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Characterization of the Nuclear Polyhedrosis Virus DNA of *Adoxophyes orana* and of *Barathra brassicae*

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Circular double-stranded DNA was isolated from nuclear polyhedrosis virus (NPV) of *Adoxophyes orana* (virus particles singly embedded in the polyhedral matrix) and NPV of *Barathra brassicae* (virus particles multiply embedded in the polyhedral matrix), and some of their physical properties were determined. The molecular weights of *A. orana* and *B. brassicae* NPV-DNA, 6.7×10^7 and 8.9×10^7, respectively, were determined by electron microscopy and by renaturation kinetics analysis. The latter analysis also showed that both genomes do not contain repetitive sequences. Absence of homology between DNA of these two viruses was shown by competition hybridization of *A. orana* NPV-DNA with *B. brassicae* NPV-DNA. Analysis of these DNAs with the restriction endonuclease *Eco*RI confirmed that they are different. The buoyant densities in CsCl of *A. orana* NPV-DNA and of *B. brassicae* NPV-DNA, 1.694 and 1.696 g/cm^3, respectively, are consistent with (G + C) contents of 34.5 and 37%, respectively, as determined by thermal denaturation.

INTRODUCTION

Since the need to develop alternatives to chemical insecticides is urgent, the potential use of nuclear polyhedrosis viruses as entomopathogenic biocontrol agents for Lepidoptera has aroused much interest (Summers *et al.*, 1975). Insect viruses, although they seem to be logical substitutes for chemical agents, require fundamental and applied studies, to remove doubts about their utilization. The use of insect viruses as biological agents for insect control and possible hazards have been discussed by the World Health Organization (1973). There are few data available on the genetic material of these biological agents (Davies, 1975). Biochemical and biophysical properties of insect viruses can provide much information, but caution is required since the viruses of some invertebrates can be cross-infective (Vail *et al.*, 1973; Beavers and Reed, 1972; Webb *et al.*, 1974; Stairs and Lynn, 1974). Therefore, insect viruses have to be identified and compared mutually as well as with viruses of vertebrates before they can be used for biological control. In our laboratory we are studying the suitability of the nuclear polyhedra of *Adoxophyes orana* and *Barathra brassicae* for biological control in the field. This article describes part of this work: the characterization and comparison of the genetic material of these two nuclear polyhedrosis viruses.

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MATERIALS AND METHODS

Viruses. The sample of the *A. orana* NPV was obtained from Dr. M. B. Ponsen of our laboratory. The *B. brassicae* NPV was obtained from Dr. L. P. S. van der Geest, Laboratory of Entomology, University of Amsterdam. Virus was further produced by inoculation of homologous insect hosts with their own nuclear polyhedrosis virus.

Marker DNAs. DNA of bacteriophage

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PM2 was provided by Dr. C. Walig, Laboratory for Hygiene, Amsterdam. DNA of adenovirus type 5 was obtained from the Laboratory of Physiological Chemistry, Utrecht.

Purification of polyhedra. Polyhedra were purified by differential centrifugation according to the procedure of Van der Geest (1968). Filtered homogenates of NPV-diseased larvae were centrifuged at 3000 *g* for 30 min at 4°. The sedimented polyhedra were suspended in distilled water and a 10-ml suspension (about 10 mg of polyhedra/ml) was layered on a 20-ml sucrose solution (61.7%, w/w) in SW-25 rotor tubes. After centrifugation at 8000 *g* for 30 min at 4°, the polyhedra accumulated at the water–sugar interface. The polyhedra were collected and resuspended in distilled water, and the suspension was placed on top of a sucrose solution (43%, w/w). Polyhedra were sedimented by centrifuging at 8000 *g* for 30 min at 4°. The polyhedra were washed free of sucrose by two cycles of centrifugation at 5000 *g* for 30 min at 4°. The purified polyhedral suspension was divided into two parts. One part was stored in distilled water at 4° and used for purification of DNA for electron microscopy. The second part of polyhedral suspension was lyophylised and used for purification of DNA for thermal denaturation.

Purification of virus. To purify the virus, the method of Summers and Paschke (1970) was modified. The virus particles were extracted from highly purified polyhedra of *A. orana* by dissolving them in 0.03 *M* Na_2CO_3, pH 10.55, for 30 min and those of *B. brassicae* in 0.015 *M* Na_2CO_3, pH 10.3, for 15 min at 4°. The virus particles were separated from the polyhedral proteins by subsequent centrifugation in a 10 to 40% sucrose gradient. Centrifuging at 23,000 rpm in a SW-25 rotor for 30 min at 4° sedimented the virus particles in a band, and the polyhedral proteins stayed in the fraction on the top of the gradient. The virus particles were collected with a drop collection unit, Model 195 obtained from ISCO.

Purification of nuclear polyhedrosis virus DNA. A modification of the methods of Gafford and Randall (1967) and Marmur (1961) was used. Virus was suspended in 8 vol of 0.015 *M* sodium citrate and 0.15 *M* NaCl (1× SSC), pH 7.4, plus 10 m*M* EDTA and 2 vol of 10% sodium dodecyl sulphate (SDS), after which the suspension was heated in a water bath at 60° for 30 min. Sufficient 5 *M* NaCl was added to give a final concentration of 1 *M*. The suspension was stored at 4°. After approximately 16 hr, the suspension was centrifuged at 15,000 *g* in a Serval RC-2 centrifuge to remove the precipitate. The supernatant was layered onto a solution of CsCl (initial density, 1.7 g/ml) in SW-50 rotor tubes and centrifuged in a Beckman LB 5-65 preparative ultracentrifuge at 30,000 rpm at 25°. After 24 to 36 hr of centrifugation the tubes were fractionated with a drop collection unit, Model 195 obtained from ISCO. The DNA fraction was dialysed against 0.2 *M* Tris–HCl buffer plus 3 m*M* EDTA, pH 8.5.

To increase the quantity of supercoiled DNA, the method described above was shortened by omitting the addition of NaCl and isopycnic centrifugation in CsCl.

Caesium chloride equilibrium centrifugation. The buoyant density measurements were made in a Beckman analytical ultracentrifuge according to Mandel *et al.* (1968). Ten microliters of reference *Escherichia coli* B-DNA or bacteriophage T4 DNA (40 μg/ml) and 40 μl (20 μg/ml) of DNA of a density to be determined were added to 840 μl of a stock solution (13 g of CsCl in 7 ml of 0.02 *M* Tris–HCl buffer, pH 8.5). The refractive index of the solution was adjusted with about 200 μl of Tris–HCl buffer to a value of 1.3996. Centrifugation was at 44,770 rpm for 20 hr at 25°.

Thermal denaturation. Thermal denaturation curves (T_m) were made in 1× SSC, pH 7.0, according to Mandel and Marmur (1968) in a Gilford Model 2400 spectrophotometer. The DNA samples at a final concentration of 30 μg/ml were dialysed against 1× SCC, at 4°, with three changes of 16 to 18 hr each. The DNA preparations did not contain covalently closed DNA because they were prepared from lyophylised virus (Summers and Anderson, 1972b) and sheared

twice by vortexing for 15 sec. During melting, the temperature was raised by 0.1°/min and the absorbance was recorded every minute. The base composition was calculated from T_m values according to Marmur and Doty (1961).

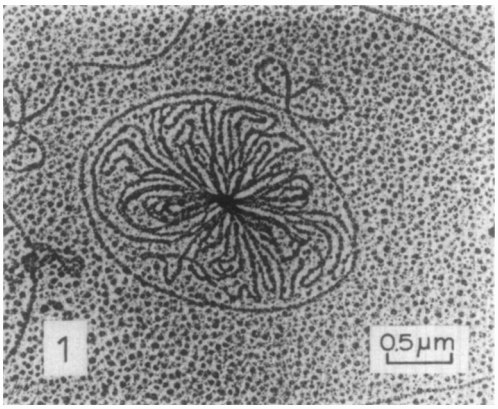
Preparation of DNA samples for electron microscopy. A modification of the Kleinschmidt technique (Kleinschmidt, 1968; Davis *et al.*, 1971) was used. The spreading solution contained 25 μl of 99% distilled formamide, 10 μl of water, 10 μl of DNA (25 μg/ml), 5 μl of 1 *M* Tris–HCl buffer plus 0.1 *M* EDTA, pH 8.5, and 2.5 μl of cytochrome *c* (1 mg/ml in 0.02 *M* Tris–HCl buffer plus 0.002 *M* EDTA, pH 8.5). The mixture was spread on a hypophase containing 20% formamide. A carbon-coated grid was used to collect a small drop from the hypophase surface. The grid was stained with 50 m*M* uranyl acetate in 90% ethanol for 30 sec, dehydrated in 2-methylbutane for 10 sec, and air-dried. The grids were rotary shadowed at an angle of 5 to 7° with platinum–iridium (90–10%).

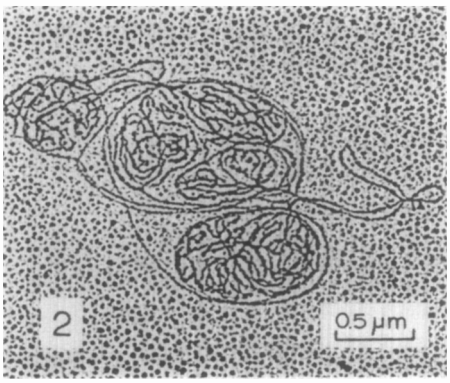
Electron microscopy of DNA. Electron micrographs were obtained with a Siemens Elmiskop 101 electron microscope, at a magnification of 8000 to 16,000 (80 kV, at 50-μm objective aperture). Magnification was calibrated with a carbon replica of a diffraction grating (2160 lines per mm). The plates were optically enlarged four times by projection. The images were traced on paper and measured by a Hewlett–Packard Model 10 calculator.

Labelling of DNA for reassociation kinetics. For labelling of DNA, the nick-translation technique described by Rigby *et al.* (1977) was modified. Isolated DNA was labeled to a high specific radioactivity with DNA polymerase I, after introduction of single-stranded nicks with DNase I. Reactions were carried out at 13.5° for 90 min in 50-μl volumes containing 200 pmol of [^{32}P]dATP, 200 pmol of [^{32}P]dCTP. The specific activity of the ^{32}P substrates was 350 Ci/mmol. After evaporation, the following substances were added: 2 μl of 0.05 m*M* dGTP, 2 μl of 0.25 m*M* dTTP, 5 μl of 10× nick buffer (500 m*M* Tris–HCl buffer, 50 m*M* MgCl, 100 m*M* β-mercaptoethanol, 500 μg/ml bovine serum albumin), 2 μl of 0.5 *M* NaCl, 25 μl of *A. orana* or *B. brassicae* NPV-DNA at a concentration of 20 μg/ml, 8 μl of water, 5 μl of DNase, 10^{-8} g/ml, and 1 μl of DNA polymerase I. After nick–translation the reaction mixture was extracted once with phenol and once with a mixture of chloroform–isoamyl alcohol (24:1). After these extractions, the mixture was passed over a Sephadex G-50 column to separate the DNA from the remaining substrates.

Reassociation kinetics. Reassociation was based on the method of Sharp *et al.* (1974). Before annealing, the labelled and unlabelled DNAs were mixed and degraded to nucleotide segments 200 to 300 bases in length by boiling in 0.3 *N* NaOH for 20 min. The solutions (500 μl) were immediately immersed in ice. After cooling, the solution was adjusted to pH 6.8 with 140 μl of ice-cold phosphate buffer (1 *M*), 40 μl of SDS (10%), 140 μl of NaCl (5 *M*) 158.4 μl of water, and 21.6 μl of HCl (6 *N*). DNA was renatured by heating fragmented DNA in a closed ampule to 68°. Renaturation was stopped by dilution of the samples with 1 ml of ice-cold 0.14 *M* sodium phosphate buffer, pH 6.8. The extent of renaturation was determined on hydroxylapatite columns at 60°. Single-stranded DNA was eluted with 0.14 *M* sodium phosphate buffer and double-stranded DNA with 0.4 *M* sodium phosphate buffer.

DNA analysis by restriction endonuclease Eco*RI.* Restriction enzyme analysis with *Eco*RI was performed according to Sussenbach and Kuijk (1977). DNA was digested in 0.09 *M* Tris–HCl buffer plus 0.01 *M* $MgCl_2$ (pH 7.9). The reaction was stopped by addition of sodium acetate to 0.2 *M* and 1 vol of chloroform–isoamyl alcohol (24:1). After extraction and low-speed centrifugation, the aqueous phase was mixed with 3 vol of ethanol and DNA was precipitated by centrifugation. The precipitated DNA was dissolved in 20 m*M* Tris–HCl buffer, 1 m*M* EDTA (pH 7.5), and analysed by electrophoresis in 1.4% agarose gels. Agarose (Bio-Rad, Richmond, Calif.) was dissolved in E buffer containing 0.04 *M* Tris–HCl buffer, 0.05 *M* sodium acetate, 0.001 *M* EDTA (pH 7.8), and 0.5 μg/ml ethium bromide. Electro-





FIGS. 1 AND 2. The *A. orana* (1) and the *B. brassicae* NPV-DNA (2) in the supercoiled form with some additional foldings. Virus was lysed with 2% SDS at 60° for 30 min. Released DNA was dialysed against 0.02 *M* Tris–HCl buffer plus 0.03 *M* EDTA (pH 8.5) and then spread on water hypophase with 20% formamide.

phoresis was at room temperature at 2 to 3 V/cm.

RESULTS

Electron Microscopy of NPV–DNAs

When DNA obtained by lysis of virus with SDS was dialysed against 0.02 *M* Tris–HCl buffer plus 0.03 *M* EDTA (pH 8.5) and then analysed by electron microscopy, it was observed that about 65% of the DNA molecules had the typical structures of supercoiled forms with some additional foldings (Figs. 1 and 2). When the DNA was purified by modification of the method of Gafford and Randall (1967) and analysed by electron microscopy, these typical forms were not found. Instead three different forms of DNA molecules were seen in the following proportions: about 40% highly twisted circles, about 50% relaxed circles, and in most preparations less than 10% linear molecules of various length. Figures 3 and 4 show different forms of the *A. orana* NPV-DNA. The same forms were also observed for the *B. brassicae* NPV-DNA.

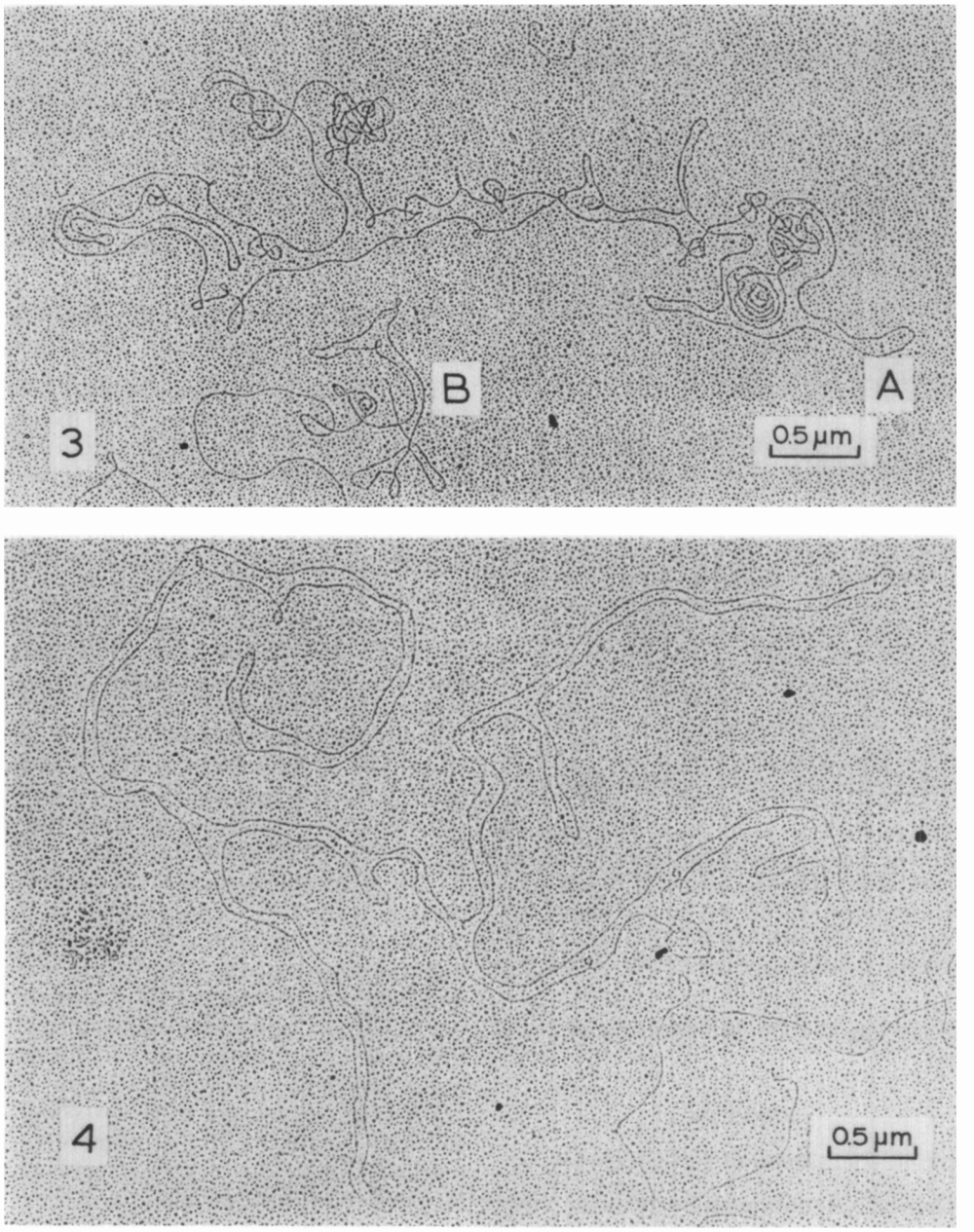
Determination of Molecular Weights of NPV-DNA by Electron Microscopy

The Kleinschmidt technique was used in this study to determine the molecular weights of NPV-DNA of *A. orana* and of *B. brassicae*. To obtain reproducible results, only the relaxed circles on the same grid with marker DNA were used for length determinations. The distribution of size classes of the *A. orana* NPV-DNA in Fig. 5 shows a peak at 34 µm and a small peak at about 130 µm. The largest molecule observed was almost five times larger than the fundamental unit. The distribution of size classes of the *B. brassicae* NPV-DNA circles in Fig. 6 shows a peak at 45 µm and peaks at 130 and 180 µm. The largest molecule observed was four times the fundamental unit.

The molecular weights (M) were calculated from the formula used by Lang (1970), $M = M'L$, where M' means daltons per micrometer and L is the length of DNA molecule in micrometers. M' calculated in our study by calibration of bacteriophage PM2 was 1.98×10^6 per µm. With this formula, the molecular weights of the NPV-DNA of *A. orana* and of *B. brassicae* are 6.7×10^7 and 8.9×10^7, respectively. The marker DNA was cospread and photographed with NPV-DNAs.

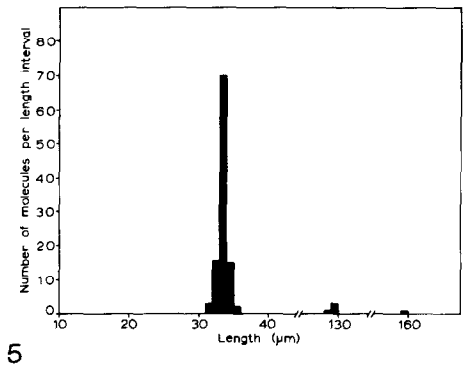
Reassociation Kinetics Analysis of the NPV-DNA of A. orana and of B. brassicae

To check the molecular weights established by electron microscopy, these were also determined by reassociation kinetics analysis. Denatured DNA from NPV of *A. orana* and of *B. brassicae* was reannealed



FIGS. 3 AND 4. Different forms of the NPV-DNA of *A. orana*. A supercoiled DNA molecule (A). A linear molecule with a free end (B) and a relaxed circular DNA molecule (C). DNA was purified by modification of the method of Gafford and Randal (1967) and spread on water hypophase with 20% formamide.

at known DNA concentrations, and the rate of reassociation was determined employing hydroxylapatite chromatography. Adenovirus type 5 DNA [23×10^6 daltons (Philipson and Lindberg, 1974)] was used as a reference DNA. Reassociation analysis was performed as described under Materials and Methods. As shown in Table 1 and Fig. 7, DNA of both viruses reassociates with kinetics approaching ideal second-order kinetics.



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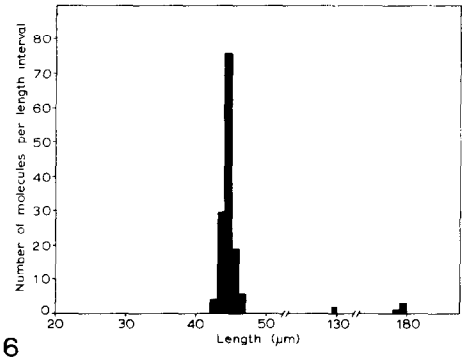

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FIGS 5 AND 6. Histograms of the length distribution of the NPV-DNA of *A. orana* and of *B. brassicae*. A total number of molecules, 112 and 139, of the *A. orana* and of the *B. brassicae* NPV-DNA, respectively, were examined. DNA of bacteriophage PM2 was used as an internal standard. Length intervals are 1 μm.

In neither preparation was a rapidly reannealing fraction detected. Thus, the genomes do not contain repetitive sequences. The genome sizes (Table 2) were derived from the linear relationship between the $C0t_{1/2}$ values of NPV-DNA and the $C0t_{1/2}$ values of adenovirus type 5 DNA which was used as marker (Sharp *et al.*, 1974). It appears that *A. orana* NPV-DNA is about 2.9 times more complex than adenovirus type 5 DNA and that the *B. brassicae* NPV-DNA is about 3.75 times more complex. Since adenovirus type 5 DNA has a molecular weight of 23 $\times 10^6$, it can be calculated that the molecular weight of *A. orana* NPV-DNA is 6.6 $\times 10^7$ and that the molecular weight of *B. brassicae* NPV-DNA is 8.6 $\times 10^7$. The molecular weights obtained by reassociation kinetics show a very good agreement with those obtained by electron microscopy. The results also indicate that the genomes contain only unique sequences.

Buoyant Density Analysis

The buoyant density of the NPV-DNA of *A. orana* and of *B. brassicae* was determined by ultracentrifugation in CsCl. As a density marker DNA, DNA of *E. coli* [$\rho = 1.710$ g/ml (Szybalski, 1968)] and DNA of bacteriophage T4 ($\rho = 1.694$ g/ml) was used (Fig. 8). The relative buoyant density of *A. orana* NPV-DNA (1.694 g/ml) and of *B. brassicae* NPV-DNA (1.696 g/ml) was calculated by the equation of Mandel *et al.* (1968). The corresponding (G + C) contests of *A. orana* NPV-DNA, 34%, and of *B. brassicae* NPV-DNA, 36.7%, were obtained from the linear relation of Schildkraut *et al.* (1962).

Thermal Denaturation Profiles

Melting profiles were made for the NPV-DNA of *A. orana* and of *B. brassicae* with DNA of T4 bacteriophage as the standard [(G + C) content, 34% (Wyatt and Cohen, 1953)]. Figure 9 shows that the approximate melting point (T_m) for the NPV-DNA of *A. orana* and of *B. brassicae* is 83.4 and 84.4°, respectively, and for the DNA of T4 bacteriophage is 83.2°. The T_m values correspond to a (G + C) content of 34.5 for *A. orana* NPV-DNA and 37% for *B. brassicae* NPV-DNA when calculated from the formula of Mandel and Marmur (1968). The values are in good agreement with those obtained from buoyant density analysis. The hyperchromicity of NPV-DNAs was about 37 to 39%.

Sequence Homology between A. orana and B. brassicae NPV-DNA

The extent of sequence homology between the DNA of these two viruses was determined by reannealing of ^{32}P-labelled *A. orana* NPV-DNA fragments in the presence of *B. brassicae* unlabelled NPV-DNA fragments (0.9, 1.8, or 2.7 μg/ml, respectively). As a positive control, ^{32}P-labelled

TABLE 1

REASSOCIATION KINETICS OF NPV-DNA OF *A. ORANA* AND OF *B. BRASSICAE*, RESPECTIVELY, AND ADENOVIRUS TYPE 5 DNA[a]

Time of sampling (hr)	^{32}P-labelled *A. orana* NPV-DNA and homologous unlabelled fragments		^{32}P-labelled *B. brassicae* NPV-DNA and homologous unlabelled fragments		^{32}P-labelled adenovirus 5-DNA and homologous unlabelled fragments	
	cpm in fss[b]	1/fss	cpm in fss	1/fss	cpm in fss	1/fss
0.0	1400	1.000	2200	1.000	1950	1.000
0.1	1388	1.008	2184	1.007	1852	1.052
0.2	1370	1.021	2164	1.016	1774	1.110
0.3	1355	1.037	2151	1.022	1696	1.150
0.4	1330	1.050	2112	1.040	1593	1.200
0.5	1302	1.070	2101	1.045	1528	1.275
1.0	1172	1.195	2000	1.100	1316	1.480
1.5	1102	1.270	1833	1.200	1146	1.700
2.0	1077	1.300	1760	1.250	1010	1.926
2.5	—	—	—	—	907	2.150
3.0	952	1.470	1599	1.375	—	—
6.0	721	1.941	1256	1.750	—	—
8.0	622	2.252	1102	1.996	—	—

[a] The reassociation mixture of *A. orana* NPV-DNA contained 1.9×10^{-3} μg of ^{32}P-labelled and 0.6 μg of unlabelled fragments. The mixture of *B. brassicae* contained 2.0×10^{-3} μg of ^{32}P-labelled and 0.6 μg of unlabelled fragments and the mixture of adenovirus type 5 DNA (marker DNA) consisted of 1.5×10^{-3} μg of ^{32}P-labelled and 0.6 μg unlabelled fragments. All reaction mixtures had a volume of 1 ml. For the analysis of the *A. orana* and of the *B. brassicae* two samples of 1 ml were used.

[b] cpm = counts per min; 1/fss = 1/single-stranded fraction.

A. orana NPV-DNA was reannealed with unlabelled homologous fragments (0.9, 1.8, or 2.7 μg/ml, respectively) while as negative control a mixture of ^{32}P-labelled *A. orana* NPV-DNA and unlabelled *E. coli* DNA fragments (0.9, 1.8, or 2.7 μg/ml, respectively) was used. The value of $C0t_{1/2}$ of *A. orana* NPV-DNA, which reannealed in the presence of *E. coli* DNA fragments, was the same as that of *A. orana* NPV-DNA, which reannealed in the presence of *B. brassicae* NPV-DNA (3.8×10^{-2}). The rate of reassociation of *A. orana* NPV-DNA, which reannealed in the presence of *B. brassicae* NPV-DNA, did not increase when the concentration of *B. brassicae* NPV-DNA fragments was increased. The data are given in Table 3 and Fig. 10. There is no sequence homology between *A. orana* and *B. brassicae* NPV-DNA.

Comparison of the A. orana *and of the* B. brassicae *NPV-DNAs by Cleavage with Restriction Endonuclease* EcoRI *Enzyme*

In addition to the comparison of the two DNAs by competition hybridization the *A. orana* and the *B. brassicae* NPV-DNAs were also digested by *Eco*RI enzyme. As shown in Fig. 11, the NPV-DNA of *A. orana* and of *B. brassicae* give completely different patterns when the digestion products were fractionated on 1.4% agarose gels. Both DNAs are cleaved into at least 13 fragments, but no fragments of corresponding electrophoretic mobility are observed.

DISCUSSION

In the past, the NPV of *A. orana* and of *B. brassicae* were identified by the shape

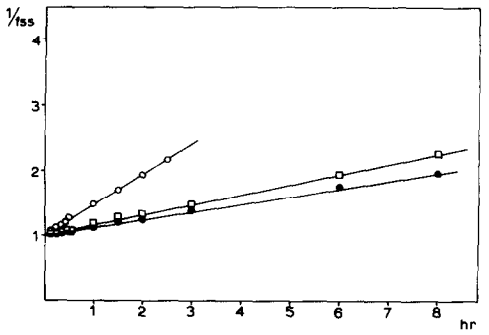

FIG. 7. Reassociation of the *A. orana* and of the *B. brassicae* NPV-DNA, respectively. Reassociation of fragments of *A. orana* (□ —— □). The reassociation mixture contained 1.9×10^{-3} μg (1400 cpm) of ^{32}P-labelled *A. orana* NPV-DNA and 0.6 μg of unlabelled homologous fragments. Reassociation of *B. brassicae* NPV-DNA (● —— ●). The reassociation mixture contained 2.0×10^{-3} μg (2200 cpm) of ^{32}P-labelled NPV-DNA of *B. brassicae* and 0.6 μg of unlabelled homologous fragments. Reassociation of adenovirus type 5 DNA (marker DNA) (○ —— ○). The mixture contained 1.5×10^{-3} μg (1950 cpm) of ^{32}P-labelled adenovirus type 5 DNA and 0.6 μg of unlabelled homologous fragments. All samples had a volume of 1 ml. The single-stranded fraction (fss) was determined by hydroxylapatite chromatography.

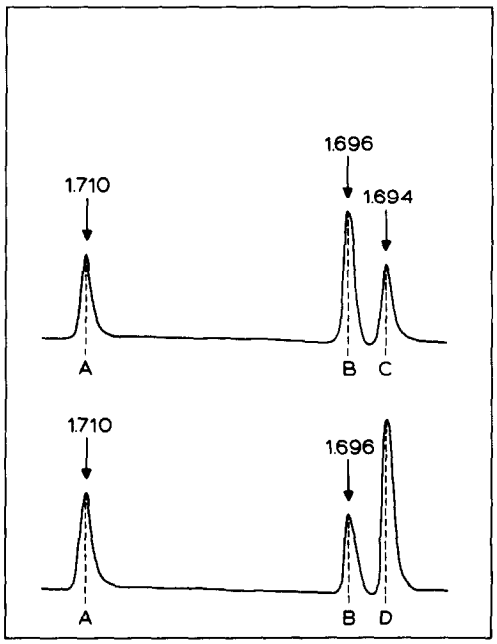

FIG. 8. Analytical buoyant-density analysis of the *B. brassicae* NPV-DNA (B) [the *E. coli* DNA (A) and the bacteriophage T4 DNA (C) were used as markers (top)]; and the *A. orana* NPV-DNA (D) [the *E. coli* DNA (A) and the *B. brassicae* NPV-DNA were used as markers (bottom)].

of the polyhedral bodies and by the morphology of their viruses (Ponsen *et al.*, 1965; Ponsen and de Jong, 1964; Ponsen and Bruinvis, 1963). However, the differences in shape and morphology do not seem to be uniquely associated with a particular biological characteristic of different NPVs (Shigematsu and Suzuki, 1971). To elucidate further the relationship between these viruses, we determined some physical properties of their DNAs.

TABLE 2

THE MOLECULAR WEIGHTS OF *A. ORANA* AND OF *B. BRASSICAE* NPV-DNA

DNA	$C_0t_{1/2}$	Equivalent molecular weight[a]
A. orana NPV	$3.8 \times 10^{-2} \pm 0.0020$	6.6×10^7
B. brassicae NPV	$4.9 \times 10^{-2} \pm 0.0022$	8.6×10^7
Adenovirus type 5	$1.3 \times 10^{-2} \pm 0.0009$	2.3×10^7

[a] The molecular weights of *A. orana* and *B. brassicae* NPV-DNA were calculated assuming a linear relationship between the $C_0t_{1/2}$ values and genome sizes. The molecular weight of adenovirus 5 DNA was taken as a standard [2.3×10^7 (Philipson and Lindberg, 1974)]. Standard deviations of *A. orana* and *B. brassicae* NPV-DNA are calculated from six determinations and of adenovirus type 5 DNA from three determinations.

As shown by electron microscopy and thermal denaturation, nucleic acid material isolated from NPV of *A. orana* and of *B. brassicae* larvae (resistent to ribonuclease A—type XI) consists of circular double-stranded DNA. Electron microscopy of DNA released from nucleocapsids further revealed that the NPV-DNAs were obtained in highly supertwisted relaxed circular and double-stranded linear structures. The observations of different forms of NPV-DNAs agree with the results of caesium chloride analysis as reported by Summers and Anderson (1973) and Harrap *et al.* (1977) for *Spodoptera* sp. NPV-DNAs and by Summers and Anderson (1972b) for granulosis

virus of *Trichoplusia ni* and *Spodoptera frugiperda*. They are also consistent with electron microscopic observations of Bud and Kelly (1977) for *Spodoptera* sp. NPV-DNAs and of Brown *et al.* (1977) for granulosis virus of *Pieris brassicae*.

From experience gained during this work and according to Bud and Kelly (1977), we think that the environmental conditions of the DNA outside the virus particle induce either supercoiling or relaxation of the DNA molecule. On the other hand, the observation of supercoiled molecules in DNA preparations obtained by the shortened purification method suggests that the molecules may be packed in the virus in the supercoiled form with additional foldings. The formation of a supercoil is probably the first step in fitting the large DNA molecules into the small nucleocapsid.

To distinguish the DNAs from the NPV of *A. orana* and of *B. brassicae*, their lengths were studied by electron microscopy. These two DNAs differ in length but they are both large molecules similar to those observed for granulosis or other NPV-DNAs (Kok *et al.*, 1972; Burgess,

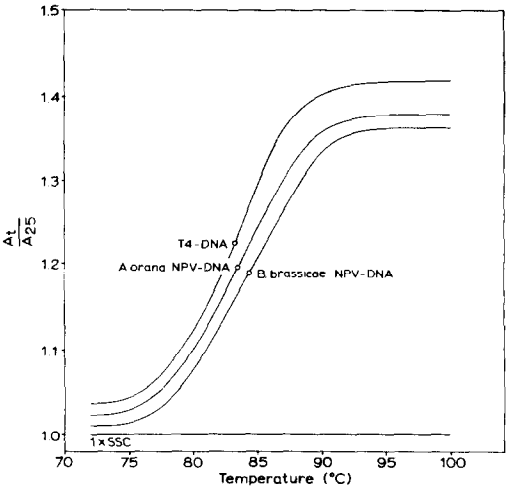

FIG. 9. Thermal melting profiles (T_m) of the nuclear polyhedrosis DNA of *A. orana* and of *B. brassicae*. Measurements were made in 1× SSC in a Gilford Model 2400 recording spectrophotometer. T_m values were calculated from the formula of Mandel and Marmur (1968), using T4 bacteriophage DNA [34% (G + C) content (Wyatt and Cohen, 1953)] as the standard. The DNA preparations did not contain covalently closed DNA; they were sheared by vortexing two times for 15 sec. The experiment was repeated four times.

TABLE 3

REASSOCIATION KINETICS OF THE *A. ORANA* NPV-DNA IN THE PRESENCE OF *B. BRASSICAE* NPV-DNA[a]

Time of sampling (hr)	^{32}P-labelled *A. orana* NPV-DNA and unlabelled *B. brassicae* NPV-DNA		^{32}P-labelled *A. orana* NPV-DNA and unlabelled *A. orana* NPV-DNA		^{32}P-labelled *A. orana* NPV-DNA and unlabelled *E. coli* DNA	
	cpm in fss[b]	1/fss	cpm in fss	1/fss	cpm in fss	1/fss
0	1529	1.000	1507	1.000	1537	1.000
1	1525	1.000	1200	1.250	1536	1.000
2	1527	1.000	1017	1.474	1536	1.000
3	1518	1.001	856	1.751	1528	1.001
4	1518	1.001	769	1.950	1526	1.002
6	1515	1.002	624	2.403	1526	1.002
8	1516	1.002	—	—	1515	1.010
54	1497	1.015	—	—	1508	1.014
78	1494	1.017	—	—	1505	1.016

[a] The competition reaction mixture contained 2.06×10^{-3} μg of ^{32}P-labelled *A. orana* NPV-DNA and 0.9 μg of unlabelled *B. brassicae* NPV-DNA. The positive control contained 2.03×10^{-3} μg of ^{32}P-labelled *A. orana* NPV-DNA and 0.54 μg of unlabelled homologous fragments. The negative control contained 2.07×10^{-3} μg of ^{32}P-labelled *A. orana* NPV-DNA and 0.9 μg of unlabelled *E. coli* DNA fragments.

[b] cpm = counts per min; 1/fss = 1/single-stranded fraction.

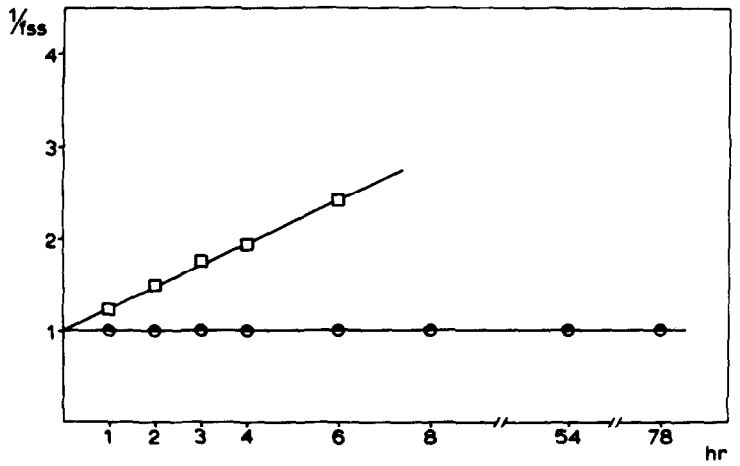

FIG. 10. Reassociation of *A. orana* NPV-DNA in the presence of *B. brassicae* NPV-DNA (● —— ●). The reaction mixture contained 2.06×10^{-3} μg (1529 cpm) of ^{32}P-labelled *A. orana* NPV-DNA and 0.9 μg of *B. brassicae* unlabelled NPV-DNA. Positive control (□ —— □). The reaction mixture contained 2.03×10^{-3} μg (1507 cpm) of ^{32}P-labelled *A. orana* NPV-DNA and 0.54 μg of homologous unlabelled fragments. Negative control (○ —— ○). The reaction mixture contained 2.07×10^{-3} μg (1537 cpm) of ^{32}P-labelled *A. orana* NPV-DNA in the presence of 0.9 μg of unlabelled *E. coli* DNA. All reaction mixtures had a volume of 1 ml.

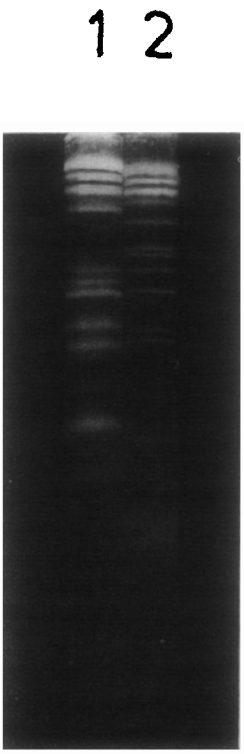

FIG. 11. Patterns of DNAs cleaved by restriction endonuclease *Eco*RI in 1.4% agarose gels: the *Adoxophyes orana* NPV-DNA (1); the *Barathra brassicae* NPV-DNA (2). Digestion and electrophoresis are described under Materials and Methods.

1977; Rohrmann and Beaudreau, 1977; Scharnhorst *et al.*, 1977; Tweeten *et al.*, 1977). Electron microscopy has also shown that DNA of the NPV of *A. orana* and of *B. brassicae* are homologous in size, in contrast to the DNA molecules isolated from some other NPVs, which were found to be heterogenous in size (Kok *et al.*, 1972; Scharnhorst *et al.*, 1977). However, a small fraction of DNA molecules of both viruses were oligomers. The genome sizes of DNA of these viruses were also determined by reassociation kinetics. A rapidly annealing fraction was not detected, indicating that within the range of detection of the hydroxylapatite columns analysis, no repetitive sequences are present. Kelly (1977) reported about 2 to 3% of intragenome homology for the NPV-DNAs of *Spodoptera* sp. However, this amount can be indicated as the range of detection for this method. The molecular weights obtained by reassociation kinetics analysis of the NPV-DNA of *A. orana* and of *B. brassicae* are in agreement with the values obtained from the length measurements.

To elucidate further the relationship between these NPV-DNAs, we performed competition hybridization to determine the nucleotide sequence homology between both DNAs using ^{32}P-labelled *A. orana* NPV-

DNA and increasing amounts of unlabelled *B. brassicae* NPV-DNA (0.9 to 2.7 μg/ml). In contrast to Kelly (1977), who found sequence homology between four NPV-DNAs of *Spodoptera* sp., we found no competition between the NPV-DNAs of the *A. orana* and of the *B. brassicae*, indicating that no homology exists between both DNAs.

As for NPV-DNAs of *Orgyia pseudotsugata* (Rohrmann *et al.*, 1978), also for NPV-DNA of *A. orana* and of *B. brassicae*, completely different *Eco*RI cleavage patterns were obtained. However, the comparison of these NPV-DNAs by *Eco*RI cleavage patterns is less sensitive than the comparison by reassociation kinetics. The only purpose of the gel patterns of restricted DNA is to make our viruses comparable with the viruses employed in other laboratories.

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