



## Phylogenetic Relationship of *DpNPV* (*Diaphania pulverulentalis* Nuclear Polyhedrosis Virus) with Other Lepidopteran NPVs based on Polyhedrin Gene

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The mulberry leaf-roller, *Diaphania pulverulentalis* (Hampson) is a major defoliator pest of mulberry in sericultural tracts of Karnataka, India. A nuclear polyhedrosis virus isolated from *D. pulverulentalis* larvae was analysed via its polyhedrin gene, which was amplified from *DpNPV* DNA in polymerase chain reaction using degenerate set of primers and the sequence was analysed phylogenetically with other polyhedrin genes. The analysis revealed that *DpNPV* was closely related to *Galleria mellonella* NPV, *Plutella maculipennis* NPV and *Autographa californica* NPV and *Autographa biloba* NPV and distinct from *Bombyx mori* NPV.

**Keywords:** *Diaphania pulverulentalis*, nuclear polyhedrosis virus, polyhedrin gene, phylogenetic analysis.

Baculoviruses (Baculoviridae) are a diverse group of insect viruses, with good potential as biological insecticides (Moscardi, 1999) mainly used to control insect pest populations due to their high species-specificity, efficiency against certain pests and safety (Blissard and Rohrmann, 1990). Traditional approaches for classification of organisms using morphological features or molecular sequences such as ribosomal DNA (Woese *et al.*, 1990) do not easily transfer to viruses. For lack of fossil records for viruses, their streamlined gene complements and the plasticity of their genomes compared to cellular organisms. Nevertheless, viral gene sequences useful for reconstructing phylogenies have been identified. DNA polyhedrin gene homologs have been used to infer phylogenetic relationships among DNA viruses, but other genes such as DNA polymerase, egt, gp41, lef2 and gp37 have also been used (Chen *et al.*, 1999; Jin *et al.*, 1999; Kang *et al.*, 1998).

Productivity in sericulture is mainly based on the quality and yield of mulberry leaves, which are the only source of food for rearing of silkworms. The occurrence of NPV on mulberry leaf-roller, *Diaphania pulverulentalis*, a major pest of mulberry in South India has been reported (Rajadurai *et al.*, 1999). In the present study, a polyhedrin gene was sequenced from *DpNPV* (*Diaphania pulverulentalis* nuclear polyhedrosis virus) and a phylogenetic tree based on polyhedrin gene sequence was constructed and the phylogenetic position of *DpNPV* was resolved.

### Materials and Methods

The *DpNPV* polyhedrin gene (*Diaphania pulverulentalis* nuclear polyhedrosis virus) was

amplified in PCR reactions using degenerate set of primers for polyhedrin. The forward and reverse primers used for PCR were GTTAAA CCCGACACC ATGAAGC and AACTTCTACAA GCCCATCGTTTAC. Reaction products were cloned into pGEM-T easy plasmids (promega) and automatically sequenced (Bangalore Genei, Bangalore). The sequence obtained was deposited in GenBank under the accession number of FJ 901339.

In order to assess the relationship of *DpNPV* polyhedrin with other lepidopteran polyhedrin NPVs, phylogenetic analysis of baculovirus occlusion matrix proteins was carried out. Multiple sequence comparison and phylogenetic inferences were performed with CLUSTAL W package and trees were constructed using Neighbourhood method. For this analysis, lepidopteran NPVs and those with the highest amino acid identity with *DpNPV* polyhedrin genes were used. The *DpNPV* polyhedrin gene sequence was compared with other polyhedrin genes and granulin gene sequences such as *Bombyx mori* NPV (DQ231336), *Galleria mellonella* MNPV (AY706696), *Lymantria monacha* NPV (AY706702), *Lymantria xyliana* NPV (AY706703), *Malacosoma neustria* NPV (AY706708), *Mamestra brassicae* NPV (AY706705), *Peridroma margaritosa* NPV (AY706710), *Actias selene* NPV (AY706680), *Agrotis segetum* NPV (AY706683), *Anagrapha falcifera* NPV (AY706686), *Antheraea pernyi* NPV (AY706687), *Autographa biloba* NPV (AY737724), AY706681 *Autographa californica* NPV (AY706681), *Buzura suppressaria* NPV (DQ231342), *Plutella maculipennis* NPV (AY706713), *Samia cynthia* NPV (AY706711), *Spodoptera litura* NPV (AY706715), *Spodoptera littoralis* NPV (AY706717), *Agrotis segetum granulovirus* (AY706661), *Choristoneura*

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*murinana granulovirus* (AY706663), *Cydia pomonella granulovirus* (AY706667), *Hyphantria cunea granulovirus* (AY706669), *Pieris brassicae granulovirus* (DQ235253), *Pieris rapae granulovirus* (AY706673) and *Spodoptera frugiperda granulovirus* (AY706677) downloaded from GenBank.

## Results and Discussion

The application of PCR combined with molecular phylogeny provides an excellent method for fast and reliable baculovirus identification and needs only tiny traces of a sample even if the virus cannot be recovered (Lange *et al.*, 2004). The

degenerate oligonucleotides used in the present study remained unchanged in their gene specific binding region. The partial sequence of *DpNPV* polyhedrin gene was determined and its length was 396 bp representing 63 per cent of the complete polyhedrin open reading frame. Comparison of the multiple sequences revealed that there was high degree of DNA sequence similarity between *DpNPV* with *AcNPV*, *GmNPV*, *PmNPV* and *AbNPV* and the base pair substitution took place at particular nucleotide through the entire length of the 396 bp. Even though, these NPVs showed variability with other NPVs and GVs, bases starting from 58 to 122

**Table 1. Per cent identity of *DpNPV* with other NPV polyhedrin genes**

		Percent Identity																											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
Divergence	1	100.0	87.9	89.4	87.9	85.6	90.9	84.0	82.4	85.6	100.0	89.4	87.9	88.6	88.6	100.0	85.6	100.0	90.9	53.8	55.3	56.8	51.5	53.4	54.9	53.0	1	<i>DpNPV</i>	
	2	0.0	100.0	87.9	89.4	87.9	85.6	90.9	84.0	82.4	85.6	100.0	89.4	87.9	88.6	88.6	100.0	85.6	100.0	90.9	53.8	55.3	56.8	51.5	53.4	54.9	53.0	2	<i>AbNPV</i>
	3	13.3	13.3	100.0	87.9	100.0	96.2	90.2	84.0	84.0	85.6	87.9	88.6	100.0	85.6	85.6	87.9	93.9	87.9	90.2	55.3	56.1	56.8	51.5	54.2	55.7	53.0	3	<i>ApNPV</i>
	4	11.5	11.5	13.3	100.0	87.9	85.6	95.5	82.4	81.7	90.9	89.4	93.9	87.9	87.9	89.4	84.1	89.4	92.4	56.8	56.8	58.3	52.3	55.7	57.4	55.3	4	<i>AsNPV</i>	
	5	13.3	13.3	0.0	13.3	100.0	96.2	90.2	84.0	84.0	85.6	87.9	88.6	100.0	85.6	85.6	87.9	93.9	87.9	90.2	55.3	56.1	56.8	51.5	54.2	55.7	53.0	5	<i>AseNPV</i>
	6	16.0	16.0	3.9	16.0	3.9	100.0	87.1	82.4	83.2	83.3	85.6	85.6	96.2	83.3	83.3	85.6	93.9	85.6	87.1	54.5	54.5	56.1	50.8	52.7	54.1	52.3	6	<i>BmNPV</i>
	7	9.7	9.7	10.6	4.7	10.6	14.2	100.0	85.5	85.5	92.4	90.9	98.5	90.2	88.6	88.6	90.9	84.8	90.9	97.0	56.1	56.8	57.6	53.0	55.7	57.4	56.1	7	<i>BsNPV</i>
	8	18.1	18.1	18.1	20.1	18.1	20.1	16.2	100.0	94.7	79.4	83.2	83.2	84.0	82.4	82.4	83.2	79.4	83.2	84.7	55.0	55.7	56.5	51.1	51.1	51.6	54.2	8	<i>LmNPV</i>
	9	20.1	20.1	18.1	21.1	18.1	19.1	16.2	5.6	100.0	78.6	81.7	83.2	84.0	80.2	80.2	81.7	80.2	81.7	84.7	51.1	53.4	54.2	50.4	49.6	50.0	51.9	9	<i>LxNPV</i>
	10	16.0	16.0	16.0	9.7	16.0	18.9	8.0	23.1	24.2	100.0	85.6	90.9	85.6	86.4	86.4	85.6	81.8	85.6	90.9	55.3	53.8	55.3	50.0	53.4	53.3	53.0	10	<i>MnNPV</i>
	11	0.0	0.0	13.3	11.5	13.3	16.0	9.7	18.1	20.1	16.0	100.0	89.4	87.9	88.6	88.6	100.0	85.6	100.0	90.9	53.8	55.3	56.8	51.5	53.4	54.9	53.0	11	<i>PmNPV</i>
	12	11.5	11.5	12.4	6.3	12.4	16.0	1.5	18.1	18.1	9.7	11.5	100.0	88.6	88.6	88.6	89.4	83.3	89.4	95.5	54.5	55.3	56.1	53.0	54.2	55.7	54.5	12	<i>PmaNPV</i>
	13	13.3	13.3	0.0	13.3	0.0	3.9	10.6	18.1	18.1	16.0	13.3	12.4	100.0	85.6	85.6	87.9	93.9	87.9	90.2	55.3	56.1	56.8	51.5	54.2	55.7	53.0	13	<i>ScNPV</i>
	14	12.4	12.4	16.0	13.3	16.0	18.9	12.4	19.1	22.1	15.1	12.4	12.4	16.0	100.0	88.6	83.3	88.6	88.6	55.3	53.8	55.3	51.5	51.9	53.3	51.5	14	<i>SinNPV</i>	
	15	12.4	12.4	16.0	13.3	16.0	18.9	12.4	19.1	22.1	15.1	12.4	12.4	16.0	0.0	100.0	88.6	83.3	88.6	88.6	55.3	53.8	55.3	51.5	51.9	53.3	51.5	15	<i>SINPV</i>
	16	0.0	0.0	13.3	11.5	13.3	16.0	9.7	18.1	20.1	16.0	0.0	11.5	13.3	12.4	12.4	100.0	85.6	100.0	90.9	53.8	55.3	56.8	51.5	53.4	54.9	53.0	16	<i>AcMNPV</i>
	17	16.0	16.0	6.3	17.9	6.3	6.3	17.0	24.2	23.1	20.9	16.0	18.9	6.3	18.9	18.9	16.0	100.0	85.6	84.8	51.5	51.5	53.0	48.5	50.4	51.6	49.2	17	<i>AMNPV</i>
	18	0.0	0.0	13.3	11.5	13.3	16.0	9.7	18.1	20.1	16.0	0.0	11.5	13.3	12.4	12.4	0.0	16.0	100.0	90.9	53.8	55.3	56.8	51.5	53.4	54.9	53.0	18	<i>GmMNPV</i>
	19	9.7	9.7	10.6	8.0	10.6	14.2	3.1	16.2	16.2	9.7	9.7	4.7	10.6	12.4	12.4	9.7	17.0	9.7	100.0	53.0	55.3	56.1	51.5	54.2	55.7	54.5	19	<i>MbMNPV</i>
	20	70.3	70.3	66.7	63.3	66.7	68.5	65.0	65.8	74.9	66.7	70.3	68.5	66.7	66.7	66.7	70.3	75.9	70.3	72.1	100.0	92.4	92.4	77.3	85.5	86.1	87.1	20	<i>AsGV</i>
	21	66.7	66.7	65.0	63.3	65.0	68.5	63.3	64.1	69.3	70.3	66.7	66.7	65.0	70.3	70.3	66.7	75.9	66.7	66.7	8.0	100.0	94.7	80.3	88.5	89.3	89.4	21	<i>CmGV</i>
	22	63.3	63.3	63.3	60.0	63.3	65.0	61.7	62.4	67.5	66.7	63.3	65.0	63.3	66.7	66.7	63.3	72.1	63.3	65.0	8.0	5.5	100.0	80.3	88.5	89.3	87.9	22	<i>CpGV</i>
	23	75.9	75.9	75.9	74.0	75.9	77.9	72.1	74.9	76.8	79.9	75.9	72.1	75.9	75.9	75.9	84.0	75.9	75.9	27.1	22.9	22.9	100.0	76.3	77.0	76.5	23	<i>HcGV</i>	
	24	69.3	69.3	67.5	64.1	67.5	71.1	64.1	68.3	72.0	71.1	69.3	67.5	67.5	73.0	69.3	76.8	69.3	67.5	15.2	11.6	11.6	28.5	100.0	99.2	81.7	24	<i>PbGV</i>	
	25	67.6	67.6	65.7	62.1	65.7	69.5	62.1	66.6	70.5	71.5	67.6	65.7	65.7	71.5	71.5	67.6	75.6	67.6	65.7	15.5	11.5	11.5	27.4	0.8	100.0	81.1	25	<i>PrGV</i>
	26	72.1	72.1	72.1	66.7	72.1	74.0	65.0	67.5	73.0	72.1	72.1	68.5	72.1	75.9	75.9	72.1	81.9	72.1	68.5	14.2	11.5	13.3	28.2	20.1	20.7	100.0	26	<i>SiGV</i>
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26			

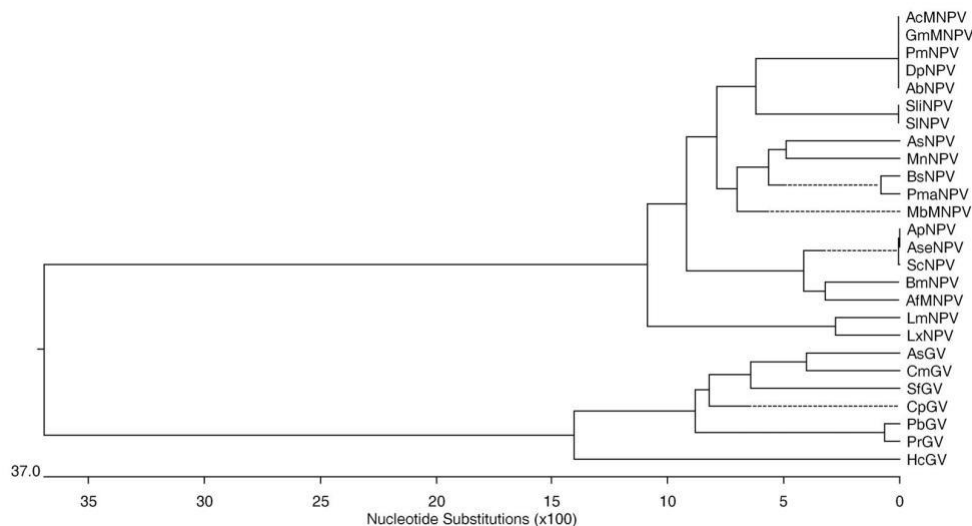
showed similarity with consensus sequence. GVs were highly variable from NPVs through the entire length of nucleotides. But, nucleotides of 58 to 69 showed similarity between NPVs and GVs.

The results also showed that the NPVs used in this analysis were clustered along at least four branches named in this study as group I, II (II-A, II-B, II-C), III (III-A, III-B) and IV. The granulovirus are placed as a sister group to the lepidopteran NPV. Within the NPV group, Group IV was used as an outgroup based on its polyhedrin gene sequence divergence from the remaining polyhedrins. Granuloviruses are used as an outgroup V which included *Cydia pomonella* GV, *Spodoptera frugiperda* GV, *Agrotis segetum* GV, *Choristoneura murinana* GV, *Pieris brassicae* GV, *Pieris rapae* GV and *Hyphantria cunea* GV which are highly different from *D. pulverulentalis* NPV (Fig. 1). The present study clearly indicated that *D. pulverulentalis* NPV was closely related to *Galleria mellonella* NPV, *Plutella maculipennis* NPV

and *Autographa californica* NPV and *Autographa biloba* NPV which showed 100 per cent identity (Table 1). The phylogenetic tree in Fig.1 implied that *G. mellonella* NPV, *D. pulverulentalis* NPV, *P. maculipennis* NPV, *A. californica* NPV shared a common ancestral distinct from the other groups. The *D. pulverulentalis* NPV also clustered with *Mamestra brassicae* NPV (90.9%) and *Buzura suppressaria* NPV (90.9%) followed by *Agrotis segetum* NPV (89.4%), *Peridroma margaritosa* NPV (89.4%), *Spodoptera litura* NPV (88.6 %) and *Spodoptera littoralis* NPV (88.6%).

The *D. pulverulentalis* NPV also showed identity with *Malacosoma neustria* NPV (85.6%) and *B. mori* (85.6%). *D. pulverulentalis* NPV was distantly related to *Lymantria monacha* NPV and *Lymantria xyliana* NPV which showed identity of 84.0 and 82.4 per cent. The analysis also indicated that the same genus had close similarity which is confirmed with *Autographa californica* NPV and *Autographa biloba*

NPV, *Lymantria monacha* NPV and *Lymantria xyliana* NPV, *Spodoptera litura* NPV and *Spodoptera littoralis* NPV which corresponds closely to the host species separation within the host genus. This relationship apparently reflects a tight co-evolution between the viruses and their hosts. The GVs showed least similarity with NPVs ranging from 51.5 to 56.8 per cent. The information obtained from the comparison of the phylogenies of the associated species is



**Figure 1. Phylogenetic tree analysis of DpNPV with other polyhedrin genes and Granuloviruses**

of their natural interrelationships, time and mode of evolution (Ludwig *et al.*, 1991; Gorman *et al.*, 1991; Dolja and Koonin, 1991; Hillis *et al.*, 1992).

It was concluded that the phylogenetic analysis based on polyhedrin gene sequences of *DpNPV* was closely related to *Galleria mellonella* NPV, *Plutella maculipennis* NPV and *Autographa californica* NPV and distantly related to *Bombyx mori*. This molecular and biological information of *DpNPV* elucidated in the present study could be useful in understanding the relationships within viruses and to promote the use of baculoviruses in insect pest control strategies.

## References

- Blissard, G.W. and Rohmann, G.F. 1990. Baculovirus diversity and molecular biology. *Annu. Rev. Entomol.*, **35**: 127-155.
- Brooks, D.R. and Mclennan D.A. 1991. *Phylogeny Ecology and Behavior. A Research Program in Comparative Biology*. The University of Chicago Press, Chicago, IL, pp. 32-68.
- Chen, X., Ijkel, W.F.J., Dominy, C., Zanotto, P. and Hashimoto, Y. 1999. Identification, sequence analysis and phylogeny of the *lef-2* gene of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus genome. *Virus Genes* **65**: 21-32.
- Dolja, V.V. and Koonin, E.V. 1991. Phylogeny of capsid proteins of small icosahedral RNA plant viruses. *J. Gen. Virol.* **72**: 1481-1486.
- Gorman, O.T., Bean, W.J., Kawaoka, Y., Donatelli, I., Guo, Y. and Webster, R.G. 1991. Evolution of Influenza A virus nucleoprotein genes: Implications for the origins of H1N1 human and classical swine viruses. *J. Virol.*, **65**: 3704-3714.
- Hillis, D.M., Bull, J.J., White, M.E., Badgett, M.R. and Molineux, I.J. 1992. Experimental phylogenies: Generation of a known phylogeny. *Sci.*, **255**: 589-592.
- Jin, T., Qi, Y., Liu, D. and Su, F. 1999. Nucleotide sequence of a 5892 base pairs fragment of the LsMNPV genome and phylogenetic analysis of LsMNPV. *Virus Genes* **18**: 265-276.
- Kang, W., Tristem, M., Maeda, S., Crook, N.E. and O'Reilly D.R. 1998. Identification and characterization of the *Cydia pomonella* granulovirus *cathepsin* and *chitinase* genes. *J. Gen. Virol.*, **79**: 2283-2292.
- Lange, M., Wang, H. and Zhihong, H. 2004. Towards a molecular identification and classification system of lepidopteran-specific baculoviruses. *Virology*, **325**: 36-47.
- Ludwig, S., Schultz, U., Mandler, J., Fitch, W.M. and Scholtissek, S. 1991. Phylogeny relationship of the nonstructural (NS) genes of influenza A viruses. *Virology*, **183**: 566-577.
- Moscardi, F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. *Annu. Rev. Entomol.*, **44**: 257-289.
- Rajadurai, S., Manjunath, D., Katiyar, R.L., Prasad, K.S., Sen, A.K., Shehar, M.A., Ahsan, M.M. and Datta, R.K. 1999. Leaf roller- a serious pest of mulberry. *Indian Silk*, **37**: 9-12.
- Woese, C.R., Kandler, O. and Wheelis, M.L. 1990. Towards a natural system of organisms-proposal for the domains Archaea, bacteria and eucarya. *In: Proc. Natl. Acad. Sci. USA* **87**: 4576-4579.