

Restriction Enzyme Analysis of the Genomes of *Plodia interpunctella* and *Pieris rapae* Granulosis Viruses¹

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The DNAs from the granulosis viruses of *Plodia interpunctella* and *Pieris rapae* were clearly distinguishable based on restriction endonuclease fragment patterns. By sizing single- and double-digestion products approximate molecular weights of 72×10^6 and 75×10^6 , respectively, were estimated for the viral genomes.

Granulosis viruses (GV) are insect viruses belonging to the family Baculoviridae. These structurally complex viruses exhibit wide variations in host specificity, serological properties, and biochemical characteristics. The GV genome consists of a covalently closed, supercoiled double-stranded DNA molecule. The conformation and size of the viral DNA have been studied by a number of techniques including sedimentation in neutral and alkaline sucrose gradients (1, 2), equilibrium centrifugation in cesium chloride-propidium iodide gradients (3), electron microscopy (3, 4), reassociation kinetics (4a), and restriction enzyme digestion (6). The GV DNA has been estimated to range in molecular weight from 69×10^6 to 111×10^6 depending on the viral isolate being investigated.

Recent studies with nuclear polyhedrosis viruses (NPV) have demonstrated the usefulness of restriction endonucleases in analysis of the structure of the large baculovirus genome. Restriction fragment patterns of NPV DNAs have been utilized to estimate genome size (5, 6), to identify viral isolates from various hosts (6-8), and to distinguish between closely related genomic variants (6, 9, 10, 12). Also, cleavage sites of a num-

ber of restriction enzymes have been localized on the genomes of *Autographa californica* NPV and other NPVs in order to construct physical maps of the viral DNA (11, 12).

Restriction endonuclease data on the GV are comparatively sparse and little information is available on genetic differences between GVs isolated from different host species. The only report of restriction enzyme analysis of GVs was the digestion of *Spodoptera frugiperda* and *Trichoplusia ni* GV DNAs by *EcoRI*. Whereas 23 *EcoRI* restriction sites were observed in *T. ni* GV DNA, 15 were present in *S. frugiperda* GV DNA. Molecular weights of 11.0×10^6 and 77.3×10^6 , respectively, were calculated for the viral DNAs based on sizes of the restriction fragments. In the present study, we have characterized restriction enzyme digestion products from two additional granulosis viruses, one infecting *Plodia interpunctella*, the other, *Pieris rapae*. This was accomplished to investigate the genetic relatedness of these viruses, particularly because of similarities observed in their structural polypeptide compositions. Polyacrylamide gel analysis of the structural polypeptides of nucleocapsids from these GVs revealed proteins having approximate molecular weights of 49,000, 39,500, 37,000, 31,500, and 12,500 in each viral isolate (13). Restriction fragments resulting from *BamHI*, *HindIII*, and *EcoRI* cleavage were identified and used to estimate the molecular

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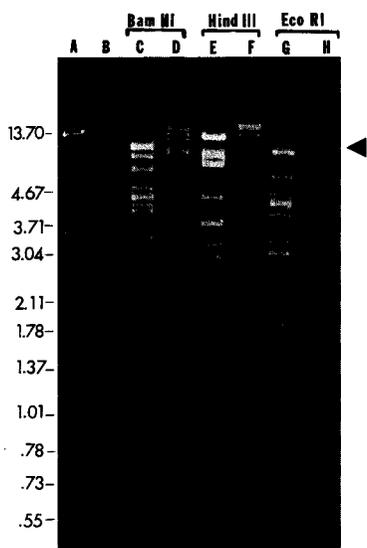


FIG. 1. Comparison of restriction fragments of *P. interpunctella* and *P. rapae* granulosis virus DNAs by electrophoresis in agarose gels. DNA (1–2 μ g) was digested to completion with 10 units of each enzyme in a 50- μ l reaction mixture. The enzymes and their digestion buffers were: *Eco*RI (0.1 M Tris-hydrochloride, pH 7.2, 0.05 M NaCl, 0.005 M MgCl₂, 2 mM mercaptoethanol), *Bam*HI (0.1 M Tris-hydrochloride, pH 7.5, 0.01 M MgCl₂), and *Hind*III (6 mM Tris-hydrochloride, pH 7.5, 6 mM MgCl₂, 50 mM NaCl). After incubation for 3 hr at 37° the reaction was stopped by addition of 10 μ l of 50% glycerol, 5% SDS, 0.2 M EDTA, 0.01% bromophenol blue. Samples were heated at 70° for 10 min, cooled on ice, and electrophoresed on 1.4% agarose slab gels using 40 mM Tris-hydrochloride, pH 7.8, 5 mM sodium acetate, and 1 mM EDTA as the buffer. (A, B) *Eco*RI and *Hind*II, respectively, digestion fragments of λ DNA. Numerical designations refer to molecular weight ($\times 10^3$) of the marker fragments. (C, E, and G) *Bam*HI, *Hind*III, and *Eco*RI restriction fragments, respectively, of *P. interpunctella* GV DNA. (D, F, and H) *Bam*HI, *Hind*III, and *Eco*RI restriction fragments, respectively, of *P. rapae* GV DNA.

weights of the viral DNA molecules. As an initial step in analysis of the structure and organization of the genome of *P. interpunctella*, GV, results of digestion of this DNA with several other enzymes and combinations of restriction enzymes are also presented.

P. interpunctella GV was produced in a laboratory colony of *P. interpunctella* larvae and purified as previously described (14, 15).

Pieris rapae GV, produced in *P. rapae* larvae, was obtained from Dr. R. P. Jacques (Canadian Department of Agriculture, Harrow, Ontario) in the form of an insecticide preparation. The *P. rapae* GV formulation was made 0.5% with respect to sodium dodecyl sulfate (SDS) and the inert ingredients were allowed to settle out. The preparation was filtered through Whatman No. 1 paper and the virus was then purified by the method utilized for *P. interpunctella* GV.

To isolate viral DNA, the purified GVs were disrupted with 0.05 M sodium carbonate, pH 10.6, for 30 min at room temperature and enveloped nucleocapsids were recovered by centrifugation on 30–70% glycerol (v/v in 0.01 M Tris-hydrochloride, pH 7.5) gradients at 25,000 rpm (SW 41 rotor) for 1 hr. The enveloped nucleocapsids were incubated in 0.15 M NaCl, 0.01 M ethylenediaminetetraacetic acid (EDTA) containing 2% Sarkosyl (v/v) and 200 μ g/ml of proteinase K for 3 hr at 37°. The preparations were extracted three times with phenol and dialyzed extensively against 0.01 M Tris-hydrochloride, 0.001 M EDTA, pH 7.5. The purified viral DNAs were digested with various restriction enzymes and the fragments were resolved by electrophoresis in 0.75 and 1.4% horizontal agarose slab gels. Following electrophoresis for 12–15 hr at 56 V, gels were stained for 1 hr in 0.5 μ g/ml of ethidium bromide and then photographed under uv illumination. *Eco*RI, *Sal*I, *Bgl*II, *Hpa*I, *Sst*II, *Sma*I, *Hind*II, and λ DNA were purchased from Bethesda Research Laboratories, Inc. *Bam*HI and *Hind*III were obtained from Miles Laboratories, Inc.

Fractionation of restriction enzyme cleavage products of the DNA from the GVs of *P. interpunctella* and *P. rapae* by gel electrophoresis revealed a large degree of genetic diversity between these viruses. The fragments generated from the viral DNAs by digestion with *Eco*RI, *Hind*III, or *Bam*HI differed markedly in number and size (Fig. 1). Fewer *Bam*HI restriction sites were present in *P. rapae* GV DNA as compared to *P. interpunctella* GV DNA. Whereas 11 fragments resulted from cleavage of *P. rapae* GV DNA with *Bam*HI, 18

fragments were produced from *P. interpunctella* GV DNA. Although approximately 12 fragments resulted from cleavage of both DNAs with *Hind*III, the fragments generated from each viral DNA had distinct electrophoretic mobilities. Several clearly distinguishable fragments also were produced by digestion of the viral DNAs with *Eco*RI. Whereas 15 fragments were released from *P. rapae* GV DNA, at least 27 fragments including several low molecular weight fragments resulted from *P. interpunctella* GV DNA. The molecular weights of the various fragments were estimated by comparison with the *Eco*RI, *Hind*II, and *Hind*III fragments of λ DNA (16, 17). Densitometer tracings of the gels showed that several of the bands contained more than one fragment. The total number of fragments detected and their molecular

weights are summarized in Table 1. The differences observed in the majority of the DNA fragments suggest that the GVs of *P. interpunctella* and *P. rapae* are not closely related. These DNAs also were observed to be clearly unique from the genomes of the GVs of *Spodoptera frugiperda* and *Trichoplusia ni* based on *Eco*RI restriction fragment patterns. By summation of the molecular weights of the DNA fragments, molecular weights of 70.2×10^6 and 74.6×10^6 , respectively, were calculated for the genomes of the GVs of *P. interpunctella* and *P. rapae* (Table 1). This compares to molecular weight values of 77.3×10^6 and 111.0×10^6 previously determined by restriction enzyme analysis of *S. frugiperda* and *T. ni* GV DNAs. (6). The average molecular weight of 75×10^6 estimated for *P. rapae* GV DNA is very similar to that of

TABLE 1
MOLECULAR WEIGHTS OF RESTRICTION FRAGMENTS OF THE DNAs FROM
THE GVS OF *P. interpunctella* AND *P. rapae*

<i>P. interpunctella</i> GV DNA ^a			<i>P. rapae</i> GV DNA ^a		
<i>Bam</i> HI	<i>Hind</i> III	<i>Eco</i> RI	<i>Bam</i> HI	<i>Hind</i> III	<i>Eco</i> RI
10.8	12.4	9.4	15.5	15.9	15.4
9.3	10.3 (2)	6.3	14.1	15.0	12.4
7.5	8.1 (2)	4.7	13.1	13.4	7.3
5.3	4.7	4.5 (2)	10.5 (2)	9.6	6.0
4.7 (2)	3.9 (2)	4.3	8.6	3.5	5.0
4.4	3.4	4.1	2.2	3.3	4.6
4.2	3.2	3.6	1.7	2.9	4.4
3.6	1.1	3.3 (2)	1.5	2.4 (2)	3.6 (2)
2.7	0.61	1.9	1.2	1.5	3.0
2.4	0.55	1.8 (3)	1.1	1.2	2.5
2.0		1.7			1.4 (2)
1.8		1.5			1.1
1.6		1.44			0.95
1.5		1.42			
1.2		1.32			
1.1		1.25			
0.93		0.98 (2)			
		0.95 (2)			
		0.86			
		0.65			
		0.57			
70 (± 2)	70.6 (± 4)	69.9 (± 2)	80 (± 4.7)	71.1 (± 5)	72.7 (± 5)

^a Molecular weight ($\times 10^6$) based on average of three separate determinations.

^b Values in parentheses are numbers of molar equivalents present; determined from densitometer tracings of the gels by plotting logarithm of the area under each peak versus migration distance (6).

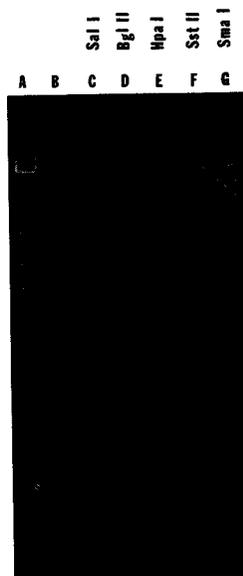


FIG. 2. Agarose gel electrophoresis of restriction fragments of DNA from the GV of *P. interpunctella*. GV DNA was digested to completion with 5 units of each restriction enzyme in a 50- μ l reaction mixture. The enzymes utilized and their digestion buffers were: (C) *Sal*I (8 mM Tris-hydrochloride, pH 7.6, 6 mM $MgCl_2$, 0.2 mM EDTA, 150 mM NaCl); (D) *Bgl*II (20 mM Tris-hydrochloride, pH 7.4, 7 mM $MgCl_2$, 7 mM 2-mercaptoethanol); (E) *Hpa*I (20 mM Tris-hydrochloride, pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol, 6 mM KCl); (F) *Sst*II (14 mM Tris-hydrochloride, pH 7.5, 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 90 mM NaCl); (G) *Sma*I (15 mM Tris-hydrochloride, pH 8.0, 6 mM $MgCl_2$, 15 mM KCl). Digestion and electrophoresis was as described in the legend of Fig. 1. (A) *Eco*RI digestion fragments of λ DNA. (B) *Hind*III digestion fragments of λ DNA.

74.6×10^6 determined for *Pieris brassicae* GV DNA by reassociation kinetics (4a). It would be most interesting to further examine the relatedness of these GVs by restriction enzyme analysis and cross-hybridization studies.

To further characterize *P. interpunctella* GV DNA, it was digested to completion with five additional restriction endonucleases. The resulting fragments were resolved by electrophoresis in agarose gels (Fig. 2). The *Sal*I and *Bgl*II digests of the DNA contained 23 and 15, respectively, well-separated fragments ranging in molecular weight from 0.74×10^6 to 10.7×10^6 . The

*Hpa*I, *Sst*II, and *Sma*I digests, on the other hand, consisted primarily of several high molecular weight fragments which could not be resolved. For example, *Sst*II cleavage of the DNA produced a few low molecular weight fragments, a 14.3×10^6 fragment, and 6 fragments having molecular weights between 7.0×10^6 and 9.2×10^6 . Similarly, the digestion products of *Sma*I included 9 fragments, 6 of which ranged in size from 9.2×10^6 to 14.9×10^6 . Utilizing the molecular weights of the fragments generated by each of these restriction endonucleases, values ranging from 72.1×10^6 to 77.1×10^6 were obtained for the genome size of the GV (Table 2).

To obtain a more reliable estimate of molecular weight, *P. interpunctella* GV DNA was digested by a combination of restriction enzymes. In these digestions, the majority of the high molecular weight fragments observed in the single enzyme digests, whose size is difficult to precisely determine,

TABLE 2

MOLECULAR WEIGHTS OF *P. interpunctella* GV DNA RESTRICTION FRAGMENTS

Molecular weight ($\times 10^6$)				
<i>Sal</i> I	<i>Bgl</i> II	<i>Hpa</i> I	<i>Sst</i> II	<i>Sma</i> I
10.4	10.7	14.5 (2) ^a	14.3	14.9
8.0	8.8 (2)	12.4 (2)	9.2 (3)	11.5 (3)
7.6	8.0	10.2	7.0 (3)	9.2 (2)
5.6	7.0	3.53	2.89	1.85
4.6 (2)	4.7	2.88	2.50	1.24
3.9 (2)	3.52	2.10	2.33	1.18
3.5	3.41 (2)	1.34	1.44	
3.18	2.68		1.30	
2.65	2.43			
2.29 (2)	2.37 (2)			
1.90	2.10			
1.86	1.85			
1.78 (2)				
1.44				
1.23				
0.98				
0.85				
0.74				
77.1	72.1	73.9	73.4	72.1

^a Values in parentheses are numbers of molar equivalents present.

were cleaved to lower molecular weight fragments. The distribution of fragments resulting from the combined digestion of GV DNA with *Bam*HI-*Hind*III, *Eco*RI-*Hind*III, or *Eco*RI-*Bam*HI is shown in Fig. 3. Each combined digest consisted of a complex pattern of over 25 fragments ranging in molecular weight from 0.57×10^6 to 10×10^6 . The relative molecular weights of the fragments from the *Eco*RI-*Hind*III and *Bam*HI-*Hind*III double digests were determined, and when summed, provided estimates of 75.9 and 72.3×10^6 , respectively, for the DNA. Combined digestion should also provide a more reliable estimate of molecular weight for the *P. rapae* genome.

All the fragments obtained by restriction endonuclease cleavage of the GV DNAs appeared to be present in molar amounts except for a minor 12×10^6 dalton band observed in *Hind*III digestion product patterns of *P. interpunctella* GV DNA (Fig. 1, arrow). These results may indicate that the wild-type isolates of these GVs contain minimal levels of the genetic variation found in other uncloned baculovirus preparations (6, 9, 10). Since most of the gel patterns are complex, it is also possible that other fragments were present in submolar quantities but were not detected due to co-migration with major fragments. Detailed analysis of the genetic variation present in GV isolates awaits development of tissue culture systems for the growth and plaque purification of these viruses.

There was close agreement between the molecular weight estimates obtained for *P. interpunctella* GV DNA from independent determinations by several restriction enzymes. The average value of 73×10^6 (± 5) was consistent with the 78×10^6 estimate previously determined by sedimentation analysis and electron microscopic length measurements (3). The lower molecular weight detection limit of fragments in the electrophoresis system used in this study was 0.5×10^6 . The presence of very small, but undetectable, cleavage products may account for the slightly larger estimate based on electron microscopic and sedimentation data.

The results of the single and double

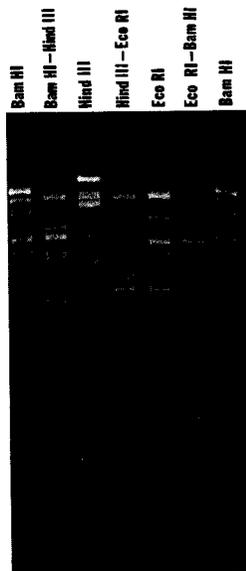


FIG. 3. Single- and double-digestion fragments of *P. interpunctella* GV DNA. Combined digestion with *Bam*HI and *Eco*RI was in 50- μ l reaction mixture containing 0.1 M Tris-hydrochloride, pH 7.5, 0.05 M NaCl, 0.01 M MgCl₂. For *Hind*III-EcoRI or *Hind*III-*Bam*HI digestion, GV DNA was incubated with *Hind*III for 3 hr at 37° in 30 μ l of *Hind*III digestion buffer. Other reagents were then added as necessary for subsequent digestion with *Eco*RI or *Bam*HI. Single digestion with *Hind*III, *Bam*HI, and *Eco*RI and electrophoresis was as described in the legend to Fig. 1.

digestion of *P. interpunctella* GV DNA represent the initial step in analysis of the fine structure and organization of the GV genome. *Bam*HI, *Eco*RI, and *Sal*I especially should be useful in mapping this viral DNA as they generated a workable number of well-separated fragments. As physical maps of this and other GV DNAs are developed and specific genome sites are related to viral functions, direct comparisons with mapped nuclear polyhedrosis viral DNAs can be made. Such comparisons will be useful in defining which regions of the genome are responsible for phenotypic and host range differences observed among closely related baculoviruses and genotypic variants (6, 9, 10, 12). It also will be possible to determine if the organization of viral genomes is similar among baculoviruses which, based on restriction endonuclease digestion (6-8)

and DNA reassociation studies (7, 18), display extensive genetic divergence.

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