

Key words: *Oryctes rhinoceros/baculovirus/restriction endonuclease map/reiterated sequences*

A Physical Map of the *Oryctes* Baculovirus Genome

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(Accepted 28 August 1985)

SUMMARY

The restriction endonucleases, *Bam*HI, *Eco*RI, *Hind*III and *Pst*I, cleave *Oryctes* baculovirus (strain PV505) DNA into 21, 43, 23 and seven fragments respectively. A large number of these fragments were cloned into the bacterial plasmids pUC8 and pBR328. These clones encompass 96% of the genome. The restriction sites for the four endonucleases were mapped using double and partial digestions of cloned fragments as well as hybridization of labelled fragments to Southern transfers of cleaved DNA. When *Hind*III fragment D and *Bam*HI fragment D were hybridized to Southern transfers six regions containing reiterated sequences were found. The physical map for *Oryctes* baculovirus could not be orientated with respect to other published baculovirus maps because the *Bam*HI fragment F of *Autographa californica* nuclear polyhedrosis virus which contains conserved polyhedrin gene sequences common to occluded baculoviruses did not bind to *Oryctes* baculovirus DNA.

INTRODUCTION

Oryctes baculovirus (Huger, 1966) is the type species of the subgroup C baculoviruses (Matthews, 1982). To date, this subgroup contains approximately 15 members isolated from a wide variety of arthropods including arachnids and crustaceans. With the exception of *Oryctes* baculovirus and the Hz-1 virus found in the IMC-Hz-1 cell line none has been characterized beyond its appearance in thin sections viewed with an electron microscope (Crawford & Granados, 1982). Three major differences have been identified between *Oryctes* baculovirus and the more intensively studied subgroup A baculoviruses: the absence of unenveloped nucleocapsids during virus morphogenesis in the nucleus, the acquisition of a second unit membrane by those particles budded from the plasma membrane, and the absence of any inclusion body production including late protein synthesis (Crawford & Sheehan, 1985).

Physical maps for a small but growing number of baculoviruses have now been published: *Autographa californica* nuclear polyhedrosis virus (NPV) and variants (Miller & Dawes, 1979; Smith & Summers, 1979), *Spodoptera frugiperda* NPV (Loh *et al.*, 1981), *Mamestra brassicae* NPV (Wiegert & Vlak, 1984), *Heliothis zea* NPV (Knell & Summers, 1984), *Orygia pseudotsugata* NPV (Leisy *et al.*, 1984) and *Choristoneura fumiferana* NPV (Arif & Doerfler, 1984). To orientate these circular genomes with respect to one another, the polyhedrin genes, which contain conserved sequences, have been designated as the starting points of baculovirus maps (Vlak & Smith, 1982).

The cloning and mapping of *Oryctes* baculovirus is described in this paper. Evidence is also presented for the existence of six regions within the genome containing reiterated sequences.

METHODS

Viruses. *Oryctes* baculovirus, strain PV505, was cloned by endpoint dilution as previously described (Crawford & Sheehan, 1985). *Oryctes* baculovirus was grown in DSIR-HA1179 cells (Crawford, 1982) or adult *Oryctes rhinoceros*. *A. californica* NPV strain HR3 (Brown *et al.*, 1979) and *S. frugiperda* NPV, obtained from Dr K. Harrap

(formerly of NERC Institute of Virology, Oxford, U.K.), were grown in IPLB-SF-21 cells (Vaughn *et al.*, 1977).

Purification of DNA. Adult *O. rhinoceros* infected with strain PV505 virus were kindly supplied by Dr B. Zelazny, FAO/UNDP Coconut Pest and Diseases Project, Albay Research Centre, Philippines. *Oryctes* baculovirus DNA was purified either from pelleted extracellular virus derived from tissue culture or from the midguts of infected adult *O. rhinoceros*. Five midguts were homogenized together in 2 ml 0.01 M-Tris-HCl pH 7.2, 1 mM-EDTA then centrifuged at low speed (1500 g) for 10 min. The supernatant was filtered through a 100 × 1.5 cm controlled pore glass column (Sigma; mesh size 120 to 200 µm, pore diameter 70 nm) and the virus collected from the void volume. The virus was concentrated by centrifugation (100000 g, 30 min) and the pellet disrupted by overnight incubation in a solution of 0.1 M-Tris-HCl pH 8.0, 10 mM-EDTA, 0.1% SDS, and proteinase K (100 µg/ml). The digested virus was then extracted three times with phenol:chloroform:isopropanol (24:24:1), and precipitated with ethanol using standard methods (Maniatis *et al.*, 1982).

A. californica NPV and *S. frugiperda* NPV DNA was obtained by pelleting tissue culture-produced extracellular virus (100000 g, 30 min). The pellet was digested, extracted and precipitated exactly as described above.

Restriction and cloning of Oryctes baculovirus DNA. All digestion of *Oryctes* baculovirus DNA with *Bam*HI, *Eco*RI, *Hind*III and *Pst*I restriction endonucleases (Amersham) was carried out in TA buffer (33 mM-Tris acetate pH 7.9, 66 mM-potassium acetate, 10 mM-magnesium acetate, 0.5 mM-dithiothreitol). All cloning was carried out using standard methods (Maniatis *et al.*, 1982). All *Hind*III, *Bam*HI and *Eco*RI fragments were cloned in the pUC8 vector with *Escherichia coli* strain JM83 as host cells. The *Pst*I fragments were cloned into pBR328 in *E. coli* strain HB101 cells. Plasmids were purified using the base/acid procedure of Birnboim (1983).

DNA labelling and hybridization. DNA was labelled by nick translation (Rigby *et al.*, 1977) using either [α -³²P]dCTP or [α -³²P]dATP (Amersham). Typical specific activities obtained were 10⁷ c.p.m./µg. DNA was transferred onto Gene Screen (New England Nuclear) using the procedure described by Smith & Summers (1980). All hybridizations used to obtain the physical map were performed under stringent conditions at 42 °C in a solution containing 50% formamide, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.2% Ficoll, 0.05 M-Tris-HCl pH 7.5, 0.1% sodium pyrophosphate, 10% SDS, 10% dextran sulphate and 100 µg/ml denatured, sonicated calf thymus DNA. Blots were washed using the following protocol: two washes using GS buffer (1 × GS buffer is 0.3 M-NaCl, 0.06 M-Tris-HCl pH 8.0, 2 mM-EDTA) for 5 min at room temperature, followed by two washes with GS buffer + 1% SDS for 30 min at 60 °C then two washes with 0.1 × GS buffer for 5 min at room temperature. Filters were then autoradiographed against Kodak X-Ray film using X-Omatic intensifying screens.

RESULTS

Restriction endonuclease digestion

The electrophoresis profiles of *Oryctes* baculovirus DNA digested with *Bam*HI, *Eco*RI, *Hind*III and *Pst*I restriction endonucleases are shown in Fig. 1. While most fragments could be resolved on 0.7% agarose gels, a 2% agarose gel was needed to resolve the smallest *Bam*HI, *Eco*RI and *Hind*III fragments. The sizes of the fragments, the method(s) used to determine their position on the physical map and what bacterial plasmids they were cloned into, are shown in Table 1.

Physical map of Oryctes baculovirus DNA

The physical map of *Oryctes* baculovirus DNA is shown in linear form in Fig. 2. A total of 94 restriction sites have been mapped on the genome. Details of the experiments performed in order to construct the map are given in Fig. 3. Twenty-five Southern hybridizations using cloned restriction fragments as probes were performed (dark shaded fragments in Fig. 3). Also, six uncloned fragments were excised from agarose gels, labelled, and used as probes (lined shading in Fig. 3). To map the fragments more precisely and to identify and determine the position of very small fragments, restriction of all the cloned fragments with each of the four enzymes was performed. The large *Pst*I fragments B, C, D, E and F cloned in pBR328 (stippled fragments in Fig. 3) were very useful in this regard. It was also necessary to use partial digestion experiments of cloned *Hind*III fragments E, G and H to order the *Eco*RI sites within these fragments (data not shown).

Orientation of the physical map

To orientate the genome as suggested by Vlak & Smith (1982) the *Bam*HI fragment F of *A. californica* NPV was used to probe for a related sequence in *Oryctes* baculovirus DNA digests. This fragment contains the polyhedrin gene minus its promoter and 171 bases at the 5' end. Five

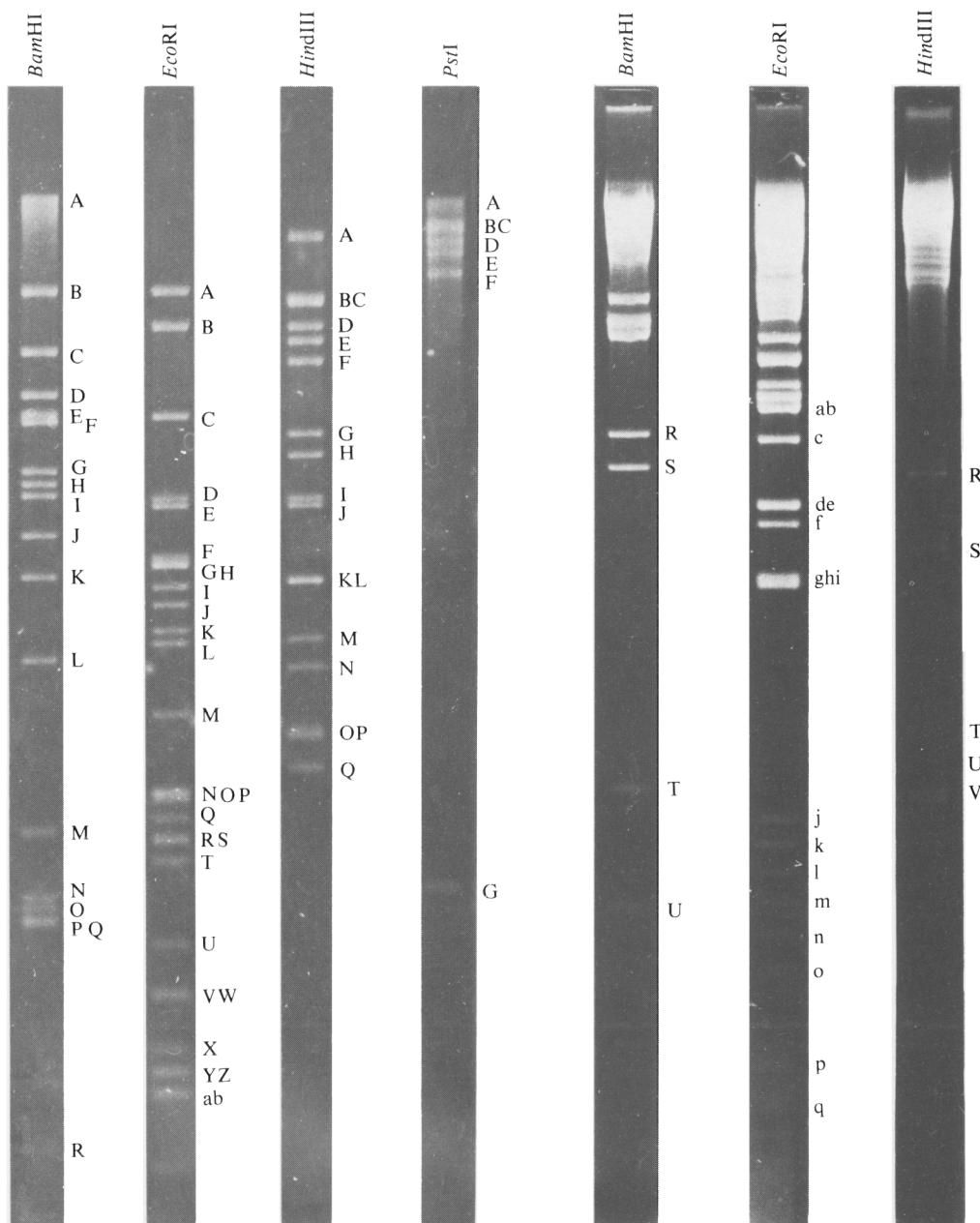


Fig. 1. Restriction endonuclease digestion of *Oryctes* baculovirus strain PV505 DNA. DNA was digested as described in Methods and electrophoresed on horizontal submerged gels of either 0.7% (first four lanes) or 2.0% (second three lanes) agarose at a constant 35 V for 16 h using TBE running buffer (0.89 M-Tris, 0.89 M-boric acid, 0.002 M-EDTA).

attempts were made to find related sequences on Southern transfers of digested *Oryctes* baculovirus under both high (50% formamide) and low (25% formamide) stringency hybridization conditions. A *Hind*III digest of *S. frugiperda* NPV was included on the Southern transfer as a positive control. Even when the hybridized Southern transfers were greatly

Table 1. *Restriction endonuclease fragments of Oryctes baculovirus DNA (strain PV505)*

<i>Bam</i> HI			<i>Eco</i> RI			<i>Hind</i> III		
Fragment	Size (kbp)	Mapping methods*	Fragment	Size (kbp)	Mapping methods*	Fragment	Size (kbp)	Mapping methods*
A	35.4	ABC	A†	12.5	AB	A	17.5	BC
B	11.1	AB	B	10.5	BC	B	11.5	AB
C	9.6	ABC	C	7.1	ABC	C	10.8	AB
D†	8.5	ABC	D	5.8	AB	D†	9.7	AB
E	7.0	ABC	E	5.8	AB	E†	9.5	AB
F	6.8	BC	F	5.1	AB	F†	9.3	AB
G†	5.8	AB	G	5.0	ABC	G†	6.8	AB
H†	5.6	A	H†	4.7	AB	H†	6.3	AB
I	5.5	BC	I	4.6	AB	I†	5.9	AB
J	5.0	AB	J†	4.5	AB	J	5.9	BC
K	4.8	AB	K	4.4	AB	K†	5.0	AB
L†	4.1	ABC	L	4.3	AB	L	5.0	BC
M	3.0	AB	M	3.8	AB	M†	4.2	AB
N	2.7	ABC	N	3.3	ABC	N†	3.9	AB
O	2.7	ABC	O†	3.2	ABD	O	3.5	BC
P	2.6	B	P	3.2	ABC	P†	3.5	AB
Q	2.6	A	Q	3.1	AB	Q	3.3	AB
R†	1.6	ABC	R	2.9	BC	R†	1.5	AB
S	1.5	BC	S†	2.9	AB	S†	1.2	AB
T	0.6	B	T	2.5	AB	T†	0.7	AB
U	0.43	B	U	2.2	AB	U†	0.7	AB
Total 126.93			V	2.2	AB	V	0.6	BC
			W	2.2	AB	W	0.6	BC
<i>Pst</i> I			X	2.0	ABD	Total 126.90		
Fragment	Size (kbp)	Mapping methods*	Y	2.0	ABD			
A	36.7	ABE	Z	2.0	AB			
B‡	21.1	AE	a	1.9	ABC			
C‡	20.1	AE	b	1.9	ABD			
D‡	17.7	ABE	c	1.5	BC			
E‡	15.3	ABE	d	1.2	AB			
F‡	13.3	AE	e	1.2	ABD			
G‡	2.7	AB	f	1.2	ABD			
Total 126.90			g	1.1	AB			
			h	1.0	AB			
			i	1.0	ABD			
			j	0.55	B			
			k	0.52	B			
			l†	0.48	AB			
			m	0.44	B			
			n	0.40	BD			
			o	0.36	BD			
			p	0.26	BD			
			q	0.22	B			
Total 127.03								

* Code for mapping methods: A, Southern hybridization with cloned probes; B, digestion of cloned fragments; C, Southern hybridizations with fragments excised from the gels as probes; D, partial digestion experiments of cloned fragments; E, double digestion of virus DNA.

† Fragment cloned in pUC8.

‡ Fragment cloned in pBR328.

overexposed (Fig. 4) no evidence of the *A. californica* NPV *Bam*HI fragment F probe binding to any *Oryctes* baculovirus fragment was found. The probe did however bind to the *S. frugiperda* NPV *Hind*III fragment L as well as the homologous *A. californica* NPV *Eco*RI fragment I (Fig. 4). The physical map therefore cannot as yet be orientated with other baculovirus maps. At present, the map arbitrarily begins with the largest *Eco*RI fragment.

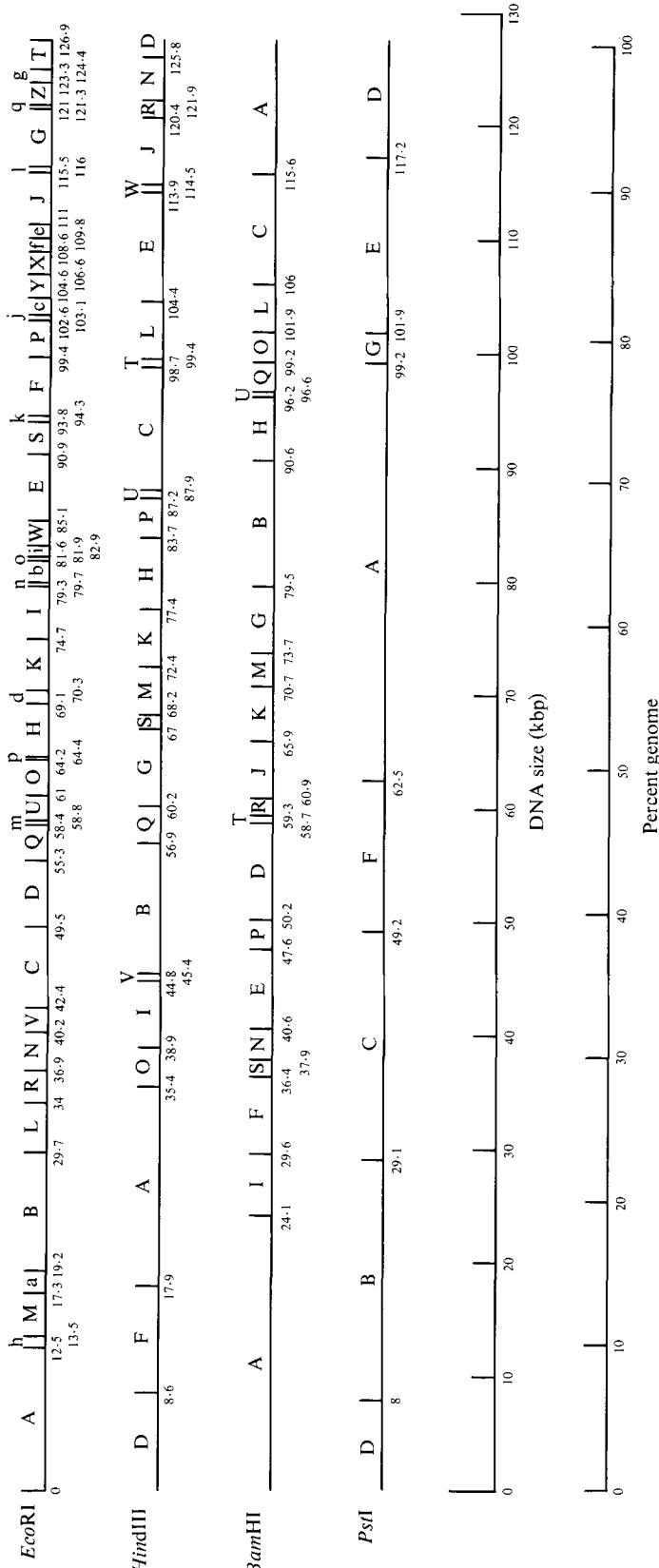


Fig. 2. Physical map of *Oryctes* baculovirus strain PV505 DNA.

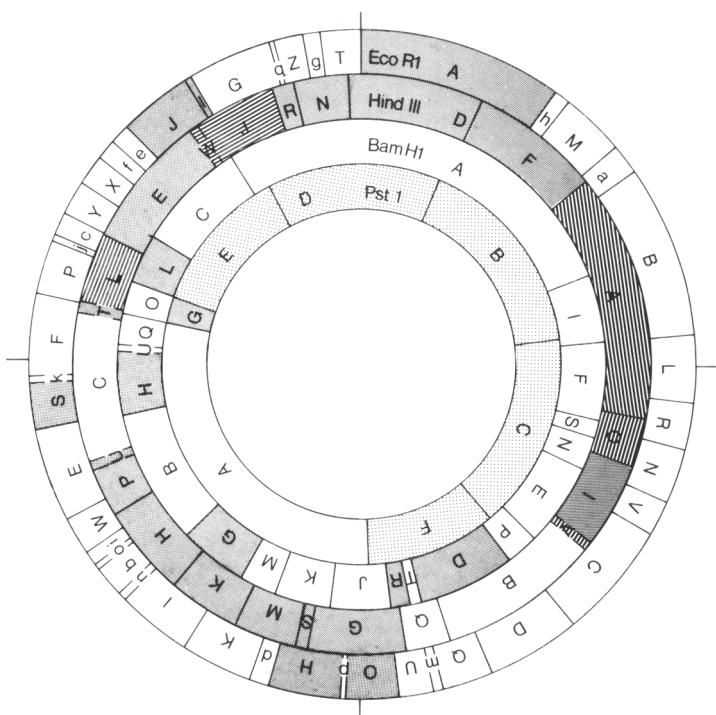


Fig. 3. Physical map of *Oryctes* baculovirus strain PV505 DNA in circular form showing the methods used to elucidate the map. Dark shaded areas indicate cloned fragments (pUC8) used for double digestion experiments and as probes for hybridization. Areas with lined shading show those fragments excised from gels, labelled, then used as probes for hybridization. The stippled *Pst*I fragments were also cloned (pBR328) but were only used in double digestion experiments.

Repeated sequences within the Oryctes baculovirus genome

Evidence for reiterated sequences within the genome was obtained from Southern hybridizations of the *Oryctes* baculovirus using the cloned *Hind*III fragment D and *Bam*H1 fragment D as probes. The hybridization results (Fig. 5) show the fragments not only binding to their homologous sequences but to five other sites within the *Oryctes* baculovirus genome. From these data the approximate position of these areas of reiterated sequences could be determined (Fig. 6). The exact size of the homologous regions is not however defined by these data.

DISCUSSION

Oryctes baculovirus DNA appears to have all the features of a typical subgroup A or B baculovirus except it appears to lack the conserved sequences associated with the polyhedrin gene. This is not surprising as these viruses do not have any form of inclusion body. There is also no late gene expression analogous to that of the polyhedrin/granulin gene (Crawford & Sheehan, 1985). Unfortunately it is these conserved sequences which have been used to orientate the previously published maps (Vlak & Smith, 1982). We therefore cannot as yet align our map with those previously published. Smith & Summers (1982) have reported that sequences in addition to the polyhedrin gene are conserved between baculoviruses. Leisy *et al.* (1984) showed that the DNA from two baculoviruses, *A. californica* NPV and *O. pseudotsugata* NPV, is predominantly colinear by mapping the shared conserved sequences. Smith & Summers (1982) also showed that slight DNA homology existed between Hz-1 baculovirus and *A. californica* NPV, suggesting that there may be some gene sequences shared between subgroup C and subgroup A baculoviruses. Efforts are currently being made to find these common sequences.

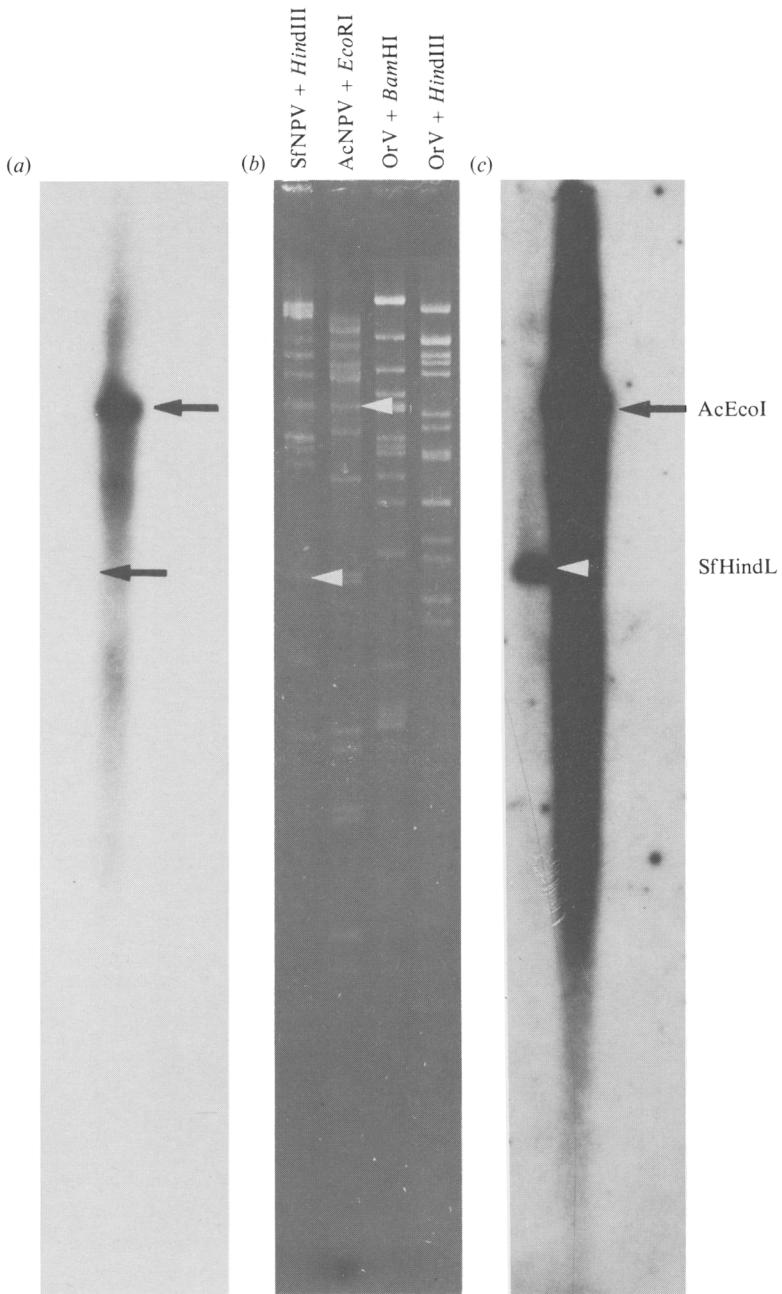


Fig. 4. Hybridization of the *Bam*H1 fragment F of *A. californica* NPV to digested DNA from *A. californica* NPV (AcNPV), *S. frugiperda* NPV (SfNPV) and *Oryctes* baculovirus (OrV). As well as binding to the homologous *A. californica* NPV *Eco*RI fragment I (AcEcoI) the conserved polyhedrin gene sequences in *S. frugiperda* NPV *Hind*III fragment L (SfHindL) were identified. However, even when the hybridization membrane was overexposed, binding to *Oryctes* baculovirus DNA could not be detected. (b) Restriction enzyme digests; (a) short exposure of blot; (c) long exposure of blot.

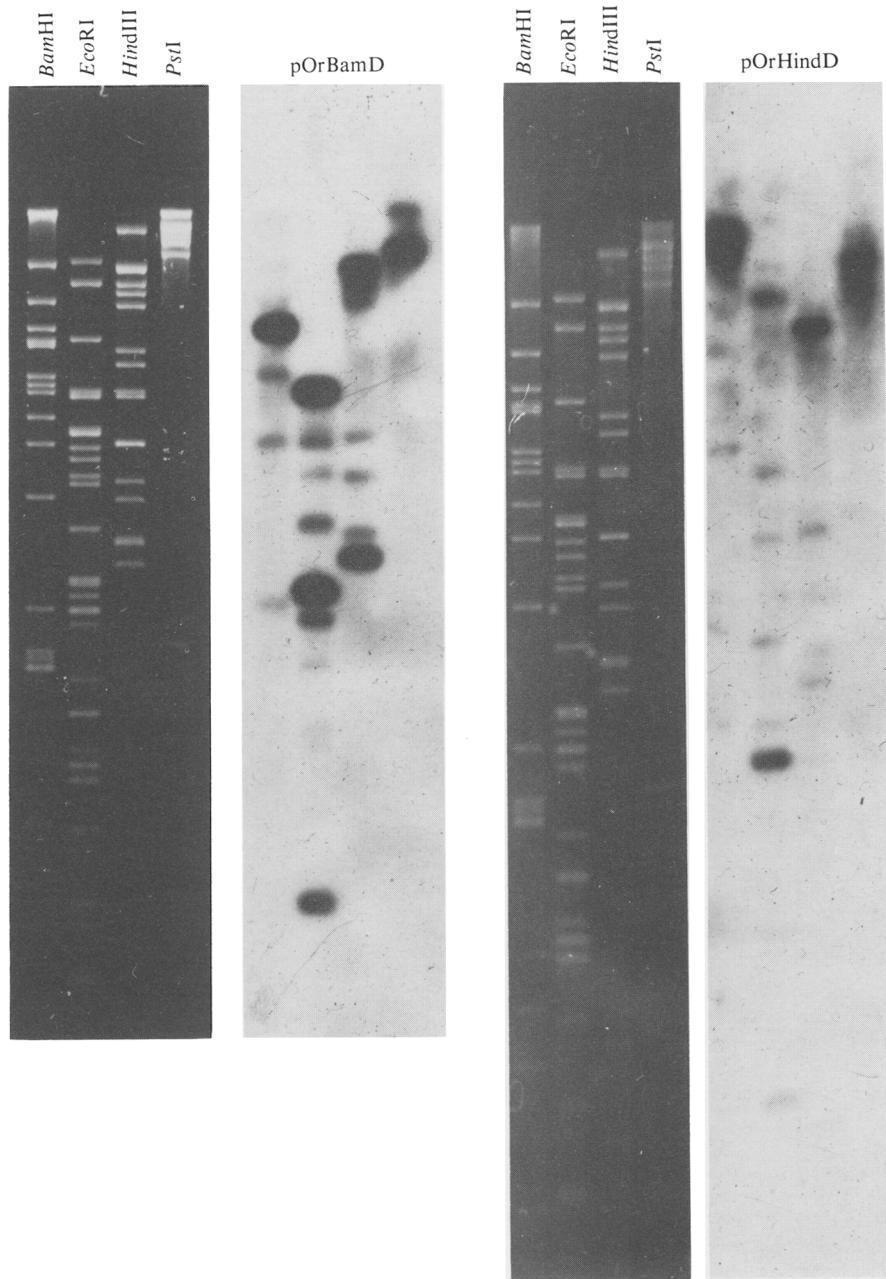


Fig. 5. Hybridization to *Bam*HI, *Eco*RI, *Hind*III and *Pst*I digests of *Oryctes* baculovirus DNA using the cloned *Oryctes* baculovirus DNA *Bam*HI fragment D (pOrBamD) and *Hind*III fragment D (pOrHindD) as probes.

Ours is the third report of reiterated sequences in a baculovirus genome. *A. californica* NPV was reported to have five regions of reiteration (Cochran & Faulkner, 1983) and *C. fumiferana* four regions (Arif & Doerfler, 1984). As yet, no function has been found associated with these regions. The fact that they have now been found in baculoviruses of subgroup C as well as

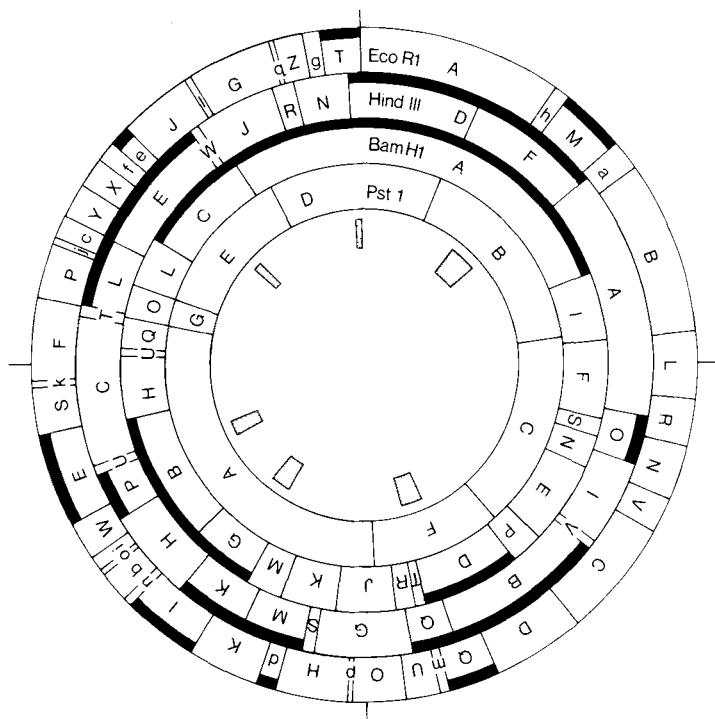


Fig. 6. Physical map of *Oryctes* baculovirus DNA showing the DNA hybridization results from Fig. 5. Fragments to which both probes bound are indicated by a wide black line at the top of the segment. Probe binding to the *Hind* III K/L band, the *Hind* III O/P band or the *Eco* R1 d/e band could not identify which of the fragments in these double bands contained the reiterated sequence. When probe binding to all the digests is examined, however, it is clear which of these fragments contains the reiterated sequence. The location of regions containing reiterated sequences was defined as those regions where binding occurs to colinear *Bam* H1, *Eco* R1 and *Hind* III fragments. The six regions identified are shown by six shaded segments in the centre of the map.

subgroup A suggests they may be common to most if not all the group and perhaps play a role in the replication of baculoviruses.

The authors wish to thank S. Fredericksen for providing the tissue culture fluid used in this study.

REFERENCES

- ARIF, B. M. & DOERFLER, W. (1984). Identification and localization of reiterated sequences in the *Choristoneura fumiferana* MNPV genome. *EMBO Journal* **3**, 525-529.
- BIRNBOIM, H. C. (1983). A rapid alkaline extraction method for the isolation of plasmid DNA. *Methods in Enzymology* **100B**, 243-254.
- BROWN, M., CRAWFORD, A. M. & FAULKNER, P. (1979). Genetic analysis of a baculovirus, *Autographa californica* nuclear polyhedrosis virus. I. Isolation of temperature sensitive mutants and assortment into complementation groups. *Journal of Virology* **31**, 190-198.
- COCHRAN, M. A. & FAULKNER, P. (1983). Location of homologous DNA sequences interspersed at five regions in the baculovirus AcMNPV genome. *Journal of Virology* **45**, 961-970.
- CRAWFORD, A. M. (1982). A coleopteran cell line derived from *Heteronychus arator* (Coleoptera: Scarabaeidae). *In Vitro* **18**, 813-816.
- CRAWFORD, A. M. & GRANADOS, R. R. (1982). Non-occluded baculoviruses. In *Proceedings of the Third International Colloquium on Invertebrate Pathology* (Brighton, U.K.), pp. 154-159.
- CRAWFORD, A. M. & SHEEHAN, C. (1985). Replication of *Oryctes* baculovirus in cell culture: viral morphogenesis, infectivity and protein synthesis. *Journal of General Virology* **66**, 529-539.
- HUGER, A. M. (1966). A virus disease of the Indian rhinoceros beetle *Oryctes rhinoceros* (Linnaeus), caused by a new type of insect virus, *Rhabdionvirus oryctes* gen. n., sp. n. *Journal of Invertebrate Pathology* **8**, 38-51.

- KNELL, J. D. & SUMMERS, M. D. (1984). A physical map for the *Heliothis zea* SNPV genome. *Journal of General Virology* **66**, 445-450.
- LEISY, D. J., ROHRMANN, G. F. & BEAUDREAU, G. S. (1984). Conservation of genome organisation in two multicapsid nuclear polyhedrosis viruses. *Journal of Virology* **52**, 699-702.
- LOH, L. C., HAMM, J. J. & HUANG, E. S. (1981). *Spodoptera frugiperda* nuclear polyhedrosis virus genome: physical maps for restriction endonucleases *Bam*HI and *Hind*III. *Journal of Virology* **38**, 922-931.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory.
- MATTHEWS, R. E. F. (1982). Classification and nomenclature of viruses. *Intervirology* **17**, 52-54.
- MILLER, L. K. & DAWES, K. P. (1979). Physical map of DNA genome of *Autographa californica* nuclear polyhedrosis virus. *Journal of Virology* **29**, 1044-1055.
- RIGBY, P. W. J., DIECKMANN, M., RHODES, C. & BERG, P. (1977). Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *Journal of Molecular Biology* **133**, 237-251.
- SMITH, G. E. & SUMMERS, M. D. (1979). Restriction maps of five *Autographa californica* MNPV variants, *Trichoplusia ni* M NPV and *Galleria mellonella* M NPV DNAs with endonucleases *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xba*I and *Eco*RI. *Journal of Virology* **30**, 828-838.
- SMITH, G. E. & SUMMERS, M. D. (1980). The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. *Analytical Biochemistry* **109**, 123-129.
- SMITH, G. E. & SUMMERS, M. D. (1982). DNA homology among subgroup A, B and C baculoviruses. *Virology* **123**, 393-406.
- VAUGHN, J. L., GOODWIN, R. M., TOMKINS, G. J. & MCCAWLEY, P. (1977). The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *In vitro* **13**, 213-217.
- VLAK, J. M. & SMITH, G. E. (1982). Orientation of the genome of *Autographa californica* nuclear polyhedrosis virus: a proposal. *Journal of Virology* **41**, 1118-1121.
- WIEGERS, F. P. & VLAK, J. M. (1984). Physical map of the DNA of a *Mamestra brassicae* nuclear polyhedrosis virus variant isolated from *Spodoptera exigua*. *Journal of General Virology* **65**, 2011-2019.

(Received 4 June 1985)