IDENTIFICATION OF TWO NUCLEAR POLYHEDROSIS VIRUS FROM THE SPOTTED CUTWORM, *XESTIA C-NIGRUM* (LEPIDOPTERA, NOCTUIDAE)

Toshihiko IIZUKA*, Seiyu AOKI and Chie GOTO*****

(* Laboratory of Sericology, Faculty of Agriculture,
Hokkaido University,Sapporo 060 Japan)
(** Hiyama-regional branch of Hokkaido, Esahi 043 Japan)
(*** Hokkaido National Agricultural Experiment Station,
Memuro-cho, 082 Japan)

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Introduction

Xestia c-nigrum NPV (XcNPV) was originally isolated from the spotted cutworm, *X. c-nigrum*, an important insect pest of vegetables and sugar beet in Hokkaido. XcNPV has been considered to be a broad pathogen for lepidopteran species, such as common cutworm, *Spodoptera litura*, the cabbage moth, *Mamestra brassicae*, *Leucania separata* and *L. loreyi*[1,7,11]. Two types of XcNPV have been isolated from diseased spotted cutworm larvae. They produce polyhedra in different shapes, one tetragonal and the other trigonal under microscope. These virus particles were shown by GOTO *et al.*[1], and both viruses are known to infect the spotted cutworm larvae. Two larvae, each infected with one of two different XcNPV's show the same symptoms. Therefore, serological analysis or other methods might be necessary in order to identify the infecting virus. Restriction endonucleases have been used to identify the viruses. VLAK and GRÖNER[12] reported that two NPV's from the cabbage moth, which were isolated in two different areas in Europe, could be distinguished by restriction endonucleases analysis.

In the present study, we compare DNAs from two different XcNPV's by several restriction endonuclease digestion.

Materials and Methods

Virus strains: One strain of XcNPV forms tetragonal polyhedra (Tetra-XcNPV) and the other strain trigonal polyhedra (Tri-XcNPV). The latter

strain has been known since 1977[7] but the former strain was relatively new[1]. Both strains used in this study were isolated by GOTO *et al.*[1] from the diseased larvae of the spotted cutworm in Memuro, Hokkaido.

Isolation of polyhedra: Approximately ten larvae which had succumbed to polyhedrosis were homogenized in water and the homogenate was filtrated through a piece of cheese cloth to remove larval residues. The polyhedra were purified from the filtrate by density gradient centrifugation in NaBr (45 to 65% w/w; 1000 rpm for two hours).

Isolation of viral DNA: DNAs from both NPV were isolated according to SAKAI's procedure[8]. Five mg of dried polyhedra were suspended in 1 ml of SSC (15 mM Sodium citrate −150 mM Sodium chloride). The suspension was preincubated for 30 min at 37°C, mixed with 25 μl of 10% SDS and 100 μl of Actinase E (100 mg/ml of d. w.), and incubated at 37°C for 2 hrs. An additional 10% SDS was added until reaching to 1% final concentration. After 1.2 ml of phenol saturated with SSC was added, the mixture was shaken for 5 min and centrifuged at 10,000 rpm for 3 min. Aqueous layer was washed by 1.2 ml of the saturated phenol twice and the proteins were removed. This aqueous layer was washed with 400 μl of cold ether by centrifugation at 10,000 rpm for 3 min, and then mixed with two times volume of cold EtOH. After overnight incubation at −20°C, the solution was centrifuged at 10,000 rpm for 10 min and the supernatant was discarded with a pipett. The pellet was washed with 400 μl of cold ether two times and dried in decicator. The final pellet was suspended in 200 μl of TE buffer. Viral DNA from *Bombyx mori* NPV (BmNPV) was also isolated and it served as control.

Restriction endonucleases analysis: The viral DNA was digested with restriction endonucleases, Bgl II, Eco RI, Hpa I, Kpn I, Pst I and Xho I. DNA solution (20 μl) was diluted with 6 μl of ×5 low salt buffer (40 mM NaCl, 10 mM MgCl, 20 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, 0.01% bovine albumin) received 2 μl of restriction endonuclease and incubated at 37°C for 6 hrs. During the incubation an additional 2 μl of restriction endonucleases was added at 3 hrs in order to make a complete digestion. The reaction was stopped by addition of 6 μl of 50% glycerol-electrophoresis buffer containing 0.7% bromophenol blue and 0.7% SDS.

Agarose gel electrophoresis: Viral DNA's were separated in horizontal 0.7% agarose gels (Agarose (ME) Nakarai Co. Ltd.) by using the electrophoresis buffer consisting of 178 mM Tris-2.5 mM disodium EDTA-178 mM boric acid, pH 8.0. For analytical gel electrophoresis, a slab gel, 131 × 135 × 50 mm, was used. The samples (30 μl) were applied to the sample slots.

A marker containing λ DNA (Boehringer, Manheim, West Germany) that had been digested with Eco RI and Hind III restriction endonucleases was used as standard to estimate of relative molecular weights. The electrophoresis was carried out at 2 mA for 15 min followed by 40 mA for 5 hrs. The gel was stained in electrophoresis buffer containing of ethidium bromide (0.5

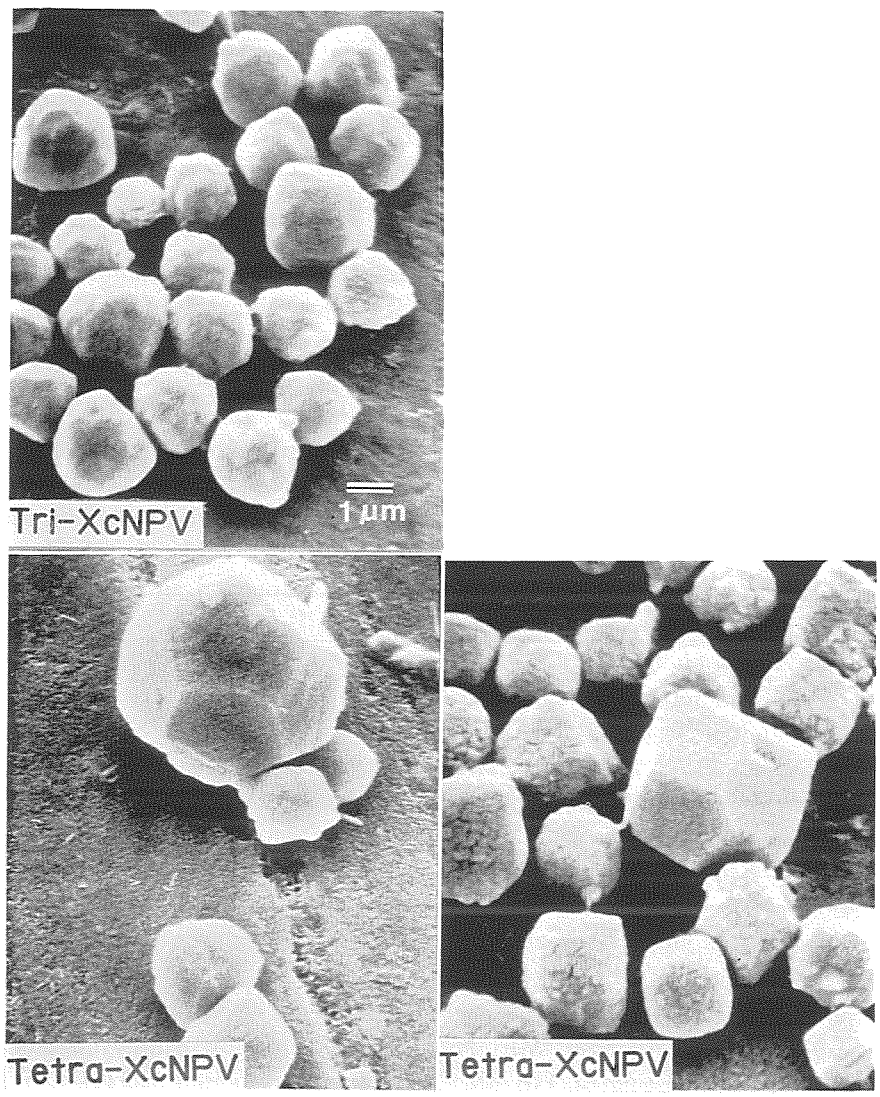

Fig. 1. Scanning electron micrographs showing the shape of polyhedra of Tri-XcNPV and Tetra-XcNPV.

μg/ml) for 30 min and photographed on a short wave transilluminator (type C62, Ultraviolet Products, Inc., San Gabriel, CA) with Polaroid high-speed type No. 55 film and a Kodak No. 23 A red filter.

Results and Discussion

The polyhedra of Tri-XcNPV and Tetra-XcNPV from *X. c-nigrum* had a trigonal and a tetragonal surface respectively (Fig. 1). Those sizes varied from 1.5 to 3 μm in diameter for Tri-XcNPV and from 1.6 to 5 μm for Tetra-XcNPV.

The DNA's of Tri-XcNPV and Tetra-XcNPV (Fig. 2) were exposed to six restriction endonucleases, Bgl II, Eco RI, Hpa I, Kpn I, Pst I and Xho

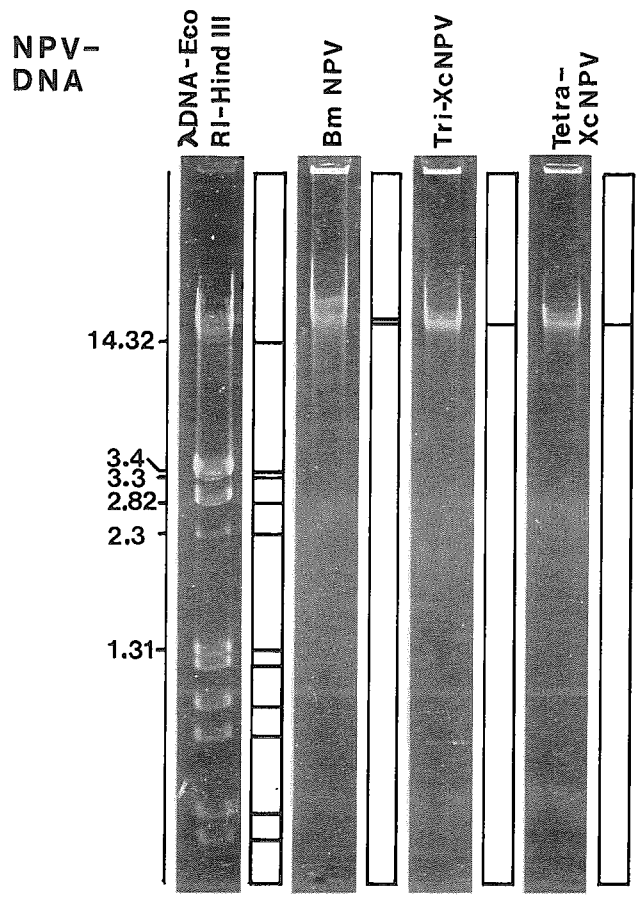

Fig. 2. Isolated viral DNA's of BmNPV, Tri-XcNPV analyzed by agarose gel electrophoresis (0.7%).

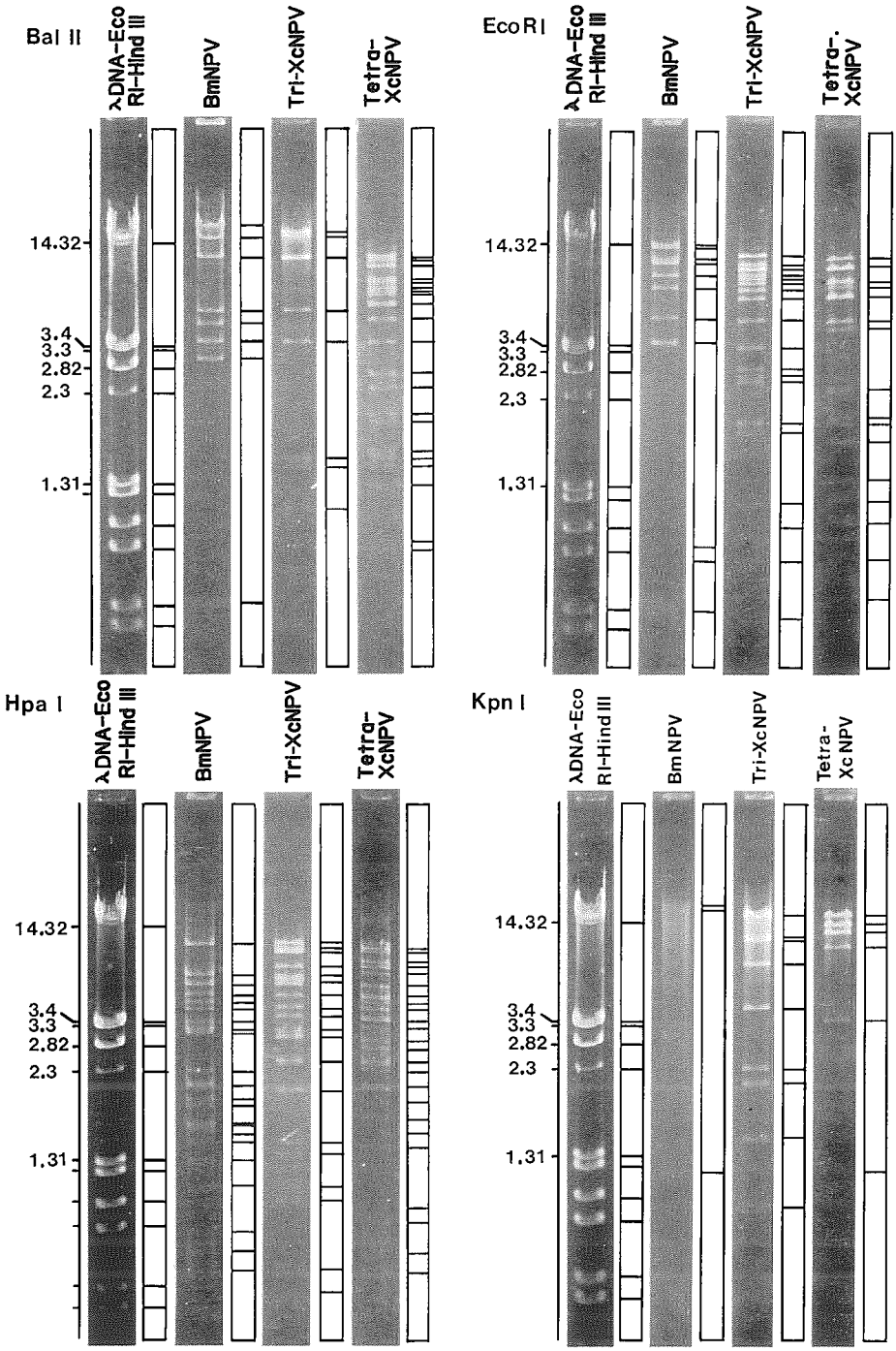

Fig. 3. Restriction endonuclease analysis of viral DNA's using Bal II, Eco RI, Hpa I and Kpn I. The gel electrophoresis was done with 0.7% agarose.

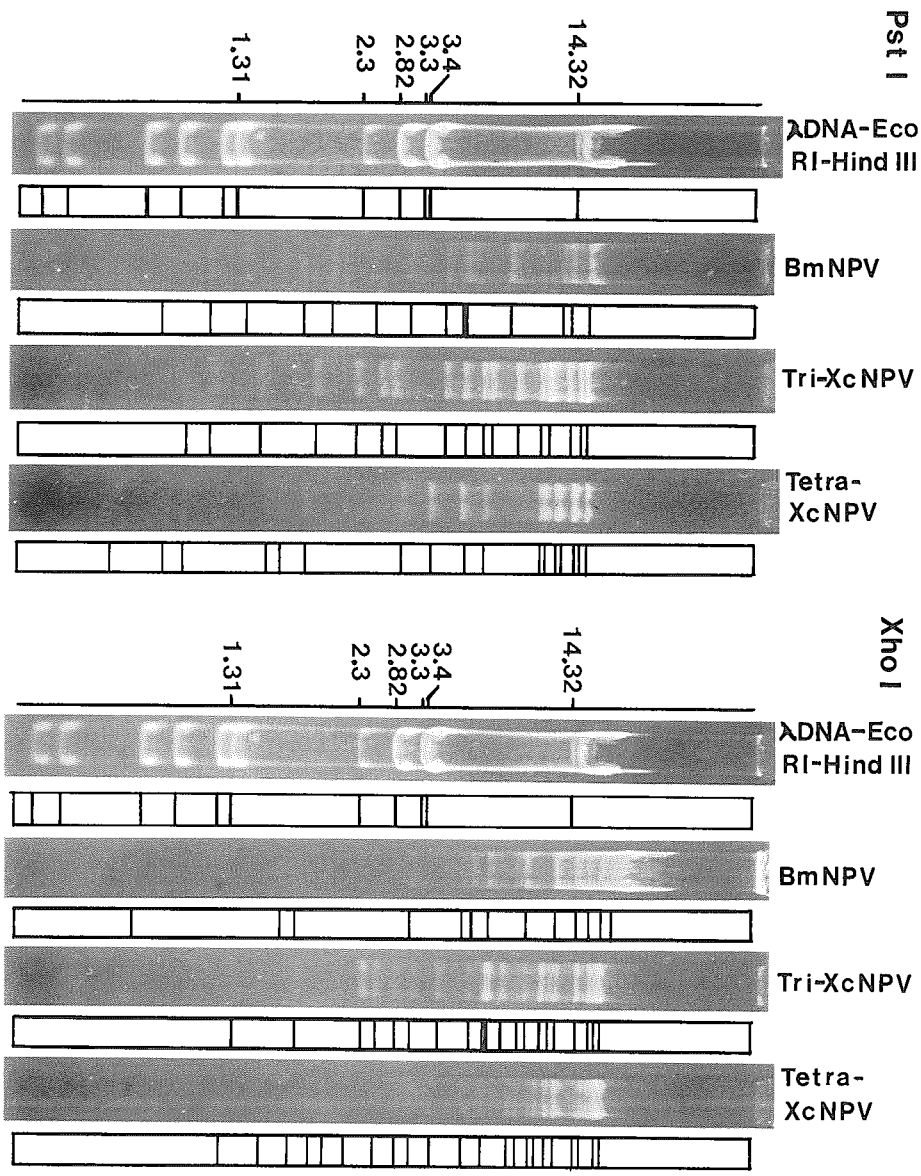

Fig. 4. Restriction endonuclease analysis of viral DNA's using Pst I and Xho I. The gel electrophoresis was done with 0.7% agarose.

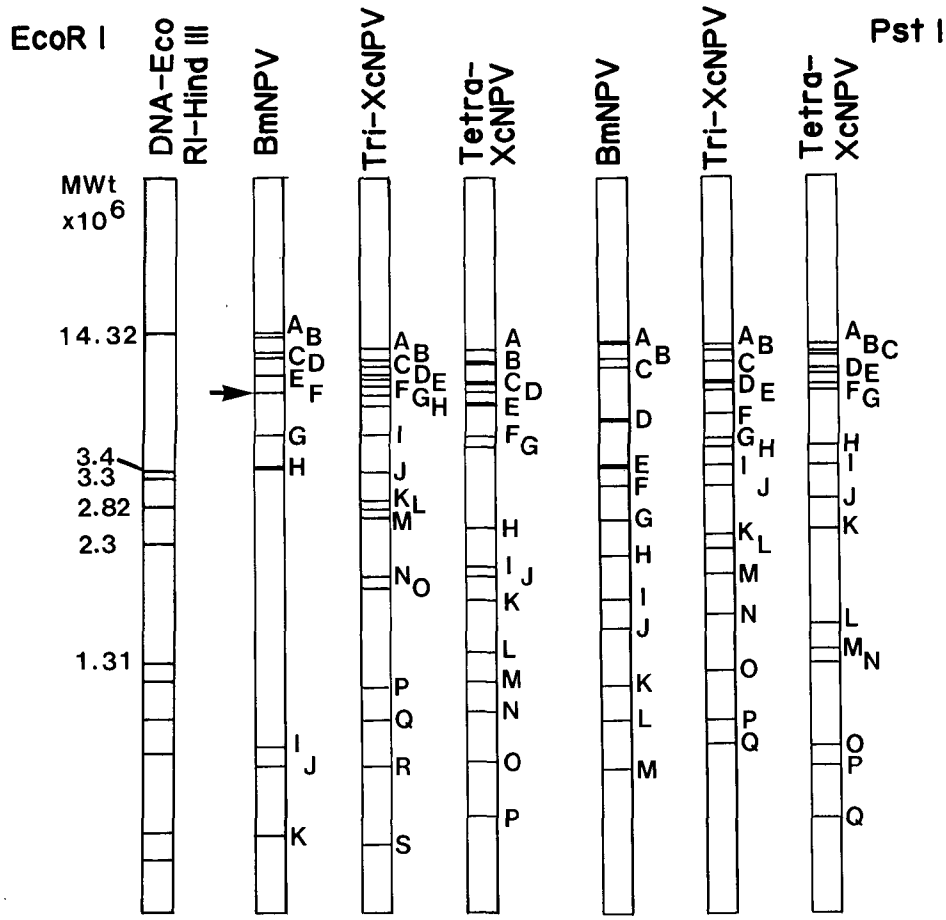

Fig. 5. Schematic diagram of restriction endonuclease produced with EcoRI and Pst I. Each band was identified alphabetically starting with A for the largest fragment. Arrow shows fragment which contains the polyhedrin gene of BmNPV.

I in order to characterize the viral DNAs (Fig. 3, 4). The fragments produced by these enzymes were identified with lines (Fig. 5). Fragments from Eco RI and Pst I digestions were referred alphabetically starting with A for the largest fragment. Molecular weights for these fragments from Eco RI and Pst I digestions, were used to estimate the molecular weight of each undigested NPV-DNA (Table 1, 2). Eco RI-B, C and E bands of Tetra-XcNPV DNA and Eco RI-H band of BmNPV contained two fragments of similar sizes. Pst I-D band of Tri-XcNPV and Pst I-A, D and E bands of BmNPV also contained two fragments. Molecular weights of BmNPV,

TABLE 1. Estimation of molecular weights of viral DNA's from their Eco RI fragments

	Molecular weight (Mdal)		
	BmNPV	Tri-XcNPV	Tetra-XcNPV
A	13.8	10.5	11.0
B	12.6	8.7	9.1*
C	9.5	7.9	7.2*
D	9.1	7.2	6.9
E	7.6	6.9	6.0*
F	6.6	6.6	5.0
G	5.0	6.0	4.6
H	4.0	5.2	2.9
I	4.0*	4.8	2.3
J	0.9	3.8	2.2
K	0.8	3.2	1.9
L	0.6	3.0	1.4
M		2.8	1.3
N		2.1	1.1
O		1.9	0.9
P		1.1	0.7
Q		1.0	
R		0.8	
S		0.6	
Total	74.5	84.1	86.8

*: counted as double fragments.

TABLE 2. Estimation of molecular weights of viral DNA's from their Pst I fragments

	Molecular weight (Mdal)		
	BmNPV	Tri-XcNPV	Tetra-XcNPV
A	12.0*	11.0	12.6
B	8.7	9.5	11.5
C	8.3	8.3	10.5
D	5.2*	6.9*	8.7
E	4.2*	6.6	8.3
F	3.8	5.5	7.9
G	3.0	4.8	7.6
H	2.5	4.6	5.0
I	2.0	4.0	4.2
J	1.7	3.6	3.5
K	1.3	2.6	3.0
L	1.1	2.5	1.7
M	0.9	2.2	1.5
N		1.7	1.4
O		1.3	1.0
P		1.0	0.9
Q		0.9	0.7
Total	76.1	83.9	90.0

*: counted as double fragments.

Tri-XcNPV, and Tetra-XcNPV calculated from Eco RI and Pst I fragments independently were in good agreement. MAEDA *et al.*[5] reported that molecular weight of BmNPV DNA was >100 Kb (>62.5 Mdal), and MAEDA and OBARA[6] reported that it is 120 Kb (75 Mdal).

In our estimation, molecular weight of BmNPV from Eco RI digestion was 74.5 Mdal, and from Pst I digestion was 76.1 Mdal. Therefore 75.3 Mdal was given as a mean molecular weight of BmNPV. Similarly 84 Mdal was given for Tri-XcNPV and 88.4 Mdal for Tetra-XcNPV. SMITH and SUMMERS[9,10] estimated the molecular weights of Baculoviruses DNA's from Eco RI digestions and determined those in the range of from 72.6 to 111.0 Mdal. Our molecular weight estimations for Tri-XcNPV and Tetra-XcNPV were within the range of these molecular weights.

Restriction endonuclease analysis indicated that the two XcNPV's studied here are different in genotype. Each XcNPV DNA contained several unique restriction endonuclease fragments regardless of the enzyme used. MAEDA *et al.*[5] reported that the Eco RI-6.6 Mdal (10.5 Kb) fragment and the Hind III-2.44 Mdal (3.9 Kb) fragment of BmNPV DNA contain the gene coding for polyhedral protein (polyhedrin gene). In our experiment, Eco RI-F band of BmNPV was identified as the Eco RI-6.6 Mdal fragment, but in the case of XcNPV, location of the polyhedrin gene was not clear.

Restriction endonuclease digestion of viral DNA appeares to be a useful tool in identifying nuclear polyhedrosis viruses[9,12]. In our present study, the digestion distinguished one XcNPV from the other. However, further studies are needed in such areas as biological activity and DNA sequencing.

In Hokkaido, new additional baculoviruses were founded by GOTO *et al.*[2]. They are a granulosis virus in *Autographa gamma*, a nuclear polyhedrosis virus in *Colias erate poliographus*, a granulosis virus in *Celaena leucostigma*, a granurosis virus in *Hydraecia amurensis*, a nuclear polyhedrosis virus in *Amphipoea burrowsi*, a granurosis virus in *Mesapamea cocinnata* and a granurosis virus in *Aletia pallens*. These new viruses, especially, granurosis viruses which appeared to have the same polyhedron shape under a scanning electron microscope have to be further characterized by other methods, such as the restriction endonucleases analysis.

Efforts for cloning the XcNPV polyhedrin gene are underway.

Summary

Two nuclear polyhedrosis viruses that had been isolated from the spotted cut-worm *Xestia c-nigrum* from polyhedra in different shape. These two NPV have shown similar biological activities and the same host range. In the present study, the viral DNA's were characterized by restriction endonuclese analysis. The analysis clearly distinguished one virus from the other.

References

1. GOTO, C., H. TSUTSUI, K. HONMA, T. IIZUKA and T. NAKASHIMA: Studies on nuclear polyhedrosis and granurosis viruses of the spotted cutworm, *Xestia c-nigrum* L. (Lepidoptera: Noctuidae) *Jpn. J. Appl. Ent. Zool.* **29**, 102-106, 1985
2. GOTO, C., H. TSUTSUI and T. IIZUKA: Some new virus diseases isolated from lepidopteran insects. *Abstract in App. Ent. Zool. at Sapporo* (In Japanese), 1986
3. ITO, J. and F. KAWAMURA: Use of restriction endonucleases in analyzing the genomes of bacteriophages ϕ29 and ϕ15. In Microbiology-1976, *American Society for Microbiology, Washington, D. C.*, pp. 367-379, 1976

4. KAWARABATA, T.: Nuclear polyhedrosis viruses of insects. *Virus* **34**, 73–88. (In Japanese), 1984
5. MAEDA, S., T. KAWAI, M. OBINATA, H. FUJIWARA, T. HORIUCHI, Y. SATO and M. FURUSAWA: Production of human α-interferon in silkworm using a baculovirus vector. Nature 315, 592–594, 1985
6. MAEDA, S. and R. OBARA: Structure of DNA and variant in the silkworm nuclear polyhedrosis virus. *Abstract in J. Seric. Soc. Jpn.* **81**, 1986
7. OKADA, M.: Studies on the utilization and mass production of *Sporoptera litura* nuclear polyhedrosis virus for control of the tobacco cutworm (*Sporoptera litura* Fabricius). *Bull. Chugoku Agr. Exp. Stn.*, Ser. **E12**, 1–66, 1977
8. SAKAI, F.: "Manual of plant gene technology". Published by Kodansha, Tokyo 15–21. (In Japanese), 1984
9. SMITH, G. E. and M. D. SUMMERS: Analysis of baculovirus genomes with restriction endonucleases. *Virology* **89**, 517–527, 1978
10. SMITH, G. E. and M. D. SUMMERS: Restriction maps of five *Autographa californica* MNPV variants, *Trichoplusia ni* MNPV, and *Galleria mellonella* MNPV DNAs with endonucleases Sma I, Kpn I, Bam HI, Sac I, Xho I and Eco RI. J. *Virology* **30**, 828–838, 1979
11. TSUDA, K., E. MIZUKI, T. KAWARABATA and K. AIZAWA: Replication of *Xestia c-nigrum* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus in continuous cell culture. *App. Ent. Zool.* **19**, 293–298, 1984
12. VLAK, J. M. and A. GRÖNER: Identification of two nuclear polyhedrosis virus from the cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae). *J. Invertebr. Pathol.* **35**, 269–278, 1980