56; Yeh *et al.*, 2007 (MV)] were cultured in TNM-FH medium (Hink & Strauss, 1976) at 28 °C. The medium contained 8 % fetal bovine serum (FBS) supplemented with 50 IU penicillin ml^{-1}, 50 μg streptomycin ml^{-1} and 1.25 μg fungizone ml^{-1}. Sf9 cells were cultured in SF900 medium (Gibco) at 28 °C. An MaviNPV clone (MV-8) was isolated using MV cells and used for mass production. In addition to the wild-type MaviNPV, the methods of Summers & Smith (1987) were used to construct an *egfp* recombinant MaviNPV (*egfp*–MaviNPV, in which the *egfp* gene is driven by the MaviNPV *pol* promoter). Virus titres were determined following the protocol described by Summers & Smith (1987). Viral occlusion bodies (OBs) and viral DNA were prepared according to a procedure of Yeh *et al.* (2007). Quantity and quality of extracted DNA were determined spectrophotometrically and by electrophoresis in 0.7 % agarose.

Nucleotide sequence determination. The MaviNPV genome was sequenced to eightfold coverage by a shotgun approach. The viral DNA was sheared by nebulization into fragments with an average size of 2000 bp (HybroShear; GeneMachines). DNA fragments were size fractionated by gel electrophoresis and cloned into the *Eco*RV site of pBluescript II SK(−) (Stratagene). Sequencing was performed by ABI 3730 DNA Analyser (Applied Biosystems) then compiled into contigs using the PHRED/PHRAP software package (Ewing & Green, 1998; Ewing *et al.*, 1998). The assembled sequences were then edited and completed using the Sun workstation interface (Bonfield *et al.*, 1995).

DNA sequence analysis. Open reading frames (ORFs) were identified using GeneWorks software (IntelliGenetics) and ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) (Wheeler *et al.*, 2003). The criterion for defining an ORF was a size of at least 150 nt (50 aa) with minimal overlap. In addition, the genome was checked in detail for the presence of any ORFs similar to AcMNPV (Ayres *et al.*, 1994) or any other baculovirus in GenBank. Homology searches were done through the National Centre for Biotechnology Information website using the BLAST program (Altschul *et al.*, 1990). Multiple alignments and percentage identities of all MaviNPV ORFs with their homologues in selected genomes were generated using CLUSTAL_X (Thompson *et al.*, 1997). Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.html) was used to locate and analyse the homologous regions (*hrs*). Gene parity plot analysis was performed on the MaviNPV genome in comparison to the genomes of AcMNPV, BmNPV, *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV) and *Cydia pomonella* granulovirus (CpGV) as described previously (Hu *et al.*, 1998).

Phylogenetic analysis. A phylogenetic tree was inferred from a dataset of combined amino acid sequences of the 29 baculovirus core genes (Herniou *et al.*, 2003; Jehle *et al.*, 2006) of the 33 baculoviruses that had been completely sequenced at the time of analysis (Supplementary Table S1 available in JGV Online). Neighbour-joining (NJ) and maximum-parsimony (MP) analyses were performed using MEGA, version 3.1 (Kumar *et al.*, 2004). *Culex nigripalpus* nucleopolyhedrovirus (CuniNPV) was selected as the outgroup. Bootstrap analyses were performed to evaluate the robustness of the phylogenies using 1000 replicates for both NJ and MP analyses.

MaviNPV infection assay. The MV, Sf9, PN, LY and LD insect cell lines (3×10^6 cells in T25 flask) were incubated with MaviNPV (m.o.i. of 10) for 1 h at 28 °C, and then washed twice with medium. After washing, 5 ml fresh medium was added to the cells which were incubated at 28 °C. Total RNA was extracted by Trizol (Invitrogen) at 0, 72 and 168 h post-infection (p.i.) according to the product manual. The cDNA samples were prepared following Liu *et al.* (2005) for real-time PCR analysis to detect the expression of the representative early, late and very late stage genes: *ie-1*, *vp39* and *polh*, respectively. Primer sets IE1-q-f: 5′-GTATTTGACTCAGAATGCGGC-3′, IE1-q-r: 5′-GTTTACATCTTTAATTTCGCCAG-3′, 5′-GAGTCCGTGCCGATG-TAAAC-3′; VP39-q-f: 5′-GCAAATAAACCATCCGTCGT-3′, VP39-q-r: 5′-CCGAGACAAATGAAACTCAATC-3′, 5′-GAAGAGCACCG-CGATAGGATT-3′; polh-q-f: 5′-CAACGAGTACAGAATTAGTCTT-GC-3′, polh-q-r and Mv18S-QPCR-F: 5′-TGTTGCGGTTAAAAAG-CTCGTA-3′, Mv18S-QPCR-R, for *ie-1*, *vp39*, *polh* and 18S, respectively, were designed by the Primer Express computer program (Applied Biosystems). The reactions were performed in the iQ5 Real-Time PCR Detection System (Bio-Rad) by using Q SYBR Green Supermix (Bio-Rad). The comparative C_t method was used to analyse the real-time PCR data (Livak & Schmittgen, 2001). All values were normalized to 18S rRNA by subtracting the mean 18S rRNA C_t from the mean target C_t for each sample to give the ΔC_t. For relative quantification, we used the $2^{-\Delta\Delta Ct}$ equation to calculate gene expression in target samples relative to the calibrator.

***egfp*–MaviNPV infection assay.** The MV, Sf9, PN, LY and LD insect cell lines (1×10^6 cells per well; six-well plate) were infected with *egfp*–MaviNPV (m.o.i of 10). The cells were checked daily for cytopathic effect (CPE), and the infection rates and virus titres were

counted and determined at 7 days p.i. by observing the fluorescent signal.

RESULTS AND DISCUSSION

Genome sequence analysis and ORFs prediction

Sequence analysis of the MaviNPV genome. The entire MaviNPV genome is 111 953 nt (Fig. 1). This size is in line with the estimate of 113.4 kbp produced by restriction-enzyme analysis (Lee *et al.*, 2007). The G+C content of the MaviNPV genome is 38.6 %, slightly lower than that of lepidopteran NPVs group I, but very close to the content recorded for a variant of AcMNPV, *Rachiplusia ou* multiple nucleopolyhedrovirus (RoMNPV) (39.1 %). A total of 126 ORFs were defined. Fifty-one per cent (64/126) of the ORFs are oriented clockwise and 49 % (62/126) anti-clockwise, with respect to the orientation of the *polh* gene (ORF1; Vlak & Smith, 1982). All 126 MaviNPV ORFs have an assigned function or homologues in other baculoviruses. The 29 genes shared by all baculovirus genomes, including the dipteran and hymenopteran baculoviruses (Herniou *et al.*, 2003; Garcia-Maruniak *et al.*, 2004), were also found in the MaviNPV genome. The 126 predicted ORFs are encoded by 92 % of the MaviNPV genome; the rest is made up of intergenic spaces and *hrs*. Five identified *hrs* were dispersed around the MaviNPV genome; the maximum distance between two *hrs* was 35 kbp. The MaviNPV genome has 29 pairs of ORFs with overlapping codons. This compares to 23 in AcMNPV, 16

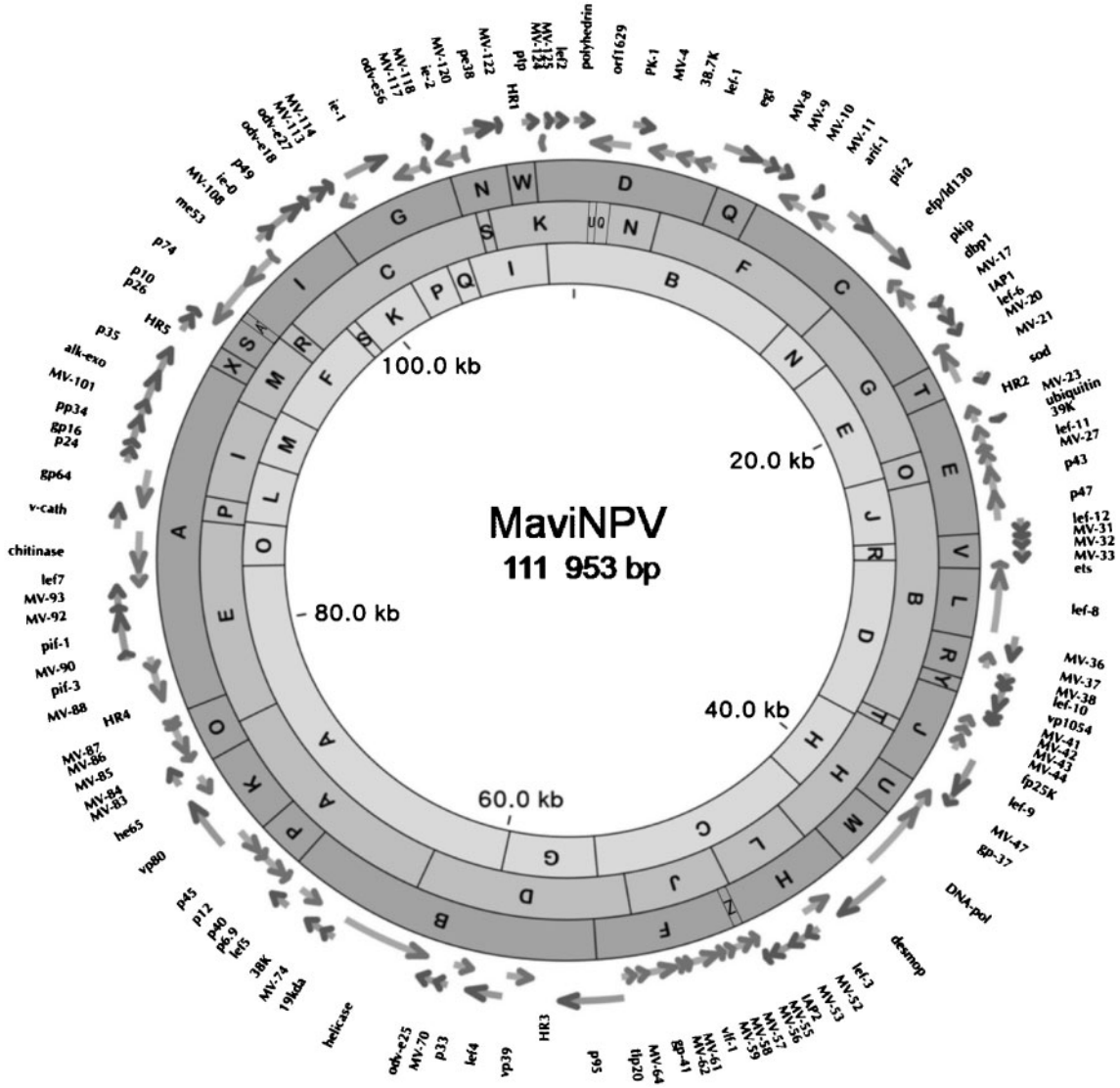

Fig. 1. Circular map of the MaviNPV genome. Locations for *Pst*I, *Hin*dIII and *Eco*RI are shown on the inner, middle and outer rings, respectively.

of which are also found in MaviNPV. In MaviNPV, the average overlap is 36 bp, and there are 12 overlaps longer than 20 bp. The maximal overlap of 153 bp exists between *lef-3* and *ac68* (Mv51/52). These two genes are in the opposite orientation, and both have homologues in AcMNPV. There are, moreover, two gene clusters packed very tightly: ORFs 36–40 and 60–66 in the MaviNPV genome (Table 1). In general, the MaviNPV genome is densely packed.

Comparison of MaviNPV ORFs to those of other baculoviruses. The gene content and organization of MaviNPV were compared with those of two group I NPVs (AcMNPV and BmNPV), a group II NPV (LdMNPV) and a GV (CpGV). MaviNPV shares 125 ORFs with AcMNPV, 123 with BmNPV, 90 with LdMNPV and 62 with CpGV, and these homologous ORFs had mean amino acid identities of 79.2, 78.6, 34.8 and 27.1 %, respectively. In the 132 genes shared between BmNPV and AcMNPV, the average amino acid identity is 93 % (Gomi *et al.*, 1999). There are fewer shared genes between MaviNPV and BmNPV (123), and between MaviNPV and AcMNPV (125); the mean amino acid identities of these shared genes are also lower (79.2 and 78.6 %, respectively). Thus, the phylogenetic relationship between BmNPV and AcMNPV is closer than the relationship that MaviNPV has to either virus.

Ninety-six ORFs show the highest percentage of identity with AcMNPV, 17 with BmNPV and 12 with identities equal to AcMNPV and BmNPV. Three pairs of adjacent AcMNPV ORFs that are in the same orientation (Ac20/Ac21, Ac58/Ac59 and Ac106/Ac107) are fused into a single ORF in MaviNPV. The homologues of these ORFs also occur in other baculovirus genomes in which they are fused into a single ORF (Harrison & Bonning, 2003).

In Fig. 2, genes of AcMNPV were renumbered manually, starting with the *polh* gene as number 1. The gene arrangement of the MaviNPV genome was completely collinear to those of AcMNPV and BmNPV, but less collinear with LdMNPV. In contrast, parity analysis of MaviNPV and CpGV ORFs displayed a much more dispersed pattern.

Transcriptional regulation. Sixteen MaviNPV ORFs possess a consensus early gene promoter motif (a TATA box followed by a CAGT motif 20–30 bp downstream) within 180 bp (Kool & Vlak, 1993; Xing *et al.*, 2005) of the initiation codon (Table 1). Of these, nine ORFs, including Mv1 (*polh*), Mv14 (*efp*/*ld130*), Mv25 (*39K*), Mv42 (*ac56*), Mv66 (*p95*), Mv89 (*pif-3*), Mv97 (*gp64*), Mv107 (*me53*) and Mv109 (*ie-0*), also possess a late gene promoter motif (A/T/GTAAG), which may allow transcription of these genes during both early and late stages of infection, which has also been reported for Ac128 (*gp64*) and Ac147 (*ie-1*) (Kool & Vlak, 1993; O'Reilly *et al.*, 1994a). Although the early gene promoter motif is present in the MaviNPV *polh*, mRNA was not detectable at the early stage of infection (data not shown). Seventy-seven MaviNPV ORFs possess a consensus late gene promoter motif within 120 bp of the initiation codon. A CGTGC motif has also been identified as an early promoter consensus sequence/transcription initiation site (enhancer-like element) in AcMNPV Ac40 (*p47*), Ac65 (*DNA-pol*) and Ac95 (*helicase*) (see Kool & Vlak, 1993). A CGTGC motif was found within 210 bp of the initiation codon of 28 MaviNPV ORFs (Table 1). Thirty-one of the MaviNPV ORFs did not possess consensus late or early promoter sequences. It seems likely that additional, as-yet-unidentified, promoter sequences might exist within the MaviNPV genome, as they do in other baculoviruses (Kool & Vlak, 1993; O'Reilly *et al.*, 1994a).

Transcription-specific genes. Baculovirus gene transcription occurs in a temporal cascade for the immediate-early, delayed-early, late and very late genes. In AcMNPV, the viral RNA polymerase comprises four subunits encoded by *lef-4*, *lef-8*, *lef-9* and *p47* (Guarino *et al.*, 1998), and these are present in all fully sequenced baculovirus genomes, including that of MaviNPV, as ORFs 68, 35, 46 and 29, respectively. Homologues of both *lef-5* and *vlf-1* were also found in MaviNPV (ORFs 76 and 60). Although the function of *lef-5* is unclear, *vlf-1* is essential for the 'burst' in very late gene expression seen for *p10* and *polh* (Yang & Miller, 1998, 1999). Two transcription-specific genes *lef-10* and *lef-12*, previously identified in AcMNPV and BmNPV, were also present in MaviNPV as ORFs 39 and 30, respectively. In addition, MaviNPV encodes homologues of *39K* (ORF25), *lef-6* (ORF19) and *lef-11* (ORF26), which are present in all sequenced lepidopteran baculoviruses (Herniou *et al.*, 2003). Although AcMNPV, BmNPV and MaviNPV are all closely related, we note that while the amino acid identity of AcMNPV *lef-6* and BmNPV *lef-6* aa is 93 %, the identity between MaviNPV *lef-6* aa and AcMNPV or BmNPV is only 34–35 %.

DNA replication genes. Six baculovirus genes, *lef-1*, *lef-2*, *lef-3*, *DNApol*, *helicase* and *ie-1*, are essential in transient assays for DNA replication in AcMNPV and *Orgyia pseudotsugata* multiple nucleopolyhedrovirus (OpMNPV) and exist in all lepidopteran baculoviruses, including MaviNPV (Kool *et al.*, 1995; Lu & Miller, 1995b; Ahrens & Rohrmann, 1995). Homologues of an additional single-stranded DNA-binding protein (*dbp*) (Mikhailov *et al.*, 1998) and an immediate-early gene, *me53*, both of which have been implicated in DNA replication, were also found in MaviNPV (ORF16 and ORF107). Homologues of the non-essential DNA replication stimulatory genes, *ie-2*, *lef-7* and *pe38* (Kool *et al.*, 1994), identified in AcMNPV, were also present in MaviNPV. However, no homologues to *rr1*, *rr2* or *dutpase* or any other gene involved in nucleotide metabolism were found in MaviNPV; MaviNPV is the only baculovirus that is so far reported to lack these genes (Ahrens *et al.*, 1997).

Table 1. ORFs predicted in the genome of MaviNPV

ORF	Name*	Position†	Length (aa)	Promoter motifs‡	Homologues (% identity)§			
					AcMNPV	BmNPV	LdMNPV	CpGV
1	*polh*	1→738	245	E, L	Ac8 (88) 245	Bm1 (93) 245	Ld1 (78) 245	Cp1 (55) 258
2	*orf1629*	735←2354	539		Ac9 (77) 543	Bm2 (74) 542	Ld2 (16) 555	
3	*pk-1*	2353→3165	270	L	Ac10 (89) 272	Bm3 (90) 275	Ld3 (39) 274	Cp3 (31) 279
4	*ac11*	3203←4201	332	E	Ac11 (82) 340	Bm4 (79) 340	Ld35 (24) 359	
5	*38.7K*	4355←5326	323	L	Ac13 (85) 327	Bm5 (81) 331	Ld122 (16) 200	Cp73 (11) 198
6	***lef-1***	5224←6018	264		Ac14 (87) 266	Bm6 (85) 270	Ld123 (35) 234	Cp74 (24) 235
7	*egt*	6072→7583	503		Ac15 (83) 506	Bm7 (80) 506	Ld125 (35) 560	
8	*ac16*	7703→8332	209		Ac16 (71) 225	Bm8 (69) 229		
9	*ac17*	8301→8948	215		Ac17 (76) 209	Bm9 (74) 210	Ld128 (18) 226	
10	*ac18*	8987←10042	351		Ac18 (87) 353	Bm10 (82) 356	Ld158 (16) 373	
11	*ac19*	10041→10370	109	L	Ac19 (87) 108 Ac20 (41) 69	Bm11 (81) 110	Ld159 (22) 118	
12	*arif-1*	10430←11650	406	E	Ac21 (65) 319	Bm12 (59) 440	Ld118 (13) 269	
13	***pif-2***	11675→12823	382	L	Ac22 (95) 382	Bm13 (90) 382	Ld119 (53) 407	Cp48 (47) 372
14	*efp/ld130*	12915→14843	642	E, L	Ac23 (66) 690	Bm14 (65) 673	Ld130 (14) 676	Cp31 (11) 601
15	*pkip*	14869←15348	159	L	Ac24 (79) 169	Bm15 (79) 169	Ld110 (22) 179	
16	*dbp1*	15429←16367	312		Ac25 (91) 316	Bm16 (91) 317	Ld47 (21) 257	Cp81 (16) 290
17	*ac26*	16443→16832	129	e, L	Ac26 (83) 129	Bm17 (83) 129	Ld36 (28) 123	
18	*IAP1*	16834→17682	282	e	Ac27 (71) 286	Bm18 (70) 292	Ld139 (15) 155	Cp17 (24) 275
19	*lef-6*	17689→18207	172		Ac28 (34) 173	Bm19 (34) 173	Ld38 (20) 159	
20	*ac29*	18301←18504	67		Ac29 (49) 71	Bm20 (47) 71	Ld39 (39) 68	Cp19 (26) 75
21	*ac30*	18531←19982	483	e, L	Ac30 (82) 463	Bm21 (80) 472		
22	*sod* *hr2*	19966→20424 20436–21162	152	L	Ac31 (88) 151	Bm23 (86) 151	Ld145 (72) 154	Cp59 (47) 132
23	*ac34*	21174←21812	212	L	Ac34 (85) 215	Bm25 (83) 215	Ld42 (27) 188	
24	*ubiquitin*	21834→22067	77	L	Ac35 (93) 77	Bm26 (94) 77	Ld43 (36) 150	Cp54 (62) 94
25	*39K*	22103←22903	266	e, E, L	Ac36 (85) 275	Bm27 (82) 277	Ld44 (25) 264	Cp57 (10) 241
26	*lef-11*	22897←23232	111	e	Ac37 (83) 112	Bm28 (82) 112	Ld45 (22) 187	Cp58 (24) 134
27	*ac38*	23198←23848	216	e, L	Ac38 (91) 216	Bm29 (92) 217	Ld46 (45) 247	Cp69 (37) 220
28	*p43*	23902←24933	343	e	Ac39 (73) 363	Bm30 (73) 362		
29	***p47***	24945←26144	399	e, L	Ac40 (92) 401	Bm31 (91) 399	Ld48 (52) 390	Cp68 (33) 460
30	*lef-12*	26143→26679	178	e	Ac41 (83) 181	Bm32 (79) 183		
31	*ac43*	26703→26918	71	L	Ac43 (80) 77	Bm34 (77) 78		
32	*ac44*	26899→27294	131	e	Ac44 (87) 131	Bm35 (86) 131		
33	*ac45*	27296→27859	187		Ac45 (77) 192	Bm36 (70) 193		
34	*ets*	27950←28207	85	E	Ac47 (82) 88	Bm38 (78) 89		
35	***lef-8***	28286←30922	878		Ac50 (93) 876	Bm39 (92) 877	Ld51 (59) 874	Cp131 (46) 873
36	*ac51*	30949→31905	318		Ac51 (84) 318	Bm40 (79) 319		
37	*ac52*	31896←32480	194	e	Ac52 (50) 123	Bm41 (84) 194	Ld53 (16) 300	
38	*ac53*	32482→32901	139	L	Ac53 (89) 139	Bm42 (88) 139	Ld54 (43) 142	Cp134 (20) 133
39	*lef-10*	32898→33134	78		Ac53a (82) 78	Bm42a (80) 78	Ld56 (37) 76	Cp137 (26) 89
40	***vp1054***	32992→34080	362		Ac54 (91) 365	Bm43 (89) 365	Ld57 (39) 332	Cp138 (24) 332
41	*ac55*	34152→34361	69		Ac55 (72) 73	Bm44 (67) 77	Ld58 (28) 64	
42	*ac56*	34363→34617	84	E, L	Ac56 (90) 84 Ac58 (59) 57	Bm45 (88) 84		
43	*ac58-59*	34697←35182	161		Ac59 (79) 69	Bm47 (74) 171	Ld61 (26) 189	
44	*ac60*	35189←35437	82	L	Ac60 (86) 87	Bm48 (78) 83	Ld60 (35) 95	
45	*fp25K*	35581←36210	209	L	Ac61 (92) 214	Bm49 (91) 214	Ld63 (49) 217	Cp118 (24) 161
46	***lef-9***	36306→37778	490		Ac62 (89) 516	Bm50 (94) 490	Ld64 (66) 496	Cp117 (51) 499

Table 1. cont.

ORF	Name*	Position†	Length (aa)	Promoter motifs‡	Homologues (% identity)§			
					AcMNPV	BmNPV	LdMNPV	CpGV
47	*ac63*	37838→38287	149	E	Ac63 (81) 155	Bm51 (80) 155	Ld117 (10) 154	
48	*gp-37*	38315←39178	287	L	Ac64 (85) 302	Bm52 (86) 294	Ld68 (41) 269	Cp13 (33) 251
49	***DNA-pol***	39220←42162	980		Ac65 (91) 984	Bm53 (89) 988	Ld83 (41) 1014	Cp111 (27) 1051
50	*desmop*	42171→44471	766	L	Ac66 (78) 808	Bm54 (76) 805	Ld82 (14) 778	Cp112 (11) 718
51	*lef-3*	44500←45618	372	L	Ac67 (80) 385	Bm55 (78) 385	Ld81 (26) 374	Cp113 (11) 353
52	***ac68***	45466→46020	184		Ac68 (79) 192	Bm56 (62) 134	Ld80 (23) 128	Cp114 (19) 126
53	*ac69*	45998→46786	262		Ac69 (32) 262	Bm57 (33) 262		
54	*IAP2*	46882→47622	246	L	Ac71 (85) 249	Bm58 (82) 249	Ld79 (28) 234	
55	*ac72*	47680→47850	56	L	Ac72 (76) 60	Bm58a (70) 61		
56	*ac73*	47861←48157	98	e, L	Ac73 (80) 99	Bm59 (78) 99		
57	*ac74*	48154←48936	260	L	Ac74 (77) 265	Bm60 (74) 268		
58	*ac75*	48983←49384	133	L	Ac75 (78) 133	Bm61 (90) 133	Ld84 (24) 128	Cp108 (15) 148
59	*ac76*	49400←49654	84	L	Ac76 (91) 84	Bm62 (91) 85	Ld85 (36) 86	Cp107 (28) 84
60	***vlf-1***	49670←50821	383	L	Ac77 (95) 379	Bm63 (94) 379	Ld86 (63) 378	Cp106 (27) 378
61	*ac78*	50828←51148	106	L	Ac78 (85) 109	Bm64 (79) 110	Ld87 (28) 113	Cp105 (15) 111
62	*ac79*	51151←51465	104	L	Ac79 (93) 104	Bm65 (94) 104		
63	***gp41***	51468←52688	406	L	Ac80 (92) 409	Bm66 (88) 403	Ld88 (44) 323	Cp104 (21) 289
64	***ac81***	52678←53361	227	e, L	Ac81 (86) 233	Bm67 (84) 234	Ld89 (46) 219	Cp103 (36) 191
65	*tlp20*	53222←53752	176	L	Ac82 (78) 180	Bm68 (74) 181	Ld90 (21) 223	Cp102 (9) 216
66	***vp91/p95***	53718→56249	843	E, L	Ac83 (84) 847	Bm69 (80) 839	Ld91 (36) 864	Cp101 (14) 665
	hr3	56264–57313						
67	***vp39***	57485←58522	345	L	Ac89 (88) 347	Bm72 (87) 350	Ld92 (38) 352	Cp96 (22) 285
68	***lef-4***	58541→59935	464		Ac90 (89) 464	Bm73 (87) 465	Ld93 (41) 485	Cp95 (26) 480
69	***p33***	59982←60752	256	e, L	Ac92 (94) 259	Bm75 (93) 259	Ld94 (48) 251	Cp93 (35) 251
70	*ac93*	60751→61239	162		Ac93 (91) 161	Bm76 (90) 161	Ld95 (43) 159	Cp92 (27) 161
71	*odv-e25*	61247→61936	229	e, L	Ac94 (87) 228	Bm77 (82) 228	Ld96 (41) 217	Cp91 (36) 213
72	***helicase***	61974←65648	1224	e, L	Ac95 (91) 1221	Bm78 (89) 1222	Ld97 (39) 1218	Cp90 (19) 1131
73	***19kda***	65635→66162	175	e	Ac96 (88) 173	Bm79 (82) 182	Ld98 (41) 173	Cp89 (30) 161
74	*op98/chch34*	66229→66903	224	e				
75	***38K***	66928←67893	321	e, L	Ac98 (86) 320	Bm82 (86) 320	Ld99 (40) 322	
76	***lef-5***	67828→68625	265	L	Ac99 (95) 265	Bm83 (92) 265	Ld100 (44) 278	Cp87 (37) 242
77	***p6.9***	68622←68789	55	L	Ac100 (100) 55	Bm84 (78) 65	Ld101 (39) 99	Cp86 (41) 49
78	*p40*	68824←69906	360	L	Ac101 (91) 361	Bm85 (89) 362	Ld102 (38) 381	
79	*p12*	69926←70270	114	L	Ac102 (83) 122	Bm86 (75) 123	Ld103 (29) 121	
80	*p45*	70251←71414	387	L	Ac103 (94) 387	Bm87 (91) 387	Ld104 (36) 389	Cp83 (29) 439
81	*vp80*	71440→73500	686	L	Ac104 (86) 691	Bm88 (85) 692	Ld105 (11) 964	
82	*he65*	73513←74316	267	L	Ac105 (41) 553 Ac106 (33) 61	Bm89 (78) 289		
83	*ac106-107*	74367→75083	238	L	Ac107 (62) 110	Bm90 (86) 249	Ld140 (53) 246	Cp52 (18) 342
84	*ac108*	75089←75400	103	L	Ac108 (92) 105	Bm91 (90) 105	Ld108 (28) 97	
85	***ac109***	75412←76575	387	e, L	Ac109 (95) 390	Bm92 (92) 366	Ld107 (46) 366	Cp55 (21) 326
86	*ac110*	76599←76769	56	L	Ac110 (95) 56	Bm92a (86) 59	Ld106 (30) 56	
87	*ac111*	76816←77031	71	e	Ac111 (75) 67	Bm93 (65) 67		

Table 1. cont.

ORF	Name*	Position†	Length (aa)	Promoter motifs‡	Homologues (% identity)§			
					AcMNPV	BmNPV	LdMNPV	CpGV
	*hr*4	77148–77878						
88	*ac114*	77902←79164	420	e, L	Ac114 (86) 424	Bm94 (82) 424		
89	***pif-3***	79185←79799	204	E, L	Ac115 (93) 204	Bm95 (87) 204	Ld143 (43) 203	Cp35 (30) 199
90	*ac117*	79873→80154	93		Ac117 (82) 95	Bm96 (77) 95		
91	***pif-1***	80215→81807	530	L	Ac119 (88) 530	Bm97 (81) 527	Ld155 (44) 530	Cp75 (34) 538
92	*ac120*	81812→82060	82	L	Ac120 (77) 83	Bm98 (76) 82		
93	*ac124*	82131→82859	242	e, L	Ac124 (81) 247	Bm101 (72) 244		
94	*lef-7*	82876←83532	218		Ac125 (63) 225	Bm102 (67) 226		
95	*chitinase*	83522←85177	551	L	Ac126 (91) 551	Bm103 (89) 551	Ld70 (64) 558	Cp10 (55) 594
96	*v-cath*	85222 → 86196	324	e	Ac127 (92) 323	Bm104 (90) 323	Ld78 (60) 356	Cp11 (39) 333
97	*gp64*	86258←87799	513	E, L	Ac128 (90) 512	Bm105 (86) 530		
98	*p24*	87928→88506	192	L	Ac129 (81) 198	Bm106 (80) 195	Ld133–135 (32) 223	Cp71 (19) 203
99	*gp16*	88537→88842	101	L	Ac130 (82) 106	Bm107 (82) 106		
100	*pp34*	88906→89850	314	L	Ac131 (64) 252	Bm108 (78) 315	Ld136 (23) 313	
101	*ac132*	89853→90509	218	L	Ac132 (79) 219	Bm109 (78) 220		
102	***alk-exo***	90537→91793	418		Ac133 (92) 419	Bm110 (88) 420	Ld157 (35) 420	Cp125 (29) 398
103	*p35*	91969→92862	297	E	Ac135 (80) 299	Bm112 (75) 299		
	*hr*5	92877–93652						
104	*p26*	93707→94429	240		Ac136 (83) 240	Bm113 (80) 240	Ld40 (19) 253	
105	*p10*	94500→94721	73	L	Ac137 (55) 94	Bm114 (55) 70	Ld41 (24) 77	
106	***p74***	94724←96661	645	L	Ac138 (89) 645	Bm115 (86) 645	Ld27 (52) 672	Cp60 (38) 688
107	*me53*	96789←98186	465	E, L	Ac139 (69) 449	Bm116 (65) 451	Ld23 (17) 342	Cp143 (10) 303
108	*ac139a*	98330←98482	50		Ac139a (50) 67			
109	*ie-0*	98434→99201	255	E, L	Ac141 (87) 261	Bm117 (86) 261	Ld21 (26) 258	
110	***p49***	99216→100649	477	L	Ac142 (94) 476	Bm118 (94) 477	Ld20 (46) 483	Cp15 (28) 457
111	*odv-e18*	100651→100920	89	L	Ac143 (53) 62	Bm119 (71) 101	Ld19 (44) 88	
112	***odv-e27***	100936→101802	288	L	Ac144 (96) 290	Bm120 (96) 290	Ld18 (47) 283	Cp97 (20) 288
113	*ac145*	101815→102102	95	L	Ac145 (70) 77	Bm121 (85) 95	Ld17 (46) 92	Cp9 (26) 101
114	*ac146*	102016←102693	225		Ac146 (80) 201	Bm122 (78) 201	Ld16 (26) 208	Cp8 (17) 192
115	*ie-1*	102759→104486	575	E	Ac147 (87) 582	Bm123 (85) 584	Ld15 (23) 566	Cp7 (17) 488
116	***odv-e56***	104525←105661	378	L	Ac148 (85) 376	Bm124 (77) 375	Ld14 (50) 356	Cp18 (39) 355
117	*ac149*	105703←106002	99		Ac149 (38) 107	Bm125 (36) 106		
118	*ac150*	106040→106381	113		Ac150 (38) 99	Bm126 (34) 115		
119	*ie-2*	106373←107374	333	E	Ac151 (56) 408	Bm127 (48) 422		
120	*ac152*	107491←107676	61		Ac152 (50) 90			
121	*pe38*	107781→108884	367	e, L	Ac153 (52) 321	Bm128 (50) 309		Cp24 (8) 382
122	*ac154*	109016→109228	70	L	Ac154 (56) 81	Bm129 (51) 77		
	*hr*1	109272–110071						
123	*ptp*	110137→110676	179	e, L	Ac1 (78) 168	Bm130 (78) 168		
124	*ac4*	110745←110909	54	e	Ac4 (25) 151	Bm133 (26) 151	Ld29 (6) 146	
125	*ac5*	110938→111246	102	L	Ac5 (74) 109	Bm134 (69) 101		
126	***lef-2***	111215→111847	210	e, L	Ac6 (83) 210	Bm135 (82) 210	Ld137 (35) 216	Cp41 (17) 171

*Baculovirus core genes are printed in bold and underlined; additional genes common to lepidopteran NPVs and GVs are underlined.

†Nucleotides in the MaviNPV genome were numbered sequentially, beginning with the A (position 1) of the initiation codon (ATG) of the *polh* gene, in the direction of transcription of the *polh* gene. The directions of the transcripts are indicated by arrows.

‡Promoter motifs present upstream of ORF. E, Early promoter motif [TATA box TATA(A/T)A(A/T) followed by CA(G/T)T motif 20–30 bp downstream] within 180 bp of the initiation codon; e, enhancer-like element (CGTGC) within 210 bp; L, late promoter motif (A/T/G)TAAG within 120 bp.

§The ORF number of a putative homologue is shown with the per cent amino acid identity in parentheses, followed by the length of the homologue.

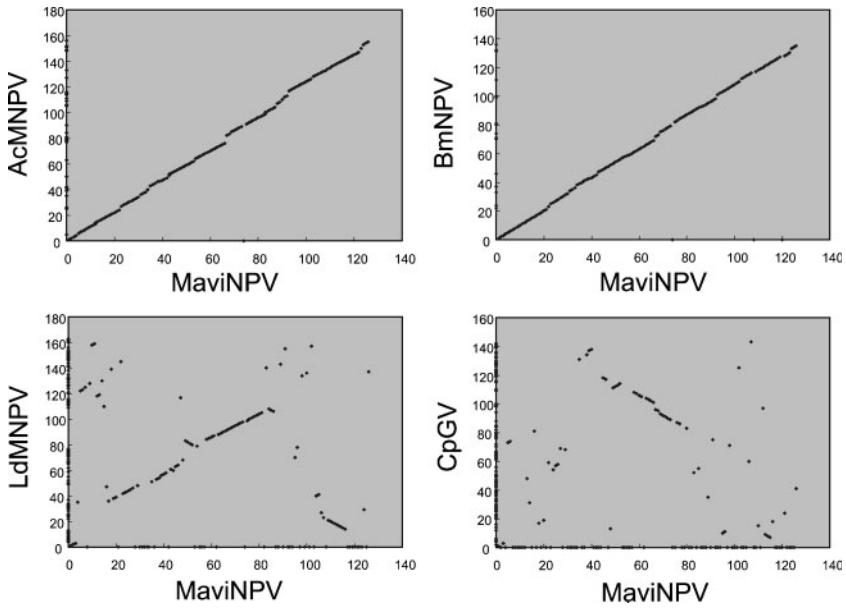

Fig. 2. Gene parity plot analysis of MaviNPV in comparison with AcMNPV, BmNPV, LdMNPV and CpGV, as indicated. Axes: the relative position of each ORF; dots: ORFs.

Structural protein genes. Eighteen structural protein genes are conserved in 26 lepidopteran baculoviruses that have been sequenced (Herniou *et al.*, 2003; Jehle *et al.*, 2006). Only 17 of these genes were identified in the MaviNPV genome, including basic DNA-binding protein *p6.9* (ORF77); capsid-associated proteins *vp39* (ORF67), *vp1054* (ORF40) and *vp91*/*p95* (ORF66); occlusion-derived virus (ODV)-associated proteins tegument protein *gp41* (ORF63) and *p74* (ORF106); ODV envelope proteins *odv-e18* (ORF111), *odv-e25* (ORF71), *odv-e27* (ORF112) and *odv-e56* (ORF116); occlusion body matrix *polh* (ORF1); per os infectivity factors *pif-1* (ORF91), *pif-2* (ORF13) and *pif-3* (ORF89); as well as protein kinase-1 (*pk-1*; ORF3), *efp/ld130* (ORF14) and *fp25K* (ORF45). An *odv-e66* homologue was not identified. An additional seven structural protein genes – including *protein tyrosine phosphatase* (*ptp*), *orf1269*, *vp80*, *p24*, *pp34*, *p10* and group I NPV-specific *gp64* – were also found in MaviNPV, as ORFs 123, 2, 81, 97, 98, 100 and 105, respectively. Sequence identity among the 24 structural proteins of MaviNPV and AcMNPV was generally 83.6 % (Table 1), suggesting that they may be structurally similar. The p6.9 protein was even more conserved than the polyhedrin (polh) protein (88 % identity). In contrast, the *odv-e18*, *pp34* and *p10* genes showed the lowest levels of sequence conservation between MaviNPV and AcMNPV (Table 1).

Anti-apoptotic genes. Programmed cell death is triggered early in baculovirus infection. To counter this, baculoviruses encode proteins that inhibit apoptosis, such as P35 and IAP. A homologue for *p35* (Clem & Miller, 1994) is present in MaviNPV as ORF103. So far, the *p35* gene has also been found in AcMNPV, BmNPV and RoMNPV, three closely related group I NPVs, and it has homologues in *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) (group II NPV) and in *Choristoneura occidentalis* granulovirus (ChocGV), as well as in *Amsacta moorei* entomopoxvirus (Clem *et al.*, 1991; Du *et al.*, 1999; Escasa *et al.*, 2006; Kamita *et al.*, 1993; Means *et al.*, 2006). In contrast, *iap*s have been found in all members of the family *Baculoviridae* sequenced to date. Apoptotic inhibition has been recovered in AcMNPV *p35* deletion mutants with a variety of baculovirus *iap* homologues (Seshagiri & Miller, 1997). Two MaviNPV ORFs, MV18 and 54, show strong homology to AcMNPV *iap-1* and *iap-2*, respectively.

Auxiliary genes. Auxiliary genes are defined as being non-essential for viral replication, but they provide a selective advantage to maximize virus production/survival either at the cellular level or at the level of the organism (O'Reilly, 1997). Several of these auxiliary genes have homologues in MaviNPV, including *ptp-1*, *ubiquitin*, *p10*, superoxide dismutases (*sod*), *v-cath*, *chitinase*, ecdysteroid UDP-glucosyltransferase (*egt*), actin rearrangement-inducing factor-1 (*arif-1*), alkaline exonuclease (*alk-exo*) and *pk-1*. The only auxiliary gene conserved among all baculoviruses is *alk-exo*, which interacts with *lef-3*, possesses both endo- and exonuclease activities and can degrade both single- and double-stranded DNA in a 5′–3′ direction (Mikhailov *et al.*, 2003). Two additional auxiliary genes with unknown function, viral fibroblast growth factor (*vfgf*) and *ubiquitin*, were also considered conserved among all lepidopteran baculoviruses (Herniou *et al.*, 2003); surprisingly, no homologue of *vfgf* gene was found in MaviNPV.

MaviNPV ORF74 does not exist in the AcMNPV genome. Only one MaviNPV ORF (Mv74) had no homologue in AcMNPV (Table 1). This ORF occupied the same genomic

location as Ac97 in AcMNPV. BLASTP searches showed that the homologue of Mv74 was also found in *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchNPV, ORF34), OpMNPV (ORF98) and *Mamestra configurata* nucleopolyhedrovirus-A (MacoNPV-A, ORF28); however, with low identities (26–21 %) in amino acid sequences. The significance of Mv74 as well as of its homologues is not yet known and requires further analysis.

AcMNPV ORFs with no homologues in the MaviNPV genome. Table 2 lists the 28 AcMNPV ORFs missing from the MaviNPV genome. Genes that are important for AcMNPV replication or infection, but which are not in the MaviNPV genome are as follows: Ac42, a general transcription activator (*gta*), contains seven motifs common to the SWI2/SNF2 family of proteins, which is involved in chromatin remodelling (Pazin & Kadonaga, 1997; Tsukiyama & Wu, 1997); Ac46 (*odv-e66*) encodes an ODV envelope protein; Ac49 (*pcna/etl*) encodes a protein with similarity to eukaryotic proliferating cell nuclear antigen (O'Reilly *et al.*, 1989) and studies show that the late gene expression of a mutant AcMNPV lacking a functional *pcna* gene is markedly delayed compared with that of wild-type AcMNPV (Crawford & Miller, 1988); Ac70, host cell-specific factor-1 (*hcf-1*), has been shown to be important in host-specific virus replication (Lu & Miller, 1995a, 1996); Ac123, protein kinase 2 (*pk-2*) might be involved in the regulation of translation in infected cells (Morris *et al.*, 1994); however, an AcMNPV mutant lacking a functional *pk-2* gene displayed no noticeable phenotypic alterations compared with wild-type virus (Li & Miller, 1995).

Table 2. AcMNPV ORFs with no homologues in the MaviNPV genome

Numbers of AcMNPV ORFs were taken from Ayres *et al.* (1994). Percentage identity between AcMNPV ORFs and BmNPV homologues is shown.

AcMNPV			BmNPV		
ORF*	Length (aa)	Name	ORF	Length (aa)	Identity (%)
2	328	*bro*	131	349	77
3	53	*ctl*			
7	201	*orf603*			
12	217				
32	**181**	***vfgf***	**24**	**182**	**89**
33	182				
42	506	*gta*	33	506	96
46	**704**	***odv-e66***	**37**	**702**	**93**
48	113	*etm*			
49	285	*pcna/etl*			
57	161		46	161	94
70	290	*hcf-1*			
84	188				
85	53				
86	694	*pnk/pnl*			
87	126	*vp15*	70	126	94
88	264	*cg30*	71	267	92
91	224		74	154	–
97	56				
112	87				
113	169				
116	56				
118	157				
121	58				
122	62		99	61	90
123	215	*pk-2*	100	225	92
134	803	*p94*			
140	60				

*ORFs always found in all fully sequenced lepidopteran-specific NPV and GV (Herniou *et al.*, 2003; Jehle *et al.*, 2006) are printed in bold. ORFs not present in the genomes of any other baculoviruses and may not be expressed in AcMNPV (Harrison & Bonning, 2003) are underlined.

hrs. A novel feature of many baculovirus genomes is the presence of *hrs* that are located throughout the genome (Ayres *et al.*, 1994; Cochran & Faulkner, 1983; Garcia-Maruniak *et al.*, 1996). These regions are composed of repeated sequences encompassing both direct repeats and imperfect palindromic sequences and have closely related counterparts elsewhere in the genome. *hrs* function as enhancers of RNA polymerase II-mediated transcription of baculovirus early promoters (Guarino & Summers, 1986; Theilmann & Stewart, 1992). In both AcMNPV and OpMNPV, *hrs* can perform as *cis*-acting origins of DNA replication (Ahrens & Rohrmann, 1995; Leisy & Rohrmann, 1993; Kool *et al.*, 1995; Pearson *et al.*, 1992); it has also been suggested that *hrs* can probably substitute for each other, since it was recently shown that not all *hrs* need to be present for successful DNA replication (Carstens &Wu, 2007). In seven of the 33 baculoviruses currently sequenced [ChchNPV, *Trichoplusia ni* single nucleopolyhedrovirus (TnSNPV), *Adoxophyes honmai* nucleopolyhedrovirus (AdhoNPV), *Agrotis segetum* granulovirus (AgseGV), CpGV, *Neodiprion abietis* nucleopolyhedrovirus (NeabNPV) and *Neodiprion lecontii* nucleopolyhedrovirus (NeleNPV)] canonical *hrs* were not identified. The remaining fully sequenced baculoviruses contain between three (*Cryptophlebia leucotreta* granulovirus; CrleGV) and 17 (SpltNPV) *hrs* (Table 1).

Five homologous regions (*hrs*1–5) containing a total of 44 imperfect palindromes were found in the MaviNPV genome. The number of imperfect palindromes per *hr* ranges from 7 in *hr*2 and *hr*5 and 9 in both *hr*1 and *hr*4 to 12 in *hr*3 and accounts for 3.2 % of the genome. *hr*2, 4 and 5 have a clockwise orientation and *hr*1 and 3 are anti-clockwise in the MaviNPV genome (Fig. 3b). A similar arrangement of *hrs* is also found in the OpMNPV genome (Ahrens *et al.*, 1997). The MaviNPV *hr* palindrome consensus GTTTTACGAGTAGAATCGTACTCGTAAAGC shows 24/30 palindrome matches (Supplementary Fig. S1

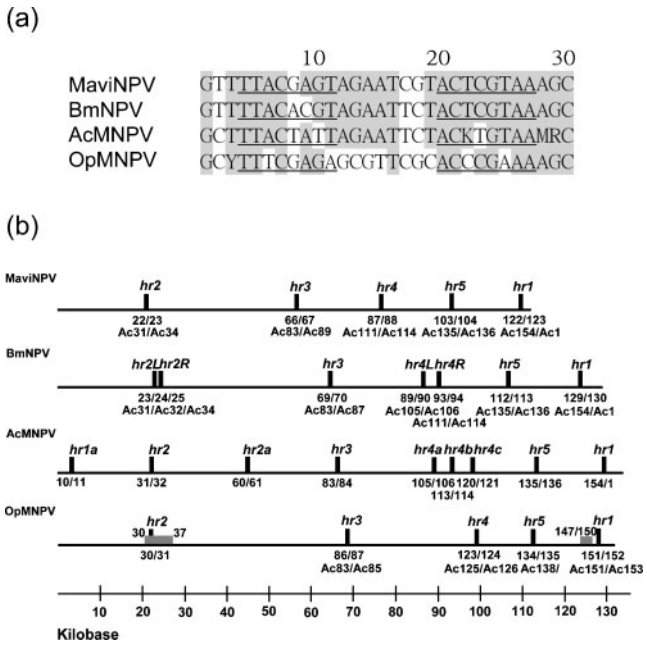

Fig. 3. Comparison of MaviNPV *hr* palindromes with *hr* palindrome consensus sequence from other baculoviruses. (a) Alignment of consensus *hr* palindrome from MaviNPV, BmNPV (Majima *et al.*, 1993), AcMNPV and OpMNPV. The two 8 bp sequences that bind the IE-1 transactivator in AcMNPV (Rodems & Friesen, 1995) are underlined, as are the equivalent regions for MaviNPV, BmNPV and OpMNPV. Y=T or C, K=G or T, M=C or A, R=A or G. (b) Comparison of the genomic context of the *hrs* and *hr* locations relative to homologous ORFs between MaviNPV, BmNPV, AcMNPV and OpMNPV in the linearized genomes. ORFs flanking the *hrs*: below the line. Grey rectangles: major inserts relative to AcMNPV and ORFs within the inserts are shown above the line. For consistency, all linearized genomes start with *polh*, but *hrs* and ORF numbers remain as in the original papers. AcMNPV *hr*2a is shown as in Possee & Rohrmann (1997).

available in JGV Online). This is similar to the 24/30 matches for the BmNPV (Gomi *et al.*, 1999) and OpMNPV (Ahrens *et al.*, 1997) palindrome sequence, and the 26/30 matches for the AcMNPV (Ayres *et al.*, 1994) palindrome consensus (Fig. 3a). In addition, the MaviNPV *hr* palindrome consensus has the highest identity to the *hr* consensus sequence from BmNPV (26/30 bases, 86 %), followed by the *hr* consensus from AcMNPV (21/30 bases, 70 %) and OpMNPV (19/30 bases, 63 %).

Major insertions and deletions were found near the MaviNPV *hrs* when we compared these regions to their counterparts in the BmNPV, AcMNPV and OpMNPV genomes (Fig. 3b). The clearest example involves *hr*3, located between ORFs 83 and 84 in AcMNPV. Homologues of AcMNPV ORF83 also flank *hr*3 on the left in BmNPV (ORF69), OpMNPV (ORF86) and MaviNPV (ORF66). Similarly, BmNPV lacks homologues to AcMNPV ORFs 84–86, and *hr*3 is therefore flanked by the BmNPV ORF70, which is an AcMNPV ORF87 homologue (Ahrens *et al.*, 1997). In contrast, MaviNPV lacks a large region comparable to AcMNPV ORFs 84–88 and therefore MaviNPV *hr*3 is flanked on the right by ORF67, an AcMNPV ORF89 homologue. MaviNPV *hr*5, BmNPV *hr*5 and AcMNPV *hr*5 are in the same genomic locations between *p35* and *p26*, while the *hr*5 of OpMNPV is between *p74* and *Op135*. The close relatedness of the *hr* sequence within each viral genome suggests that the sequences are conserved, possibly because of their interaction with a viral protein, such as IE-1, which is involved in *hr* binding (Choi & Guarino, 1995; Leisy *et al.*, 1995; Rodems & Friesen, 1995). In AcMNPV, IE-1 has been shown to bind the two 8 bp sequences that are three bases in from the ends of the palindrome sequence (Fig. 3a; underlined) (Rodems & Friesen, 1995). It would be expected that the IE-1 proteins from MaviNPV, BmNPV and OpMNPV bind the same regions of the palindrome sequences in these viruses, even though the IE-1 proteins of these viruses are not strictly conserved and show a variation in the range of 50–87 %.

Phylogenetic position of MaviNPV. The NJ and MP trees generated similar results, but the NJ tree revealed higher bootstrap values and is the only tree shown here. The results reflect the current systematic assignment of the viruses. As Fig. 4 shows, the family *Baculoviridae* consists of five major clades: the NPVs infecting Lepidoptera (including group I and group II), the GVs, the hymenopteran-specific NPVs and CuniNPV. Two subclades within the lepidopteran NPV group I resemble the AcMNPV and AgMNPV lineages as reported by Oliveira *et al.* (2006). The patterns of adjacency indicated that MaviNPV is grouped into the AcMNPV lineage with BmNPV, RoMNPV and AcMNPV. Moreover, phylogenetic analysis revealed that MaviNPV was separated from the ancestor of AcMNPV and BmNPV before these two viral species diverged, supporting the idea that MaviNPV is a distinct baculovirus species.

MaviNPV replication in cell lines

Infection assay. For wild-type MaviNPV infection, MV cells showed a typical NPV-infected CPE, and OBs in the nuclei of the infected MV cells at 3 days p.i., the infection rate of MV cells reached 98 % at 7 days p.i., while CPE in LD, PN, LY and Sf9 cells was not obvious at 3 (Fig. 5) and 7 days p.i.

For *egfp*–MaviNPV infection, the infection rates of LD, Sf9 and MV cells, with fluorescent signal at 7 days p.i., were 0.97 ± 0.135, 0.01 ± 0.00135 and 99.32 ± 1.83 %, respectively, but the fluorescent signal was not seen in the infected PN and LY cells. The progeny virus titres of the *egfp*–MaviNPV-infected MV and LD cells at 7 days p.i. were $3.98 \pm 0.37 \times 10^7$ and $3.14 \pm 1.03 \times 10^4$ $TCID_{50}$ ml^{-1}, respectively. The virus titre of the infected LD cells increased to $2.81 \pm 0.95 \times 10^5$ $TCID_{50}$ ml^{-1} at 9 days p.i.

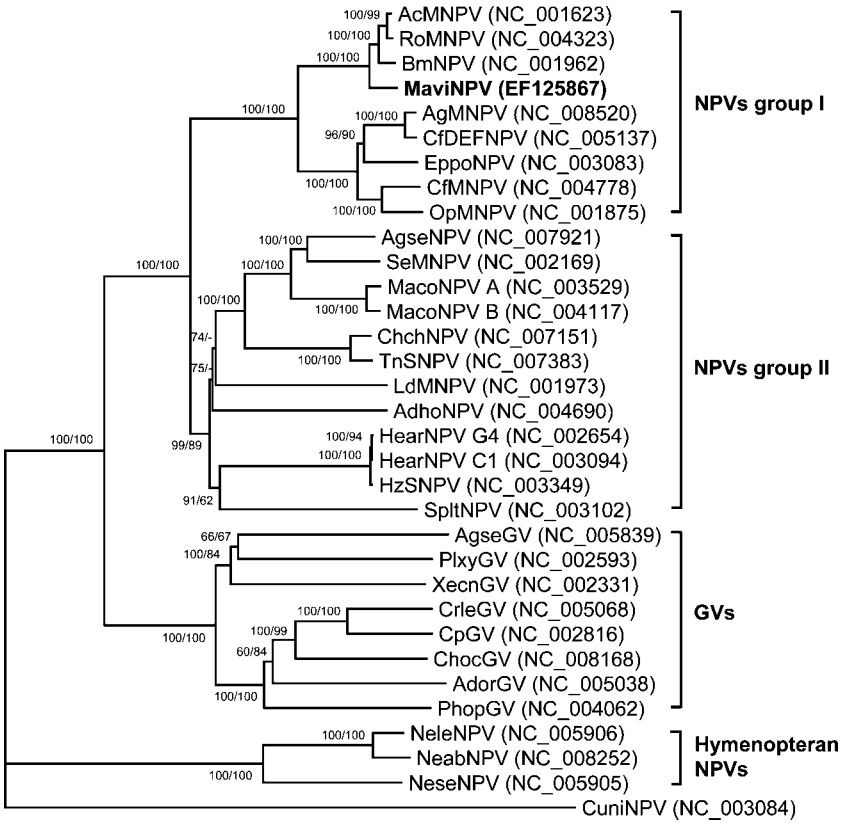

Fig. 4. Baculovirus phylogeny inferred from a combined dataset of the 29 baculovirus core protein sequences. Shown is an unrooted NJ tree. CuniNPV was selected as the outgroup. Numbers at nodes indicate bootstrap scores above 60 % for the NJ and MP analyses (1000 replicates, NJ bootstrap/MP bootstrap).

There was no progeny virus found in the infected LY, PN and Sf9 cells, although fluorescent signals were seen in a few infected Sf9 cells.

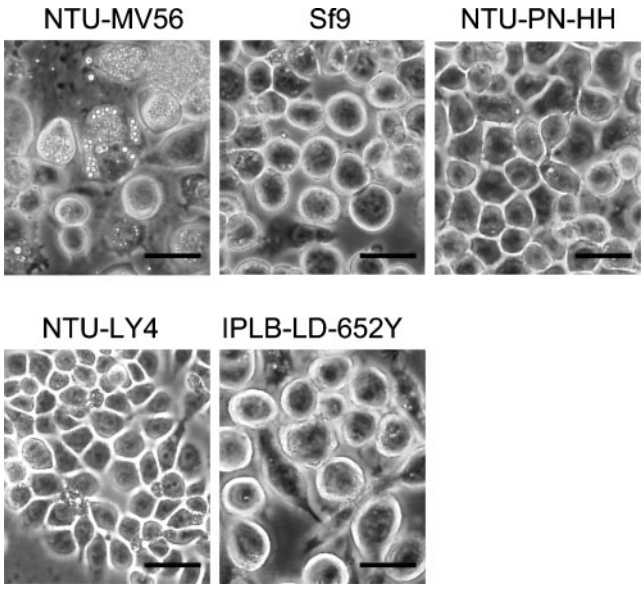

Fig. 5. CPE of MaviNPV-infected MV, Sf9, PN, LY and LD cells at 72 h p.i. Bars, 25 μm.

McIntosh *et al.* (2005) defined three types of viral susceptibility of a cell line: permissive (full replication), semi-permissive (partial replication) and non-permissive (no replication). MV cells are a permissive cell line and LY, PN and Sf9 cells are non-permissive cell lines for MaviNPV infection. Only 1 % of LD cells were susceptible to *egfp*–MaviNPV and neither obvious CPE nor OBs were observed in MaviNPV-infected LD cells (Fig. 5). This kind of baculovirus infection, with no OB formation, occurs in other baculovirus-infected cells; for example, AcMNPV-infected BmN-4 cells, *Hyphantria cunea* nucleopolyhedrovirus-infected Sf21 cells (Shirata *et al.*, 1999) and *Helicoverpa zea* single nucleopolyhedrovirus (HzSNPV)-infected *Helicoverpa zea* cells (Goodman *et al.*, 2001). Conversely, the baculovirus-infected cells that produce OBs generate progeny virus with no exception. polh proteins in NPVs and cytoplasmic polyhedroviruses (CPVs) are not homologous even though they both form similar polyhedral structures. In CPVs, hydrophobic interactions produce the main structure of the polyhedron via a trimer form of polh protein (Coulibaly *et al.*, 2007). In NPVs, aa 19–130 of polh protein are important for the formation of polh-like crystals in the nucleus. Eason *et al.* (1998) provided ultrastructural evidence that in NPVs, polyhedron formation is initiated by the nucleation of polh on the virion surface. Pilot studies showed that at 7 days p.i. the eGFP level in LD cells was about 100 times lower than in infected MV cells (data not shown), which is consistent with the

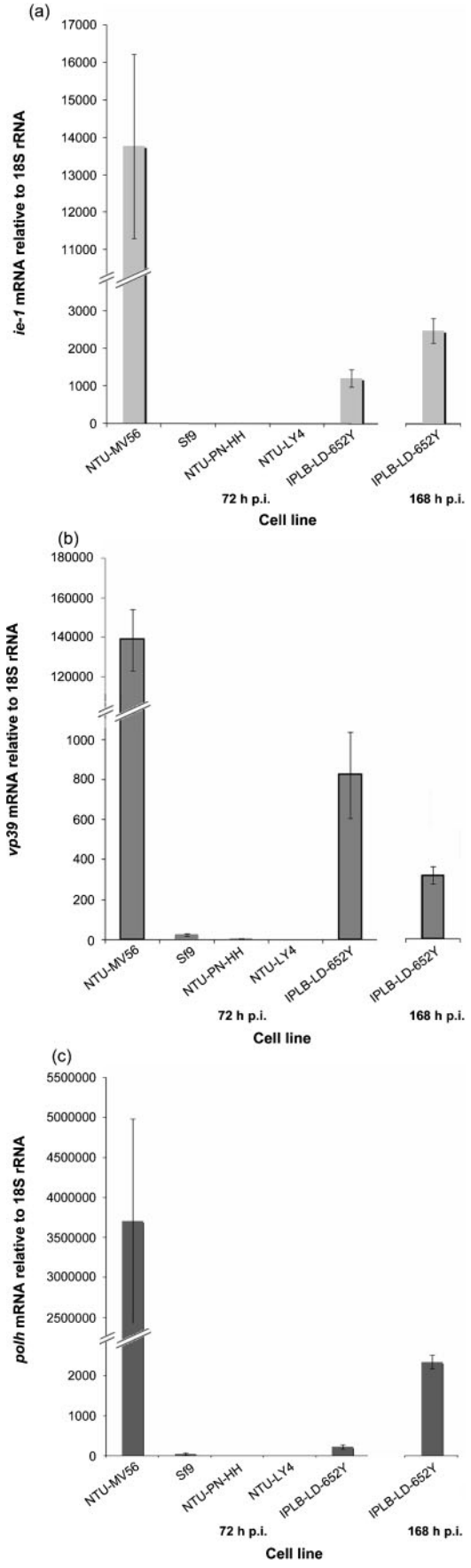

Fig. 6. MaviNPV transcription levels in MaviNPV-infected MV, Sf9, PN, LY and LD cells detected by real-time PCR analysis: (a) *ie-1*, (b) *vp39* and (c) *polh* at 72 and 168 h p.i.

hypothesis that the polh protein level may be one important factor for the formation of OBs. Two other genes may also be critical: *p10* and *pp34* encode a protein for the fibrillar structure formation and a calyx protein, respectively, and both of these proteins are important for the assembly and stability of the polyhedron structure (Lee *et al.*, 1996; Dong *et al.*, 2005).

qPCR examination. As depicted by qPCR (Fig. 6), normalized with the level of cellular 18S, MaviNPV-infected MV cells produced the highest transcription levels of three representative genes: *ie-1* (early gene), *vp39* (late gene) and *polh* (very late gene), followed by MaviNPV-infected LD cells, and then MaviNPV-infected Sf9 cells, while LY and PN cells did not produce transcription of these three genes at 3 days p.i. A very low transcription of *vp39* and *polh* in the infected Sf9 cells was found at 3 days p.i. In the MaviNPV-infected LD cells, *ie-1* and *polh* transcriptions were increased by about two- and 2000-fold, respectively, at 7 days p.i., while *vp39* transcription decreased approximately 2.5-fold at 7 days p.i. Morris & Miller (1992) examined the activities of viral and insect promoters in AcMNPV-permissive and non-permissive cell lines by CAT recombinant viruses. LD cells are non-permissive for AcMNPV and show strikingly low expression of CAT in LD cells. In particular no CAT activity of very late genes was found at 6–48 h p.i. Guzo *et al.* (1992) compared the viral and host cellular transcription of AcMNPV-infected LD cells in permissive and non-permissive cell lines, and suggested that the absence of normal AcMNPV protein synthesis was not due to a lack of virus-specific transcription, but instead could result from an inability of host LD cells to translate abundant viral mRNAs even though the virus-specific mRNAs were neither excessively destabilized nor defective with regard to protein synthesis.

Our previous reports showed that 20 % of *P. nuda* nucleopolyhedrovirus-infected LD cells contained OBs, and that LD cells are susceptible to LyxyMNPV (Chou *et al.*, 1996; Wu & Wang, 2006). However, the replication cycle of LyxyMNPV in LD cells was much slower than that of AcMNPV in Sf9 cells. Usually, the OBs in the LyxyMNPV-infected LD cells were observed at 3–5 days p.i. and the highest infection rate was found at 7–10 days p.i. (unpublished data). This delayed replication cycle was also found in the MaviNPV-infected LD cells (Fig. 6c). The qPCR assay found a higher relative amount of *polh* mRNA at 168 h (7 days) p.i. (Fig. 6c). In addition, the very late gene expression of CAT-AcMNPV-infected LD cells was detected at 6–48 h p.i. (Morris & Miller, 1992). Such a short detection time after infection was not sufficient to express the very late gene of AcMNPV, especially *polh* in

LD cells. Moreover, only about 1 % LD cells were susceptible to *egfp*–MaviNPV and no obvious CPE and OBs in wild-type MaviNPV-infected LD cells (m.o.i. of 10) were found at 168 h p.i. When LD cells were inoculated with a high dose of MaviNPV (m.o.i. of 20 or 100), cell blebbing occurred and the cells lysed at 3 days p.i. The infection dose therefore did not appear to be the main factor of the infection rate. The susceptibility of a cell line may be influenced by the age of cells, cell cycle (synchronous or asynchronous) and genetic heterogeneity of the cell types (Freshney, 2005; O'Reilly *et al.*, 1994b); the latter is more likely the reason for uneven MaviNPV susceptibility of the LD cells.

Efficient expression of the late viral genes of baculovirus in a cell line determines whether the cell line is non-permissive or semi-permissive of the virus (McIntosh *et al.*, 2005). The qPCR (Fig. 6) showed that the early, late and very late genes of MaviNPV could be transcribed. However, even though the very late viral genes of MaviNPV were expressed in some of the LD cells, the full constellation of factors that influence OB formation in LD cells remains to be determined.

A gene, *hrf-1* (host range factor 1), of LdMNPV can promote NPV infectivity (including SeMNPV, HycuNPV, BmNPV and AcMNPV) to LD cells (Du & Thiem, 1997; Ikeda *et al.*, 2005; McIntosh *et al.*, 2005, Thiem *et al.*, 1996), and *in vivo* infection experiments also revealed that the expression of *hrf-1* could increase AcMNPV infectivity to *H. zea* and *L. dispar* larvae (Chen *et al.*, 1998). No *hr* of LdMNPV *hrf-1* is found in the AcMNPV or MaviNPV genomes. We also note that the replication of MaviNPV in several cell lines is interestingly distinct from that of AcMNPV, while AcMNPV only appears to infect the MV cells with an infection rate of about 10 % (data not shown). MaviNPV can also infect LD cells, while AcMNPV could not; conversely, Sf9 cells could be infected by AcMNPV but not by MaviNPV. We will construct a DNA chip based on the MaviNPV genome to elucidate the expression of the whole MaviNPV genome in LD cells. The role of *hrf-1* on MaviNPV-infected LD cells will also be examined in our future work.

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