Characteristically distinct isolates of the nuclear polyhedrosis virus from Spodoptera litura

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More than 100 isolates were plaque-purified to examine the genetic variations in four wild stocks of Spodoptera litura nuclear polyhedrosis virus (NPV) collected in Japan. These isolates were characterized by their in vitro host range in three established insect cell lines, growth characteristics, polyhedral protein, DNA restriction endonuclease pattern and DNA hybridization. The isolates were separated into four distinct groups: (I) isolates corresponding to Autographa californica NPV, (II and IV) two different groups of isolates of S. littoralis NPV which had been previously characterized and (III) isolates with no correspondence to any reported virus group. Of the S. litura NPV wild stocks, two were mixtures of more than two different groups of NPVs. We have discussed the advantage of having a mixture of different NPV groups in the same wild virus stocks.

Introduction

The nuclear polyhedrosis virus (NPV) (Baculoviridae, subgroup A) is an enveloped rod-shaped virion carrying a circular dsDNA genome. NPV can produce proteinaceous crystal inclusion bodies, which contain a large number of progeny virus particles, in the infected host cell nuclei at a late stage of larval infection. NPVs have been isolated from many insect species and they have potential for use in insect pest control (Granados & Federici, 1986).

With technological advances in DNA analysis, NPV genomes have been analysed and their relationships to each other have been investigated. DNA restriction enzyme analysis and hybridization experiments have shown genetic relatedness between baculoviruses (Knudson & Tinsley, 1978; Smith & Summers, 1978, 1979; Miller et al., 1980; Knell & Summers, 1981; Gettig & McCarthy, 1982; Kislev & Edelman, 1982; Cherry & Summers, 1985). These methods have proven useful for the comparison of geographical isolates of NPVs from the same or similar insect species (e.g. Vlak & Gröner, 1980).

Spodoptera littoralis is a major agricultural pest in Africa, Asia and Mediterranean regions. S. littoralis NPVs have been isolated and extensively compared to each other and other baculoviruses. Two genetically distinct isolates have been identified from a large collection of wild stocks of S. littoralis NPV in Israel; they are classified as 'SINPV-T' and 'SINPV-D' by Kislev & Edelman (1982) or as 'SINPV-A' and 'SINPV-B' by Cherry & Summers (1985). They are classified as belonging to different virus groups by restriction endonuclease analysis (Kislev & Edelman, 1982; Cherry & Summers, 1985), DNA hybridization experiments (Kislev & Edelman, 1982) and their replication characteristics in established cell lines (Kislev, 1986). These two distinct S. littoralis NPVs are also different from other baculoviruses, as shown by DNA hybridization analysis (Knudson & Tinsley, 1978; Smith & Summers, 1978; Kislev, 1985). A physical map of the SINPV B-type has recently been constructed (Crozier et al., 1989).

Spodoptera litura, which is considered to be closely related to S. littoralis, is a major pest of many crops in Southwest Japan and Southeast Asia. Several isolates of S. litura NPV have been collected in Japan but, although extensive aetiological studies and insect control trials have been conducted (review by Okada, 1977), there

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have been only a few studies on their DNA characteristics and in vitro replication. Tsuda et al. (1988) have plaque-purified an isolate of *S. litura* NPV in Sf-21 cells and have shown cross-neutralization with other baculoviruses using antibodies against the viral particles of *S. litura* NPV.

In the present paper, we have used three established cell lines to plaque-purify *S. litura* NPV from four virus stocks collected from the wild in Japan and *S. littoralis* NPV from a wild stock in Egypt. We have characterized these isolates and classified them into four distinct virus groups based on their *in vitro* host range, polyhedral characteristics, DNA restriction endonuclease patterns and DNA sequence similarity.

**Methods**

**Viruses.** Four wild virus stocks of *S. litura* NPVs collected in Ogasawara, Fukuyama, Chikugo and Kanoya in Southwest Japan were used. The Ogasawara and Chikugo stocks were kindly provided by Dr T. Kawarabata (Kyushu University, Japan) and the Kanoya and Fukuyama stocks, as well as a stock of *S. littoralis* NPV collected in Egypt, were kindly provided by Dr T. Terada (Chugoku National Agricultural Experiment Station, Japan). Plaque-purified isolates of T3 *Bombyx mori* NPV (BmNPV) (Maeda, 1984; Maeda et al., 1985) and W31 BmNPV (S. Maeda, unpublished data) were used as controls.

Wild isolates of *S. litura* NPV and *S. littoralis* NPV were inoculated *per os* into third to fifth instar larvae of *S. litura*. Several days post-infection (p.i.), the haemolymph of infected larvae was collected, after piercing of the abdominal prolegs, into 1.5 ml microfuge tubes chilled on ice. The samples were centrifuged at 12,000 r.p.m. for 5 min to remove haemocytes and cell debris. The supernatants were then transferred into new tubes and 10 volumes of culture medium containing 1% foetal calf serum (FCS) and 60 μg/ml of kanamycin were added to the supernatants. The samples were stored at -80°C until used.

**Insects.** *S. litura* was collected in the field and maintained in the laboratory for several generations on an artificial diet (Hattori & Atsusawa, 1980).

**Infection of larvae with virus and purification of polyhedral inclusion bodies.** Infection of *S. litura* larvae with virus was carried out by a modification of methods previously (Maeda et al., 1985). Virus solution (20 μl; infected culture fluid) containing 10⁵ to 10⁶ p.f.u. was injected into the body cavity of fifth or sixth instar larvae. Polyhedral inclusion bodies were purified from the infected larvae 6 to 8 days p.i. by the method described previously (Maeda, 1989).

**Cell lines and plaque assay.** CLS-79 established from *S. littoralis* and Sf-21 (Knudson & Tinsley, 1974; Vaughn et al., 1977) from *S. frugiperda* were kindly provided by Dr K. Aizawa of Kyushu University, Japan; TN-368 (Hink, 1970) from *Trichoplusia ni* and BmN from *B. mori* were kindly provided by Dr L. Volkman of the University of California, Berkeley, Ca., U.S.A. CLS-79 from *S. littoralis* was maintained with IPL-41 (Dougherty et al., 1981), BmN with TC-100 (Knudson & Tinsley, 1974; Maeda, 1989) and Sf-21 and TN-368 with TNM-FH (Hink, 1970). All media were supplemented with 10% FCS and subcultured every 4 to 6 days. Plaque assays were carried out by methods reported previously (Maeda, 1984, 1989).

Polyacrylamide gel electrophoresis and peptide mapping. Polyhedral inclusion bodies purified from infected larvae were disrupted in a dissociation buffer at 100°C for 3 min and electrophoresed on a 10% polyacrylamide gel (Laemmli, 1970). MrS were estimated by comparison with the standards (Pharmacia) albumin (66K), ovalbumin (45K), trypsin inhibitor (24K), lactoglobulin (18.4K) and tyrosine (14.3K).

The methods described by Cleveland et al. (1977) were used for peptide mapping. Polyhedral proteins (polyhedrins) were separated on a 10% SDS gel and excised with a razor blade. Digestion of polyhedrins with 0.5 μg/lane *Staphylococcus aureus* V8 protease was performed in a stacking gel. The cleaved peptides were separated on a 15% SDS slab gel and stained with Coomassie blue.

**DNA restriction endonuclease analysis.** Viral DNA was prepared from purified polyhedra by the methods described previously for BmNPV (Maeda, 1989). Since viral particles of *S. litura* NPV produced multiple bands in a sucrose gradient, all major bands were collected and used for the extraction of DNA. Purified DNA was digested with EcoRI (New England Biolabs) under the conditions recommended by the supplier. Digested DNAs were electrophoresed on a 0.7% agarose gel in a Tris-acetate buffer system (Maniatis et al. 1982).

**Hybridization.** Viral DNA fragments that had been separated in an agarose gel by the methods described above were transferred onto nitrocellulose, fixed for 2 h at 80°C in a vacuum and prehybridized for 8 to 18 h at 42°C in a hybridization solution containing 50% formamide and 5 × SSC (Maniatis et al., 1982). Hybridization was carried out in the same solution for 4 to 18 h at 42°C using nick-translated labelled viral DNA as a probe. The filter was washed with 0.1 × SSC and exposed on X-ray film.

**Results**

**Isolation of plaque-purified clones of *S. litura* NPV and *S. littoralis* NPV on established cell lines**

Four wild isolates of *S. litura* NPV and one of *S. littoralis* NPV were fed initially to larvae of *S. litura*. Several days p.i. when the infected larvae showed typical symptoms of nucleopolyhedrosis, the haemolymph was collected by piercing the abdominal legs and subjected to viral plaque isolation. For the plaque isolations of *S. litura* NPV and *S. littoralis* NPV, the three cell lines CLS-79, Sf-21 and TN-368 were used. Since the CLS-79 cell line showed significant c.p.e. with all isolates and had a high production of polyhedral inclusion bodies, it was initially used for the isolation of clones from wild stocks. Plaques produced in monolayers of CLS-79 cells were picked at random with a Pasteur pipette. The plaque purification was repeated to ensure genetic homogeneity. The naming of the isolates was as follows: the first character of the isolate name refers to the first letter of the location where the wild stock was found, the second character refers to the first letter of the cell line used for the plaque isolation and the third character represents the serial number of the isolation (e.g. OC2 is from the Ogasawara stock, plaque-purified on CLS-79 cells and is the second isolate). In total, 108 isolates were plaque-purified using CLS-79 cells from the five wild stocks Ogasawara (OC1 to OC28), Fukuyama (FC1 to FC20), Kanoya (KC1 to
KC20), Chikugo (CC1 to CC20) and Egypt (EC1 to EC20).

When TN-368 cells were inoculated with infected haemolymph of the Ogasawara stock, only a small number of cells showed polyhedron production several days p.i. However, when the supernatant from the initial infection was transferred to another monolayer of TN-368, a typical c.p.e. resulted and polyhedron production occurred in almost all cells within 2 days. When the remaining four isolates of *S. litura* NPV and *S. littoralis* NPV were inoculated onto TN-368, no symptoms of viral infection appeared even after several blind passages. These results indicated that only the Ogasawara stock contained NPVs that replicated in TN-368. Plaques produced on TN-368 cells inoculated with the Ogasawara isolate were clear. Thirty purified clones (designated OT1 to OT30) were isolated in TN-368 by repeated plaque-purification. However, the titre in the original stock, calculated by the plaque numbers produced in TN-368, was at least 100-fold lower than that produced in CLS-79. This indicated that the OT isolates were extremely rare (less than 1%) in the Ogasawara stock.

When Sf-21 cells were infected with each of the wild isolates prepared from infected larvae, all four wild isolates showed typical c.p.e. and polyhedron production. The plaques, however, were not clear when compared to those formed on CLS-79 and TN-368. Five isolates from the *S. littoralis* NPV from Egypt were collected and plaque-purified in Sf-21 cells and they were designated ES1 to ES5. In total, 143 isolates were plaque-purified from the five wild stocks of *S. litura* NPV and *S. littoralis* NPV using the three different cell lines.

**Viral growth of plaque-purified isolates and classification of in vitro host range group**

Viral growth patterns of the cloned isolates were examined in detail using the four different cell lines. The same cell lines used for the initial isolation process by plaque assay were used for the titration of viral growth. When TN-368 cells were infected with the various isolates, only the OT series isolates, which had been plaque-isolated in TN-368, caused typical c.p.e. and polyhedron production. All other clones isolated in CLS-79 or Sf-21 were not infective. Although all of the OT series isolates replicated in TN-368 cells, the number of polyhedral inclusion bodies produced in the infected cells varied between the isolates from around five to more than 30 polyhedra per cell. When the number of polyhedra produced by 22 of the OT isolates in TN-368 was compared at 2 days p.i., the order of the polyhedron production rate was as follows: OT1, 2, 4, 6, 7, 10, 21 > OT9, 11, 12, 15, 16, 18, 22, 23 > OT3, 5, 8, 13, 14, 19, 20. After serial passages of *Autographa californica* NPV (AcNPV) in cell culture, few polyhedra (FP) and many polyhedra (MP) phenotypes, based on the numbers of polyhedra produced per cell, arose (Hink & Vail, 1973; MacKinnon et al., 1974; Ramoska & Hink, 1974; Potter et al., 1976; Fraser & Hink, 1982). The OT series isolates had similar phenotypic characteristics and OT2 and OT14 isolates were used mainly in our experiments as representatives of the MP and FP phenotypes, respectively.

In order to examine in detail the viral growth in TN-368, a monolayer of cells was infected with 5 p.f.u./cell of the OT2 or OT14 isolate. After 1 h adsorption, the inoculum was removed and the cell monolayer was washed twice with culture medium. Fresh medium was then added onto the monolayer and incubated at 27 °C (time zero of infection). Aliquots (10 to 50 µl) were collected at appropriate times after infection and the viral titres were calculated by plaque assay on TN-368 cells. As shown in Fig. 1(a), OT2 and OT14 infection showed a typical growth curve of viral replication with
eclipse (0 to 8 h), logarithmic (12 to 24 h) and plateau (24 to 72 h) periods with a high level of production of progeny virus, around 10^6 p.f.u./ml. No apparent difference in viral growth was observed between the MP (OT2) and FP (OT14) types. Isolates of the OT series which replicated in TN-368 were classified as host range group I.

The virus titres of the isolates FC3, OC12 and CC5, which showed no infectivity in TN-368, were determined by plaque assay using CLS-79 cells. As shown in Fig. 1(a), all of the virus titres were estimated at approximately 10^5 p.f.u./ml and did not indicate the occurrence of viral replication in TN-368. These virus titres are likely to have remained in the culture fluid after washing out of the inoculum.

When Sf-21 cells were used to study viral replication, several types of viral growth curves were observed. All the isolates in group I (for example OT2 in Fig. 1b) showed a rapid growth curve similar to that obtained in TN-368 and reached maximum titres of around 2 x 10^6 p.f.u./ml. FC3, OC6 and OC18 showed a typical viral growth curve, but it was less rapid than that observed in group I (Fig. 1b). These isolates were classified as host range group II. All tested viruses which had similar growth curves showed a typical c.p.e. and production of polyhedra (data not shown).

The existence of viruses with poor growth was detected in further analysis with Sf-21, e.g. infection with CC1, OC3 and OC12 produced a slight c.p.e. and very low numbers of polyhedral inclusion bodies in some cells. Although the plaque assay in CLS-79 showed viral growth from 10^5 to 10^6 p.f.u./ml, these growth rates were extremely low (Fig. 1b). Since the poor replication might have been caused by heterogeneity of the original Sf-21 cells, four new cell lines were recloned from Sf-21 using a 96-well plate and tested for viral replication. All recloned cells, as well as the original uncloned control Sf-21, had similar viral growth curves (data not shown). This indicated that the poor replication of the isolates was a natural characteristic of the original isolates. These viruses were classified as host range group III. About 70% of the isolates of the OC series belonged to group III, the remaining isolates belonged to group II. Although group III had the same in vitro host range as group II, the isolates in group III were separated into a different group because of their distinctly poor replication in Sf-21. Kislev (1986) also reported poor replication of S. littoralis NPV in SF-21.

Some of the isolates (e.g. CC5) did not show any viral replication in Sf-21 cells when compared to the viral growth assayed in CLS-79 cells (Fig. 1b) and they did not show a detectable c.p.e. on Sf-21 cells. These viruses were classified as host range group IV.

When CLS-79 cells were infected with the various isolates, all infections showed typical c.p.e., polyhedron production and typical viral growth curves (Fig. 1c). All tested isolates, including those isolated from Sf-21 and TN-368, replicated in CLS-79 cells. The patterns of viral growth were nearly identical for all isolates.

BmN is a cell line derived from B. mori and considered to be taxonomically distinct from the other three cell lines. As expected, none of the isolates [OT2 (group I), FC3 (II), OC12 (III) and CC5 (IV)] of S. littua NPV or S. littoralis NPV showed viral replication in BmN cells (Fig. 1d). Only BmNPV, specific for this host cell, showed typical replication upon infection. The OT2 and OT14 isolates of group I and CC5 of group IV had a relatively strong c.p.e. but no viral replication was detected. Furthermore, FC3 (group II) and OC12 (group IV) showed weaker c.p.e. than those produced by OT2 infection (data not shown).

**Replication of isolates in vivo**

Although all isolates showed replication without the helper function of other isolates in the in vitro replication studies, it is still unknown whether they can replicate in larvae without a helper virus. To examine this characteristic, these isolates were tested by injection into the larval body cavity. This type of experiment may also provide useful information about the original host of the isolates. Sixth (last) instar larvae of S. littua were injected with about 10^6 p.f.u. of each isolate. Isolates in groups II (FC3, OC6, EC3), III (OC12, CC1, KC3) and IV (CC5) killed the larvae within several days of the injection. All infected larvae had large numbers of polyhedra and there were no significant differences in the symptoms of infection among the isolates in groups II, III and IV. The polyhedral inclusion bodies were round (icosahedral) with no difference among these isolates (data not shown).

On the other hand, larvae infected with the isolates in group I (OT2, OT14) survived a few to several days longer than those with the isolates in groups II, III and IV. Although the polyhedral inclusions occurred in fat bodies and all infected larvae eventually died, some larvae were able to progress to the pupal stage. When larvae infected with OT2 (MP) or OT14 (FP) isolates were compared, the OT2-infected larvae produced significantly higher numbers of polyhedra per fat body cell than those infected with OT14. These characteristics were consistent with those obtained in vitro in the cell lines. The inclusion bodies were cuboidal for both OT2 and OT14. All tests, including those on S. littoralis NPV which had been replicated in S. littua larvae, indicated that the isolates did not require helper viruses even in the insect.
Spodoptera litura NPV phenotypic isolates

When polyhedrins were analysed on a 10% SDS-acrylamide gel, each produced a single band with an Mr of around 30K. When the bands of the various isolates were compared to each other, minor but distinct differences in their Mr's were detected (data not shown). The polyhedrin isolates fell into three groups based on their Mr's. The estimated Mr for OT2 (host range group I) was 31K, those of FC3 (II), OC6 (II), OC12 (III) and EC5 (II) were 30K, and that of CC5 (IV) was 28K. Within the isolates that belonged to the same host range group, no difference was found in the Mr of their polyhedrins.

Peptide mapping of polyhedrins

Peptide mapping was carried out to examine further the relatedness of the polyhedrins of the isolates (Fig. 2). The peptide pattern of OT2 (I) differed from the patterns of CC5 (IV) and the other two host range groups. The patterns of FC3 (II), OC6 (II), OC12 (III) and EC5 (II) were indistinguishable, but they differed from those of OT2 (I) and CC5 (IV) (Fig. 2), i.e. host range groups II and III fell into the same peptide mapping group. We did not find any difference in polyhedrin characteristics among isolates in the same host range group.

DNA restriction endonuclease analysis

The viral DNAs of the isolates of S. litura NPV and S. littoralis NPV were purified and analysed on an agarose gel after digestion with EcoRI (Fig. 3a). Submolar bands were not detected in any of the samples tested, indicating that the plaque purification was complete and that all viruses were genetically stable. The genome patterns of the isolates, based on the similarity of the fragment patterns (size and numbers), fell clearly into four groups that corresponded to the previously defined groups based on in vitro host range and viral growth. When compared to the DNA pattern of OT2 in group I, the pattern of OT14 (I) was indistinguishable; however, those of the OC6 and OC9 isolates, which belonged to different groups although they were isolated from the same wild stock, showed completely different patterns (Fig. 3a). Additional screening of the EcoRI patterns of the OT series isolates (group I) revealed that all of their patterns were identical or quite similar (data not shown).

When the pattern of FC3 of host range group II was compared to those of OC6 (II), EC5 (II) and ES4 (II), they all had similar or identical patterns (Fig. 3a) that were quite different from those of groups I, III and IV. Among the four isolates in group II showing high similarity, a minor change in the fragments was clearly identified. For example, if the patterns of FC3 and OC6 were compared to those of EC5 and ES4, FC3 had additional 11, 9 and 6 kb fragments but lacked 13 and 5-8 kb fragments, and OC6 had an additional 5-3 kb fragment but lacked 4-5 and 1-5 kb fragments.

The pattern of OC12 of group III was indistinguishable from that of OC9 (III), and similar to those of CC3 (III) and KC5 (III). The patterns of S. litura NPV and S. littoralis NPV were different from that of BmNPV. We conclude that classification based on DNA restriction endonuclease patterns was consistent with the classification based on host range.

Other researchers have used restriction endonuclease analysis to establish taxonomic differences in baculoviruses. We compared their results to the patterns that were obtained in this study. The patterns in group II and IV appeared similar to those of two S. littoralis NPVs, SINPV-B and SINPV-A (Cherry & Summers, 1985), respectively. The pattern of group I was unexpectedly similar to that of AcNPV (Lee & Miller, 1978; Smith & Summers, 1978). There was no pattern corresponding to that of group III in the published literature that was screened.

DNA hybridization analysis

EcoRI DNA fragments separated on an agarose gel (Fig. 3a) were transferred onto a nitrocellulose filter and subjected to hybridization studies. When the labelled viral DNA of OT2 of group I was used as a probe, the DNAs of OT2 and OT14 (I) hybridized strongly (Fig. 3b), whereas the viral DNAs of groups II (FC3, OC6, EC5 and ES4), III (OC12, OC9, CC3 and KC5) and IV (CC5 and CC18) hybridized poorly, indicating that group I was taxonomically distinct from the other groups. Interestingly, the probe hybridized to the DNA of BmNPV relatively strongly; the intensity was more
Fig. 3. EcoRI cleavage profiles of (a) DNA and (b to e) Southern blot hybridization of plaque-purified isolates of the NPVs of *S. litura*, *S. littoralis* and *B. mori*. Viral DNAs of OT2 (lanes 2), OT14 (lanes 3), FC3 (lanes 4), OC6 (lanes 5), OC12 (lanes 6), OC9 (lanes 7), CC3 (lanes 8), Kc5 (lanes 9), CC5 (lanes 10), CC18 (lanes 11), EC5 (lanes 12), ES4 (lanes 13), BmNPV T3 (lanes 14), and BmNPV W31 (lanes 15) after cleavage of EcoRI were separated on a 0.7% agarose gel with Mr standards of *HindIII*-cleaved lambda phage DNA (lanes 1). Names of virus groups and probes used for hybridization are shown above the pictures. DNA fragments transferred onto a filter were hybridized to nick-translated DNA of the *S. litura* NPVs OT2 (b), FC3 (c), OC12 (d) and CC5 (e).
Table 1. In vitro replication of isolates of *S. litura* and *S. littoralis* NPV

<table>
<thead>
<tr>
<th>Group</th>
<th>Name of isolate</th>
<th>Replication on cell line</th>
<th>Isolation site</th>
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<tr>
<td></td>
<td></td>
<td>TN-368</td>
<td>Sf-21</td>
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<tr>
<td>I</td>
<td>OT2, OT14</td>
<td>++ +</td>
<td>++ +</td>
</tr>
<tr>
<td>II</td>
<td>OC6, OC18</td>
<td>-</td>
<td>++ +</td>
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<tr>
<td></td>
<td>FC3</td>
<td>-</td>
<td>++ +</td>
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<tr>
<td></td>
<td>EC3, EC5, ES4</td>
<td>-</td>
<td>++ +</td>
</tr>
<tr>
<td>III</td>
<td>OC3, OC9, OC12</td>
<td>-</td>
<td>+</td>
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<tr>
<td></td>
<td>KC3, KC5</td>
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<td>CC1, CC3</td>
<td>-</td>
<td>+</td>
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<tr>
<td></td>
<td>IV</td>
<td>CC5, CC18</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>BmNPV†</td>
<td>T3</td>
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* = Not replicable.
† *B. mori* NPV as a control.

than 50% of that generated by OT14. This result was confirmed when labelled BmNPV DNA was used as a probe because it hybridized to the DNAs of *S. litura* NPV and *S. littoralis* NPV (data not shown). These two viruses with relatively high DNA similarity possessed completely different in vitro host ranges (Table 1).

When labelled FC3 (II) DNA was used as a probe, the DNAs of OC3, EC5 and ES4 of group II hybridized almost as strongly with the probe as did FC3 (Fig. 3c). This was to be expected from the high similarity of the DNA restriction patterns, however the labelled FC3 DNA also hybridized relatively strongly to OC12, OC9, CC3 and KC5 of group III with an intensity estimated to be between 30 and 50% of that observed in homologous group hybridizations using 50% formamide. The hybridization of labelled FC3 DNA (II) to the DNAs of the isolates in group I and IV was low.

Labelled DNA of OC12 in group III showed results analogous to that obtained when the labelled DNA of FC3 (II) was used. The labelled OC12 DNA hybridized strongly to OC9, CC3 and KC5 of the same group and somewhat less strongly to FC3, OC6, EC5 and ES4 of group II (Fig. 3d). These results indicated that groups II and III were taxonomically related species or variants.

When the DNA of CC5 (IV) was used for hybridization experiments, only CC18 of group IV hybridized strongly (Fig. 3e). In all hybridization experiments the entire genome was hybridized relatively equally and a specifically hybridized area (e.g. the area containing the polyhedrin gene) was not identified under the conditions used.

**Discussion**

We have plaque-purified various isolates from four wild stocks of *S. litura* NPV from Japan using three different established cell lines. Four different groups were established on the basis of in vitro host range, polyhedrin size, peptide mapping and DNA homology studies (Table 1). The classification based on in vitro host range was found to be in good agreement with the classifications based on protein and DNA characteristics. The restriction endonuclease analysis was the most sensitive method for the classification of the isolates of *S. litura* NPV into four groups. Moreover, it was able to distinguish several different genotypic variants within the same group. The DNA hybridization technique was relatively sensitive and useful in estimating the genetic distances between the different isolates of the four groups of *S. litura* NPV. In contrast to the DNA analysis, the polyhedrin characteristics were not as useful in differentiating the groups, e.g. groups II and III were indistinguishable by a comparison of the size and peptide mapping pattern of the polyhedrins.

Since *S. litura* is an insect closely related to *S. littoralis*, we expected the NPV isolates from these species to be similar or the same. The restriction endonuclease analysis and comparison with published reports revealed, however, that correspondence occurred only between group II and SINPV-B (or SINPV-T) and group IV and SINPV-A (or SINPV-D) (Kislev & Edelman, 1982; Cherry & Summers, 1985; Croizier et al., 1989). All isolates from the Egyptian wild stock of *S. littoralis* NPV were also classified as group II viruses. We could not find in the literature a species of NPV corresponding to our group III. This group might be specific for the *S. litura* NPV or might occur only in Japan. Since no viruses with DNA characteristics in between those of groups II and III were found in the isolates using DNA restriction analysis, recombination between these two virus groups does not seem to occur. This indicates that the two groups could be considered different in spite of their relatively high DNA similarity. Following the nomenclature of Cherry & Summers (1985) this group may be named SINPV-C. It seems to
be common in Japan because three of the four wild stocks contained this virus group.

Restriction endonuclease analysis indicated that group I was similar to a group of AcNPV. Variants of AcNPV have been isolated from various hosts including *Trichoplusia ni* (Miller & Dawes, 1978; Smith & Summers, 1979), *Galleria mellonella* (Smith & Summers, 1979), *Rachiplusia ou* (Summers et al., 1980) and *Spodoptera exigua* (Brown et al., 1984). Isolates of group I were found only in the Ogasawara stock and were an extremely minor proportion, less than 1% of the total isolates from this stock. In some cases, a minor isolate could have been selected due to the wide host range of the AcNPV variants. Because of the wide in vitro host range, the group I isolates were efficiently cloned using the TN-368 cell line, which was not susceptible to isolates of groups II, III or IV.

It would be difficult to identify a virus which does not have a wide host range, as is the case of the isolates in group I. Accordingly, in vitro isolation should be performed with care, especially the isolation of an associated minor virus. The group I virus replicated in larvae of *S. litura*, but the efficiency was lower than that of other groups suggesting that *S. litura* might not be the original host insect for this group. Most likely, the group I virus was carried as an accidental contaminant by other virus groups in the *S. litura* stock in the field. Since the data comparing the characteristics of uncleaved and major cloned viruses showed consistent relatedness, we concluded that a major group of *S. litura* NPV had been isolated. For the isolation of the various types of *S. litura* NPVs, the cell line CLS-79 was found to be very efficient because of the ability of these viruses to replicate in this cell line. Kislev (1986) has recommended that the cell line UIV-SL-573, established from *S. littoralis*, be used for the isolation of *S. littoralis* NPV because of its high susceptibility to the virus.

FP and MP variants were isolated in group I. Both variants seemed to exist in the original Ogasawara stock, but there was the possibility that the FP mutants had been generated during the passage of plaque-purification in TN-368 cells as reported by others (Hink & Vail, 1973; MacKinnon et al., 1974; Ramoska & Hink, 1974; Potter et al., 1976; Fraser & Hink, 1982).

Interestingly, the group I AcNPV-like viruses had relatively high DNA similarity to BmNPV, which is considered to be a completely different species according to the phenotypic (multiple or single nucleocapsid NPVs) and biological characteristics (host range) and by restriction endonuclease analysis. When the BmNPV genome was hybridized with an AcNPV (variant) DNA probe, the entire genome, rather than a specific area such as the polyhedrin gene-containing fragment (EcoRI E), was uniformly hybridized. The high DNA similarity was consistent with DNA sequence data showing about 85% identity in the polyhedrin coding sequence and more than 98% in the polyhedrin non-coding region (71 bp in the 5' region and 200 bp in the 3' downstream region) (Iatrou et al., 1985; Maeda et al., 1985). This type of novel relatedness has not been reported previously for baculoviruses. Furthermore, these results suggest that the in vitro host range or phenotypic characteristics of the two viruses might be controlled by a small region of DNA in the viral genome. Previous host range experiments in baculoviruses have attempted to locate a common DNA region in two different viruses which had low DNA similarity but the same host range characteristics (e.g. Harvey & Tanada, 1985). Since *S. litura* group I (AcNPV) isolates and BmNPV have a converse relatedness in host range and DNA characteristics to the previously studied viruses, they will provide a useful model system for studies on host range determination.

This study has shown that the wild virus stocks contain many isolates, some of which may even belong to different virus species. The Ogasawara stock contained representatives of three virus groups (I, II and III) and the Chikugo stock contained two different groups (III and IV). Although the genotypic variants had been commonly found in wild stocks of NPVs as discussed previously, this is the first report that a mixture of viruses from completely different groups of NPV may occur in the same wild virus stock. This suggests that not only genotypic diversity but also phenotypic diversity plays an important role in retaining progeny viruses under different environmental conditions. The existence of these different viruses in the wild stocks may assist in the propagation of progeny viruses when the original virus stock is ingested by different insect hosts.

We wish to thank Dr T. Kawarabata of Kyushu University for his helpful advice and discussion of the in vitro experiments, Dr Y. Tanada of the University of California, Berkeley for reviewing the manuscript, and S. G. Kamita for preparation and critical reading of the manuscript.

References


(Received 5 April 1990; Accepted 12 July 1990)