IN VIVO AND *IN VITRO* CHARACTERIZATION OF SEVERAL ISOLATES OF *SPODOPTERA EXIGUA* NUCLEAR POLYHEDROSIS VIRUS

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Received June 6, 1995; revised July 30, 1995

Summary. – *Spodoptera exigua* nuclear polyhedrosis viruses (SeNPVs), isolated form five geographically distinct regions of Japan and Thailand, were characterized by their DNA restriction endonuclease pattern, level of virus production in a continuous cell line of *S. exigua* and biological activity to *S. exigua* larvae. The *Eco*RI and *Pst*I fragments exhibited similar overall patterns with minor differences. Digestion of virus DNA from a plaque-purified isolate, SeNPV-I1, with *Pst*I yielded 14 fragments and the estimated genome size was approximately 123 kbp. The SeNPV wild isolate from Kagoshima, SeNPV-KW, showed the highest yield of extracellular virus (ECV) in the Se301 cell line of *S. exigua* among five wild isolates, but there was no significant difference in the level of polyhedral inclusion body (PIB) formation. In comparative studies of biological activity using 2nd-instar *S. exigua* larvae, SeNPV-KW had the highest virulence with an LD_{50} value of 3.0 PIBs per larva. When 16 clones, plaque-purified from the Isahaya isolate, SeNPV-IW, were examined for genetic relatedness, seven distinct *Eco*RI patterns were observed, indicating that SeNPV-IW wild isolate consisted of a mixture of different genotypes.

Key words: *Spodoptera exigua*; nuclear polyhedrosis virus; Se301 cell line; restriction endonuclease analysis; genotypic mutants

Introduction

Baculoviruses are large viruses with genome of circular, double-stranded DNA, specific for invertebrates (Francki *et al.*, 1991). The nuclear polyhedrosis virus (NPV), which is a member of the family *Baculoviridae*, has been well investigated because of the potential for use as an insect pest control agent and a vector for the expression of a diversity of heterologous genes under the control of late promoter elements (Granados and Federici, 1986; Smith *et al.*, 1983; Summers and Smith, 1987; Miller, 1988; Maeda, 1989). NPVs replicate in the host cell nucleus and produce two structurally distinst virion phenotypes: extracellular virus (ECV) and polyhedron-derived virus (PDV). The ECV is produced as the nucleocapsids in the nucleus, budding through the cell surface and acquiring envelope from the cell plasma membrane. In contrast, the nucleocapsids of PDV are enveloped *de novo* within the nucleus and then occluded within PIB composed primarily of a protein called polyhedrin.

The beet armyworm, *S. exigua*, is an immigrant and polyphagous pest with a cosmopolitan distribution (French, 1969; Hill, 1983; Trumble and Baker, 1984). Mikkola and Salmensuu (1965) reported that this insect travelled from the source area in central Asia, where the mass reproduction took place, to south-eastern Finland.

However, in the British Isles, it is considered to come from northern Africa or Spain (French, 1969), and there is little insight into the original habitat. Recently, the beet armyworm has developed the resistance to chemical insecticides and it is difficult to control effectively. Several SeNPV isolates have been isolated and extensively studied with the aim to use their biological activity as an alternative biological control agent for regulating the *S. exigua* populations (Gelernter and Frederici, 1986a; Smits and Vlak, 1988a). The SeNPV is specific for the homologous insect both *in vivo* and *in vitro* (Vlak *et al.*, 1981; Gelernter and Federici, 1986b; Hara *et al.*, 1994a). Smits and Vlak (1988b) demonstrated that the SeNPV had the greatest potential for microbial control and was more virulent than any other NPVs.

Abbreviations: AcNPV = *Autographa californica* NPV; CPE = cytopathic effect; ECV = extracellular virus; EDTA = ethylenediamine tetraacetate; FBS = foetal bovine serum; FP = few polyhedra; MP = many polyhedra; NPV = nuclear polyhedrosis virus; p.i. = post infection; PIB = polyhedral inclusion body; SeNPV = *Spodoptera exigua* NPV

On the basis of the LD_{50} value, Caballero *et al.* (1992) showed that the SeNPV isolate from Thailand was the most potent isolate for the beet armyworm among geographic isolates of SeNPV. The replication of SeNPV *in vitro* has been characterized by using a plaque assay in a continuous cell line of *S. exigua* (Hara *et al.*, 1993, 1994a,b).

The restriction endonuclease analysis of baculovirus DNA has been used to distinguish and characterize closely related genotypic variants and species (Tanada and Kaya, 1993). Studies on genetic heterogeneity of wild isolates of NPVs have often revealed that a single isolate is a mixture of genotypically different mutants (Lee and Miller, 1978; Smith and Summers, 1978; Knell and Summers, 1981; Cherry and Summers, 1985; Maeda *et al.*, 1990). The closely related genotypic mutants have been identified in a collection of geographic isolates of SeNPV (Gelernter and Federici, 1986a, 1990; Smits and Vlak 1988b) and no significant difference in their biological activity determined by LD_{50} value to *S. exigua* larvae has been found (Caballero *et al.*, 1992).

In the present paper, five wild isolates of SeNPV collected in Japan and Thailand were compared by their restriction endonuclease profiles of virus DNA, level of virus production *in vitro* and biological activity to *S. exigua* larvae. Also, the DNAs from several plaque-purified isolates were analyzed for their restriction fragment pattern to examine their genetic heterogeneity within a single wild isolate of SeNPV.

Materials and Methods

Cells and viruses. The cloned cell line, Se301 (K. Hara, unpublished data), derived from a continuous Se3FH cell line of *S. exigua* (Hara *et al.*, 1993) was cultured at 27 °C in IPL-41 medium (Dougherty *et al.*, 1981) supplemented with 10% heat-inactivated (60 °C, 30 mins) foetal bovine serum (FBS) in 25 cm^2 tissue culture flasks (Costar, 3055).

Origin and source of five SeNPV wild isolates used are listed in Table 1. *Autographa californica* NPV (AcNPV) strain E2 was obtained from Dr. Y. Hashimoto, Kyoto Institute of Technology, Kyoto, Japan. Viruses were propagated by infecting Se301 cells in logarithmic phase as described previously (Hara *et al.*, 1994a).

Table 1. SeNPV wild isolates used

Isolate	Geographic origin	Year of isolation	Source
IW	Nagasaki, Japan	1990	K.Yokomizo[a]
KW	Kagoshima, Japan	1991	K.Kusigemati[b]
SW	Kagoshima, Japan	1991	K.Tsuda[c]
OW	Oita, Japan	1992	K.Tsuda[c]
TW	Kanchanaburi, Thailand	1991	K.Tsuda[c]

[a] Nagasaki Agricultural and Forestry Experiment station, Nagasaki, Japan.
[b] Faculty of Agriculture, Kagoshima University, Kagoshima, Japan.
[c] Fukuoka Agricultural Research Center, Fukuoka, Japan.

Kinetics of virus growth and plaque assay. Se301 cells in exponential phase were infected with virus at a multiplicity of infection of 0.5 PFU per cell in 24-well culture plate (Falcon, 3014) containing 0.24 x 10^6 cells/well in IPL-41 medium supplemented with 10% FBS (Hara *et al.*, 1994b). Following the 1 hr virus adsorption period, the cells were washed, supplied with fresh medium, and incubated at 27 °C. At various time after inoculation, the culture supernatant containing ECV was removed, centrifuged at 3,000 rpm for 10 mins, and stored at -80 °C prior to plaque assay. To determine PIB concentration, SDS (final concentration 1%) was added to infected cultures and incubated at 37 °C for 30 mins.

Plaques were produced on a confluent monolayer of Se301 cells which were seeded into 24-well culture plate (Falcon, 3014) at a density 0.4 x 10^6 cells/well (Hara *et al.*, 1994b). Serial 10-fold virus dilutions in IPL-41 medium were inoculated to cells and allowed to adsorb for 60 mins at 27 °C. The cells were then overlaid with 0.3 ml/well of 0.75% (w/v) Seaplaque agarose (FMC BioProduct) in IPL-41 medium with 10% FBS at 36 °C. Plaques were counted 6 days after incubation at 27 °C without staining.

Virus DNA extraction. ECV from virus-infected Se301 cells 3 to 4 days post infection (p.i.) was used as the source of virus DNA for restriction endonuclease analysis. The supernatant was collected from the infected cell culture by centrifugation at 3,000 rpm for 20 mins. Virus was then pelleted by centrifugation at 20,000 rpm for 40 mins, (Beckman SW 28 rotor), and the pellets were suspended in TE buffer (10 mmol/l Tris-HCl pH 7.5 and 1 mmol/l ethylenediamine tetraacetate (EDTA)) at 4 °C overnight. The virus suspension was incubated with 1% SDS for 10 mins at 37 °C. Subsequently, proteinase K (Merck) was added (1 mg/ml) and incubated for 30 mins. The virus DNA was extracted with an equal volume of TE buffer-saturated phenol, phenol-chloroform (1:1, v/v) and chloroform. The extracted DNA solution was stored at 4 °C until use.

Digestion of DNA with restriction endonucleases and gel electrophoresis. The virus DNA was digested with *Bam*HI, *Eco*RI, *Hind*III, *Kpn*I, *Pst*I and *Sal*I restriction endonuclease (Nippon Gene Co.). Two µg of DNA was incubated with 10 – 20 units of enzyme at 37 °C for 60 mins. Reaction was stopped by addition of 0.1 volume of 0.1 mol/l EDTA pH 8.0, 40% glycerol and 0.25% (w/v) bromophenol blue. Horizontal slab gels (5 x 125 x 200 mm) containing 0.7% agarose (Agarose I, Dojindo Laboratories) and 0.5 µg/ml ethidium bromide dissolved in TAE buffer (40 mmol/l Tris-acetate and 1 mmol/l EDTA pH 8.0) were used for electrophoresis. A constant current of 30 mA was applied across the gel in TAE buffer containing 0.5 µg/ml ethidium bromide for 24 hrs at room temperature. The molecular size of virus DNA fragments was determined on slab gels by comparison with that of the fragments of *Hind*III-digested lambda phage DNA.

Insect rearing. The cultures of *S. exigua* were maintained on an artificial diet (Okada, 1990; H. Hayashi, personal communication) at 25 °C in constant darkness as described previously (Hara *et al.*, 1994a).

Dosage-mortality studies. PIBs from SeNPV-infected Se301 cells were fed with an artificial diet to early 4th-instar *S. exigua* larvae. The virus-infected larvae were homogenized in 1 mol/l NaCl, and the suspension was filtrated through two layers of Kimwipe. PIBs were pelleted by centrifugation at 3,000 rpm for 10 mins and then incubated with 50 mmol/l Tris containing

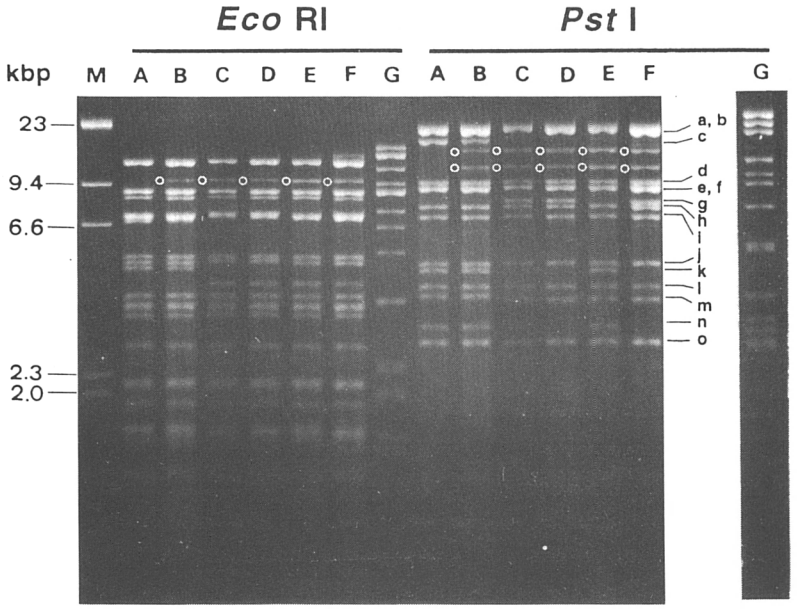

Fig. 1
Restriction patterns of the DNA from SeNPV isolates after digestion with *Eco*RI or *Pst*I
*Hind*III fragments of lambda phage DNA, their size in kbp on the left side (M). SeNPV-I1, plaque-purified isolate from SeNPV-IW (A). SeNPV-IW (B). SeNPV-KW (C). SeNPV-SW (D). SeNPV-OW (E). SeNPV-TW (F). *A. californica* NPV (G). Submolar fragments determined by density analysis are indicated by open circles to the left of the band. For locating of the viruses, see Table 1.

0.5 mol/l urea and 0.1% SDS at 37 °C for 5 mins. After washing with distilled water, PIBs were purified by 45 – 55% (w/v) sucrose density gradient centrifugation at 3,000 rpm for 30 mins. PIBs were washed twice with distilled water and stored at 4 °C.

Second-instar larvae of *S. exigua* were used to determine the biological activity of SeNPV isolates. Larvae were starved for 5 – 6 hrs and then fed on 20 mg artificial diet surface-contamined with PIBs suspended in 1.0 µl sterile distilled water at appropriate concentrations. Doses of 1, 4, 16, 64 and 256 PIBs per larva were used. The larvae were individually placed into wells of 24-well tissue culture plate (Costar, 3524) and incubated at 27 °C in constant darkness. The treated diet was completely consumed within about 24 hrs and then the fresh diet was fed to each larva. The mortality was recorded for 10 days after inoculation and the LD_{50} value was calculated by the probit analysis method (Finney, 1978).

Results

Restriction analysis of SeNPV wild isolates

The DNAs from SeNPV wild isolates were exposed to six restriction endonucleases in order to characterize the virus genome. The *Eco*RI and *Pst*I restriction profiles of five wild isolates and one cloned plaque-purified isolate are shown in Fig. 1. These SeNPV isolates produced 22 – 23 fragments after DNA digestion with *Eco*RI. The five wild isolates of SeNPV had a similar overall pattern with minor differences. The *Eco*RI patterns of SeNPV-KW, SW, OW and TW were identical, but distinct from that of SeNPV-IW in some fragments. In addition, the profile of SeNPV-I1, plaque-purified isolate from SeNPV-IW, missed the submolar 9.6 kbp fragment that was present in all SeNPV wild isolates. The *Eco*RI restriction patterns of SeNPV isolates showed distinct differences from that of AcNPV.

*Pst*I digestion of DNA from SeNPV wild isolates yielded 12 – 14 fragments with total molecular size ranging from 105.6 to 122.6 kbp (Fig. 1, Table 2). Three wild isolates, SeNPV-KW, SW and TW, had a similar *Pst*I pattern with a total molecular size of 105.6 kbp. However, this pattern was distinct from those of SeNPV-IW and OW. The *Pst*I patterns of these two isolates were nearly the same, although the fragment of 16.7 kbp was not present in SeNPV-OW. The pattern of plaque-purified SeNPV-I1 isolate was the same as that of the parental isolate SeNPV-IW, except for the absence of the 14.4 and 11.3 kbp submolar fragments. The *Pst*I patterns of SeNPV and AcNPV also differed from each other.

Additional comparison of the *Bam*HI, *Hind*III, *Kpn*I and *Sal*I patterns revealed that they were identical or quite similar for all SeNPV wild isolates (data not shown).

EcoRI analysis of SeNPV clones

We successfuly obtained 16 clones from the SeNPV-IW wild isolate by plaque purification method on *S. exigua* cells.

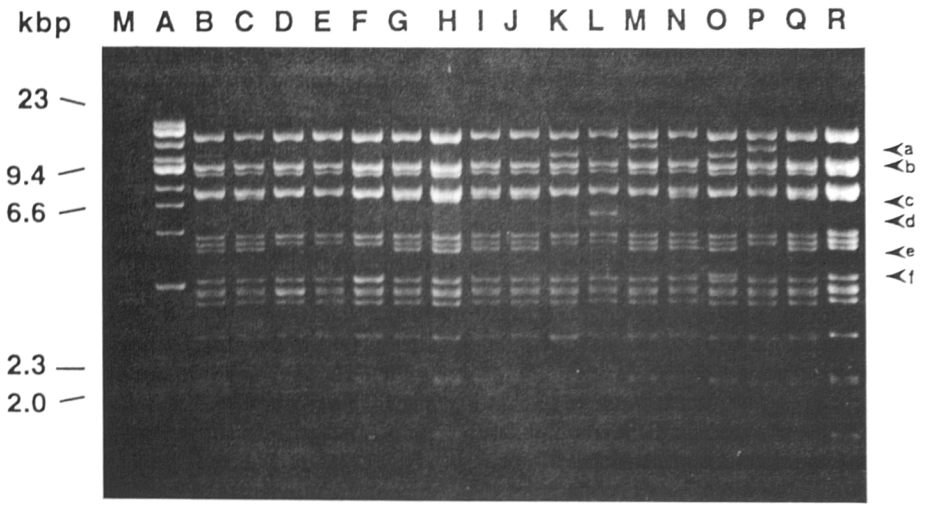

Fig. 2
***Eco*RI pattern of the DNA of plaque-purified isolates from SeNPV-IW**
AcNPV (A); SeNPV-IW (B); SeNPV-I1 to I14, plaque-purified isolates from SeNPV-IW (C to P); SeNPV-IF1 and -IF2, FP mutants from SeNPV-IW (Q and R). For the rest of legend see Fig. 1.

Table 2. Molecular size of *Pst*I restriction endonuclease fragments of the DNA from various SeNPV isolates

Fragment	Molecular size (kbp)[a]					
	I1	IW	KW	SW	OW	TW
a	21.6	21.6	21.6	21.6	21.6	21.6
b	21.6	21.6	21.6	21.6	21.6	21.6
c	16.7	16.7				
d	9.2	9.2	9.2	9.2	9.2	9.2
e	8.4	8.4	8.4	8.4	8.4	8.4
f	8.4	8.4	8.4	8.4	8.4	8.4
g			7.4	7.4		7.4
h	7.2	7.2	7.2	7.2	7.2	7.2
i	6.4	6.4	6.4	6.4	6.4	6.4
j	4.6	4.6	4.6	4.6	4.6	4.6
k	4.4	4.4			4.4	
l	4.0	4.0	4.0	4.0	4.0	4.0
m	3.8	3.8	3.8	3.8	3.8	3.8
n	3.3	3.3			3.3	
o	3.0	3.0	3.0	3.0	3.0	3.0
Total	122.6	122.6	105.6	105.6	105.9	105.6

[a]Estimation of molecular size of each *Pst*I fragment was made by comparing with standard curves obtained from lambda phage DNA *Hind*III fragments used as size markers. Submolar fragments were removed for the estimation. I1 = plaque-purified isolate from SeNPV-IW. For the designation of other isolates see Table 1.

Of the 16 plaque-purified isolates, 14 (SeNPV-I1 to I14) were many polyhedra (MP) mutants and 2 (SeNPV-IF1 and IF2) were few polyhedra (FP) mutants. Digestion of the plaque-purified virus DNAs with *Eco*RI resulted in considerable similarity to the wild isolate (Fig. 2). Seven distinct patterns were observed among plaque-purified SeNPV isolates, when 6 fragments (a, b, c, d, e and f) were emplyed for characterization of each isolate. In 8 isolates (SeNPV-I1, I5, I6, I7, I8, I12, IF1 and IF2), the patterns were similar, in which four fragments (a, b, d and f) were missing. All of the 6 fragments were missing in SeNPV-I2, I3 and I4 which showed identical patterns. The *Eco*RI patterns of other 5 isolates (SeNPV-I9, I10, I11, I13 and I14) differed one from another in some fragments.

Characteristic of virus growth in vitro

Virus growth curves of 5 wild isolates and 1 plaque-purified isolate of SeNPV in Se301 cells are shown in Fig. 3. The Se301 cells showed significant cytopathic changes and a high level of PIBs after inoculation of each SeNPV isolate. All 6 SeNPV isolates replicated well and formed distinct plaques on the monolayer of Se301 cells. The virus growth kinetics of 6 isolates revealed a typical SeNPV growth pattern with high level of progeny virus; ECV appeared as early as 6 hrs p.i. and the infectivity reached a maximum titer of about 10^7 PFU/ml 48 to 72 hrs p.i. There was a small difference in the level of ECV among different isolates. In exponential phase ranging from 6 to 48 hrs p.i., the ECV titer of SeNPV-I1 was higher than that of any other isolate. With respect to the maximum titer of ECV, SeNPV-KW showed the highest level of ECV (2×10^7 PFU/ml) among the isolates (Fig. 4), and SeNPV-TW revealed the lowest level of ECV, although it showed rapid growth in exponential phase.

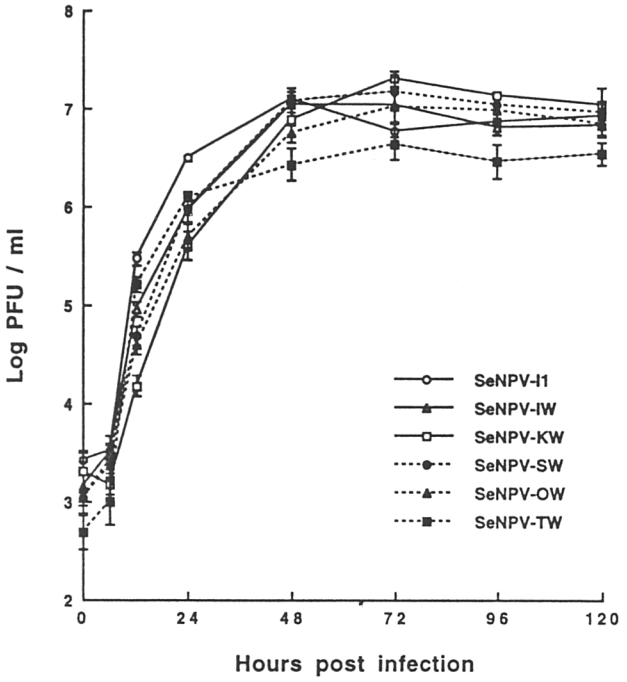

Fig 3
Replication of the wild isolates and a plaque-purified isolate of SeNPV in Se301 cells
Data represent means ± SE of a typical experiment (n = 5/sample). For the designation of virus isolates see Tables 1 and 2.

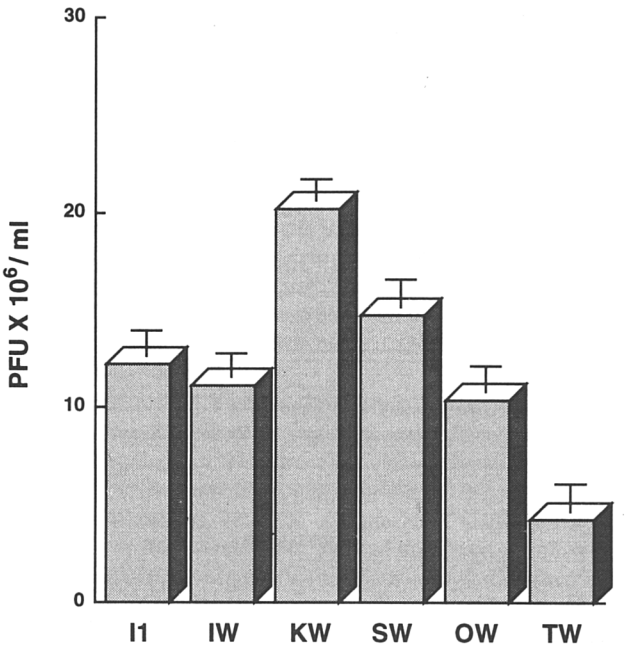

Fig. 4
Maximum ECV titers of the wild isolates and a plaque-purified isolate SeNPV in Se301 cells
Data represent means ± SE of a typical experiment (n = 5/sample). For the designation of virus isolates see Tables 1 and 2.

Observation of cytopathic effect (CPE) due to the PIB formation in Se301 cells showed no significant differences among SeNPV wild isolates. The PIBs formed in cells could be detected before 24 hrs p.i. and were mostly released into medium at late times p.i. All five wild isolates produced many PIB in Se301 cells, and there were just small significant differences in the level of PIB formation (Fig. 5). The number of PIBs formed by plaque-purified isolate SeNPV-I1, however, was three times higher than that of SeNPV-IW.

Bioassay of SeNPV isolates

To examine the virulence of the six SeNPV isolates for *S. exigua* larvae, LD_{50} values were determined by oral inoculation of PIBs into 2nd-instar larvae of *S. exigua*. All isolates killed the larvae within 5 – 7 days with the symptoms typical of NPV infection: the hemolymph turned milky and larval integuments became fragile. The infected larvae produced a large number of PIBs with no differences in the size and shape. Table 3 shows the statistics of the regression lines and the values of LC_{50} and LD_{50}. SeNPV-KW was most virulent to *S. exigua* larvae with the LD_{50} value of 3.0 PIBs per larva. Significant difference was not observed in LD_{50} values between plaque-purified isolate SeNPV-I1 and wild isolate SeNPV-IW.

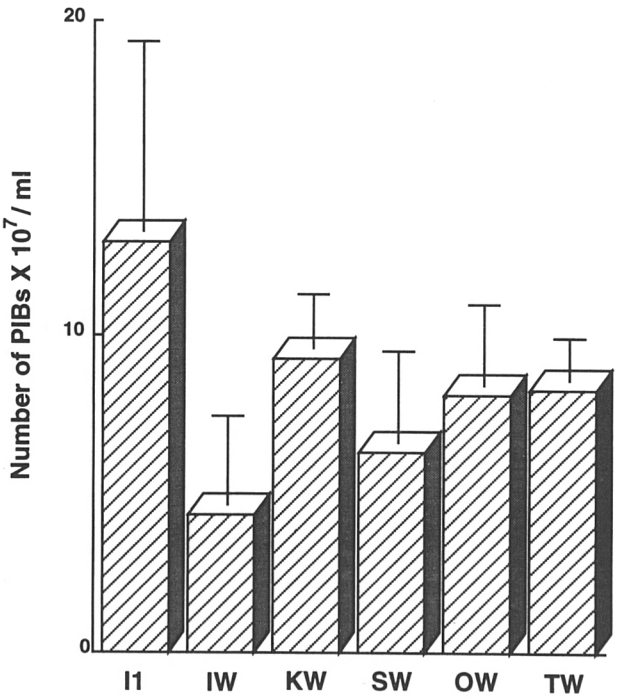

Fig. 5
PIB formation by the wild isolates and a plaque-purified isolate of SeNPV in Se301 cells 96 hrs p.i.
Data represent means ± SE of a typical experiment (n = 3/sample). For the designation of virus isolates see Tables 1 and 2.

Table 3. LC_{50} and LD_{50} values of various SeNPV isolates for 2nd-instar *S. exigua* larvae

SeNPV isolate[a]	LC_{50} (PIBs/ml)	LD_{50} (PIBs/larva)	95% fiducial limits		Slope ± SE	Intercept
			Lower	Upper		
I1	9.5×10^3	9.5	8.0	10.9	0.87 ± 0.39	4.15
IW	7.1×10^3	7.1	5.6	8.6	1.53 ± 0.12	3.70
KW	3.0×10^3	3.0	1.9	4.1	3.85 ± 0.88	3.14
SW	15.6×10^3	15.6	14.2	17.0	1.20 ± 0.14	3.57
OW	22.1×10^3	22.1	20.7	23.4	1.13 ± 0.18	3.48
TW	12.3×10^3	12.3	9.2	15.4	15.4 ± 0.06	3.51

[a] For their designation see Tables 1 and 2.
LC_{50} = 50% lethal concentration; LD_{50} = 50% lethal dose.

Discussion

In recent studies, the restriction analysis of baculovirus DNAs has been a useful technique for identification of closely related virus isolates (Smith and Summers, 1978; Miller and Dawes, 1978a,b; Vlak and Gröner, 1980; Kislev and Edelman, 1982). The restriction patterns of DNAs from wild isolates of *S. exigua* NPV indicate that these isolates are closely related but still slightly different. The patterns of SeNPV wild isolates used in this study do not resemble that of AcNPV, and we conclude that they are typical of SeNPV. There have been several reports on the isolation of SeNPV from California, Egypt, Netherlands, Spain, Thailand and Japan (Gelernter and Federici, 1990; Vlak *et al.*, 1982; Caballero *et al.*, 1992, Kondo *et al.*, 1994). The beet armyworm is an immigrant pest, coming from its homelands in subtropics, Egypt, Algeria, and Tunisia, to countries to the north as Italy, Spain and northern Europe, eventually. There it does not form persistent populations, but with these migrations NPV isolates are redistributed in temporary invaded areas. Therefore, it is interesting to compare SeNPV isolates from different areas and to look for diversity of introduced and selected isolates. Vlak *et al.* (1982) showed that SeNPV isolates from Egypt, Netherlands and California are genetically distinct. However, the results of restriction analysis indicated that the isolates from California, Thailand and Spain are closely related, and the California isolate, SeNPV-US, was proposed for the type strain of SeNPV (Caballero *et al.*, 1992). Our data clearly show that the SeNPV isolates used in the present study are closely related to the SeNPV-US reported by Caballero *et al.* (1992), although minor differences are observed.

The diversity of SeNPV isolates may offer isolates suitable for introduction or colonization in areas with adapted and compatible isolates in local sedentary population. Our studies will provide the information toward a better understanding of the diversity of SeNPV isolates eastern and south-eastern Asia.

The restriction analysis of baculovirus DNAs from wild isolates has revealed the presence of submolar fragments (Miller and Dawes, 1978a,b; Smith and Summers, 1978; Knell and Summers, 1981; Gettig and McCarthy, 1982). These results have suggested that the wild isolate is a mixture of two or more closely related genotypic mutants. E.g., the wild isolate of AcNPV was shown to have submolar fragments and subsequent plaque purification of the isolate resulted in the isolation of several different mutants (Smith and Summers, 1978). Maeda *et al.* (1990) also reported that a *S. littura* NPV wild isolate was a mixture of several different NPVs including AcNPV and *S. littoralis* NPV. Recently, several investigators (Gelernter and Federici, 1990; Caballero *et al.*, 1992) have demonstrated the presence of submolar fragments in SeNPV wild isolates. In the present study, 16 plaque-purified isolates from SeNPV-IW could be grouped into 7 distinct genotypic mutants and two phenotypic mutants. This is the first report that an SeNPV wild isolate is a mixture of genotypic and phenotypic mutants. In our preceding study, we observed that the plaque-purified SeNPV-I1 isolate consistently produced more ECV and PIBs than SeNPV-IW wild isolate in cultured cells (K. Hara, unpublished data). These findings suggest that the genetic heterogeneity influences the level of virus production. These genotypic mutants may be generated from a single original genotype by a deletion and/or an insertion of some restriction site. However, the origin and significance of these mutants are not fully understood. The preferential selection of the mutants may occur during the distribution of virus, or when the virus is ingested by different insect hosts. The appearance of these mutants could play an important role in the diversity and the evolution of baculoviruses.

The numerous serial passaging of NPVs in cultured cells causes the production of FP mutants (McKinnon *et al.*, 1974; Kumar and Miller, 1987; Granados *et al.*, 1994). The FP mutants are considered to be a result of the insertion of transposable-like elements (Miller and Miller, 1982; Blissard and Rohrmann, 1990) or host DNA (Fraser *et al.*, 1983) into the virus genome. In our study, we have isolated the FP mutants from SeNPV wild isolate after one passage through *S. exigua* larvae and one passage in cultured *S. exigua* cells, suggesting that the SeNPV wild isolate contained FP mutants. Further work is needed to determine whether FP mutants occur in nature and are relatively stable.

In this study, it was shown that SeNPV wild isolates were genetically closely related because of small differences in the restriction pattern of virus DNA, as well as in virus production *in vitro* and in virulence against *S. exigua* larvae. Gelernter and Federici (1990) demonstrated that a high degree of uniformity was observed among SeNPV isolates in Californian populations and virus epizootics were caused by a single virus or its closely related variants. In addition, there was no occurrence of apparent variation in the field

populations of Californian SeNPV over a 16-year period (Gelernter and Federici, 1986a). It is probable that a significant genetic change in the SeNPV populations has not occured. This may reflect a narrow host range of SeNPV both *in vivo* and *in vitro*. Further studies of the generation of genotypic variants will provide insight into the determination of host specificity of baculoviruses.

Acknowledgements. We thak Dr. M. Ohba, Faculty of Agriculture, Kyushu University, for reviewing the manuscript. Also, we thank Dr. K. Ueda, Kitakyushu Museum of Natural History, for his advice on the biology of *S. exigua*. This work was supported by Integrated Research Program on the Development of Insect Technology by MAFF.

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