Characterization of new baculovirus genotypes arising from inoculation of *Pieris brassicae* with granulosis viruses

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Previous studies have shown that of 15 *Artogeia (Pieris) rapae* granulosis virus isolates (ArGV1 to ArGV15) only two, ArGV1 and ArGV2, gave a normal dose-mortality response in larvae from an established colony of *Pieris brassicae*. We report here that at extremely high doses, approaching 10000 times the LD$_{50}$ for ArGV1 and ArGV2, three other ArGV isolates caused low and irregular levels of mortality in *P. brassicae*. At similar doses *Agrotis segetum* GV caused 43 % mortality in one infection, but no deaths ensued from other inoculations with this virus. Restriction endonuclease analysis of viral DNA recovered from individual larval cadavers revealed that, in most cases, progeny virus differed from the inoculum and consisted either of ArGV1 or of novel genotypes explicable as recombinants between genomes of the inoculum and of ArGV1. Field-collected *P. brassicae* inoculated with ArGV8 yielded a similar range of progeny genotypes. Physical maps were constructed for two such recombinants, based on comparative restriction analysis with reference to the published map of ArGV1 and to those of ArGV5 and ArGV8, which are presented. Replication of the inoculum genotype was observed in only two infections. The origin of ArGV1 DNA appearing among progeny from these infections and the relevance of our results to identifying ArGV DNA sequences that modulate pathogenicity for *P. brassicae* are discussed.

Introduction

Horizontal transmission of baculovirus infections in nature is initiated by the release and dispersal of viral occlusion bodies (OBs) from cadavers of diseased insect larvae. Yields of virus from dead larvae generally increase markedly with larval instar at the time of infection, and can exceed $10^{10}$ OBs per larva for nuclear polyhedrosis viruses (NPVs) or $10^{11}$ OBs per larva for granulosis viruses (GVs) (Hunter *et al.*, 1984; Teakle & Byrne, 1989). Insects of other species feeding in the vicinity of dead larvae may thus ingest massive doses of OBs, although little is known about the frequency or consequences of such ‘heterologous’ infections in field situations.

Many laboratory-based studies of heterologous infections have, however, been conducted in the context of characterizing baculovirus host range (reviewed by Gröner, 1986). In several cases, immunochemical or restriction endonuclease analysis has revealed that progeny virus from heterologous infections resembles a pathogenic ‘homologous’ genotype rather than the heterologous inoculum (Longworth & Cunningham, 1968; Jurkovičová, 1979; McKinley *et al.*, 1981; Fraser & Wang, 1986). These results have been interpreted as suggesting the existence of latent baculovirus infections in certain lepidopterous species (reviewed by Smith, 1976; Podgwaite & Mazzone, 1986), an explanation which is not, however, universally accepted (David, 1978; Evans, 1986; Woods & Elkinton, 1987).

We have been investigating genotypic relationships among 15 GV isolates, all of which are highly pathogenic for *Artogeia (Pieris) rapae* (LD$_{50}$ in third instar: 200 to 400 OBs), in relation to their variable infectivity in a second Pierid species, *Pieris brassicae* (Crook, 1981, 1986; Smith & Crook, 1988a). Only two of these isolates, designated ArGV1 and ArGV2, are pathogenic for *P. brassicae* (LD$_{50}$ in third instar: approximately $6 \times 10^8$ OBs) (Payne *et al.*, 1981; Crook, 1981, 1986) although low levels of mortality were noted in *P. brassicae* larvae fed high doses of ArGV5 (LD$_{40}$ in third instar: approximately $10^9$ OBs) (Crook, 1981). We have examined this observation in greater detail by analysing progeny viral genotypes recovered from individual larval cadavers (Smith & Crook, 1988b), and report here the results of infections of *P. brassicae* with ArGV5 and other ‘non-infectious’ GV.

Methods

*Insects and virus.* The *P. brassicae* colony used in this study has been described by Payne *et al.* (1981). Most infections were conducted with mixed offspring from indiscriminate matings. For sibling larval
infections with ArGV3a and ArGV5 (see Table 1), egg masses laid by individual females were separated prior to hatching and sibling larvae were reared in discrete groups. Sources of GV isolates were identified in the following references: ArGV5 (Crook, 1986), Agrotis segetum GV (AsGV; Allaway & Payne, 1984), and ArGV3a (Smith & Crook, 1988a), which is the predominant genotype of ArGV3 (Crook, 1986). Purified OB suspensions were counted by light microscopy under dark field illumination. The OB concentration in purified AsGV was estimated from its protein concentration using 1011 OBs = 25 mg protein (Glen & Payne, 1984).

Infections. Third instar P. brassicae were infected individually with virus applied to the surface of a small disc of cabbage leaf (Payne et al., 1981). Larvae that did not consume the entire disc within 24 h were discarded. Fourth instar A. rapae were infected by suspending OBs in 5% sucrose and allowing larvae to imbibe a 1 μl droplet (see Hughes & Wood, 1981). After infection, larvae were reared individually or in groups of 15 to 25 on cabbage leaves until death or pupation. Larvae in infected groups were transferred and maintained singly after they began to show visible signs of infection.

Occlusion body purification. Recovery of virus from individual cadavers was carried out as described by Smith & Crook (1988b). Briefly, intact cadavers were homogenized and larval debris was removed by a short centrifugation. The supernatant was centrifuged into a glycerol gradient and the OB band was recovered, washed, and suspended in 100 μl water. For DNA extraction 95 μl was used and the remainder was stored at −20 °C.

Viral DNA extraction and manipulation. OBs were dissolved in 50 mM-Na2CO3 and DNA was purified from liberated virus particles by SDS treatment, phenol/chloroform extraction, and microdialysis, all as described by Smith & Crook (1988a,b). DNA solutions were stored in 10 mM-Tris-HCl pH 8.0, 1 mM-EDTA, at 4 °C. Restriction digests and agarose gel electrophoresis were performed using standard procedures (Crook, 1986; Smith & Crook, 1988a).

Physical mapping of ArGV5 and ArGV8. Restriction maps of ArGV5 and ArGV8 were constructed by extensive reciprocal digestions of DNA fragments recovered from agarose gels by the freeze-squeeze method (Thuring et al., 1975). The methodology and approach used were as described for the construction of ArGV1 to ArGV4 physical maps (Smith & Crook, 1988a).

In vivo cloning of constituent ArGV5 genotypes. We have recently developed an effective means of isolating individual genotypes from mixed wild-type baculovirus preparations (Smith & Crook, 1988b). Low-mortality infections of A. rapae with ArGV5 were conducted, and progeny genotypes were identified by restriction analysis of viral DNA recovered from individual cadavers, using the methods described above. Screening of 14 first-passage progeny from 240 inoculated larvae yielded 12 homogeneous preparations, 10 of which contained the predominant genotype (designated ArGV5a) of ArGV5. Four additional distinct genotypes, ArGV5b to ArGV5e, were recovered from the other two homogeneous preparations and from a second low-mortality passage of one of the two heterogeneous populations obtained in the initial screen.

Results

Mortality data from the infections of P. brassicae described below are compiled in Table 1.

Restriction analysis of progeny from ArGV5 infections

Two infections of unselected P. brassicae stock larvae with ArGV5 both resulted in approximately 10% mortality (infections 1 and 2, Table 1). None of the EcoRI profiles of viral DNA extracted from progeny occlusion bodies of 13 cadavers in infection 2 (Fig. 1) was identical to that of ArGV5, indicating that productive replication of ArGV5 had not occurred. A comparative examination of EcoRI (Fig. 1), HindIII and KpnI (not shown) profiles of these 13 populations revealed that (i) three (PbA5/2, PbA5/3, PbA5/6) were indistinguishable from the pathogenic genotype ArGV1; (ii) eight (PbA5/4, PbA5/7 to PbA5/13) possessed equimolar proportions of at least one restriction fragment peculiar to ArGV5; (iii) several were genotypically heterogeneous, possessing one or more fragments in visibly submolar proportion; and (iv) five (PbA5/1, PbA5/8 to PbA5/11) possessed molar or submolar proportions of ‘offsize’ restriction fragments characteristic of neither ArGV5 nor ArGV1. EcoRI profiles of progeny viral DNA isolated from cadavers from infections 1 and 4 (not shown) were qualitatively similar to those illustrated in Fig. 1.

Analysis of progeny from ArGV5-infected sibling larvae

Infections 3 to 5 (Table 1) were carried out to determine whether there was discernible variation in mortality levels between groups of larvae reared from individual egg clusters (see Methods). For a given infection, mortality was either 0% (infection 3) or fluctuated between 0 and 38% (infections 4 and 5). EcoRI profiles of progeny viral DNAs from infection 4 (not shown) were similar to those from infections 1 and 2 (see above; Fig. 1). In contrast, profiles obtained from seven of the eight mortalities in infection 5 for Clai (Fig. 2), EcoRI and KpnI (not shown) were indistinguishable from those of ArGV5, indicating that in this instance ArGV5 replication evidently had occurred. Significantly, submolar bands visible in Clai and KpnI profiles of the ArGV5 inoculum were also present, and at similar levels, in these progeny.

Physical maps of ArGV5 and PbA5/11

As noted above, many of the individual progeny populations from infections 1, 2 and 4 possessed, in equimolar proportions, restriction fragments characteristic of both ArGV5 and ArGV1. The most likely explanation of these findings is that such genotypes arose from recombination, in infected larvae, between DNA of the ArGV5 inoculum and ArGV1 DNA of unknown origin (see Discussion). The physical map of ArGV5 is shown in Fig. 3(a), and compared with that of ArGV1 (Smith & Crook, 1988a) in Fig. 3(b). Our map is in good agreement with that of Dwyer & Granados (1987) for the same isolate (designated PrGV by those authors) except for the switched positions of HindIII-F and -G, which we
Table 1. Summary of dose–mortality data for infections of laboratory-reared P. brassicae

<table>
<thead>
<tr>
<th>Infection no.</th>
<th>Inoculum</th>
<th>Dose (OBs/larva)</th>
<th>Mortality (no. dying/no. infected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ArGV5</td>
<td>$4.4 \times 10^9$</td>
<td>5/49</td>
</tr>
<tr>
<td>2</td>
<td>ArGV5</td>
<td>$4.4 \times 10^9$</td>
<td>13/125</td>
</tr>
<tr>
<td>3(S)*</td>
<td>ArGV5</td>
<td>$4.5 \times 10^9$</td>
<td>0/144†</td>
</tr>
<tr>
<td>4(S)</td>
<td>ArGV5</td>
<td>$1.2 \times 10^9$</td>
<td>0/50, 1/48, 14/37, 1/50, 2/44</td>
</tr>
<tr>
<td>5(S)</td>
<td>ArGV5</td>
<td>$1.3 \times 10^9$</td>
<td>5/21, 1/19, 2/23, 0/137†</td>
</tr>
<tr>
<td>6</td>
<td>ArGV3a</td>
<td>$2 \times 10^7–10 \times 10^7$</td>
<td>2/17</td>
</tr>
<tr>
<td>7</td>
<td>ArGV3a</td>
<td>$5 \times 10^8$</td>
<td>0/∞ 100</td>
</tr>
<tr>
<td>8(S)</td>
<td>ArGV3a</td>
<td>$5 \times 10^8$</td>
<td>0/75†</td>
</tr>
<tr>
<td>9</td>
<td>ArGV3a</td>
<td>NR†</td>
<td>13/100</td>
</tr>
<tr>
<td>10</td>
<td>AsGV</td>
<td>$\sim 10^9$</td>
<td>26/60</td>
</tr>
<tr>
<td>11</td>
<td>AsGV</td>
<td>$\sim 10^9$</td>
<td>0/425</td>
</tr>
<tr>
<td>12</td>
<td>AsGV</td>
<td>$\sim 3 \times 10^9$</td>
<td>0/285</td>
</tr>
</tbody>
</table>

* S, Infections of sibling groups.
† Larvae infected in groups of 15 to 25 siblings.
‡ NR, Not recorded.

Fig. 1. *EcoRI* profiles of viral DNA recovered from individual ArGV5-infected *P. brassicae*. Thirteen progeny preparations, designated PbA5/1 to PbA5/13 (lanes 1 to 13, respectively), from infection 2 are shown, flanked by ArGV1 (GV1) and ArGV5 (GV5). ArGV1 restriction fragments are labelled strictly according to size; those of ArGV5 are labelled with reference to collinear ArGV1 fragments (see legend to Fig. 3).

confirmed to be in the locations shown by second-digestion of isolated *XhoI*-A and *XhoI*-D fragments with *HindIII* (not shown).

Of the eight progeny genotypes shown in Fig. 1 that possessed one or more ArGV5-specific fragments in equimolar proportion (see above), PbA5/11 appeared to contain the highest amount of ArGV5 DNA. By comparing restriction profiles of ArGV5, ArGV1 and PbA5/11 for *BamHI*, *EcoRI*, *HindIII* and *KpnI* (not shown), the recombinant genome structure of PbA5/11 was established (Fig. 3c). This map indicates that PbA5/11 consists of two regions of ArGV5 sequence separated by two regions of ArGV1 sequence. Depending on the locations of crossover sites, which we cannot map with any precision (see legend to Fig. 3), PbA5/11 apparently contains a minimum of approximately 30 kbp and a maximum of more than 70 kbp of ArGV5 sequence.
kbp

23.13
9.42 -
6.68 -
4.36 --
2.32 --
2.03 --

GV1 a b c d GV5

ClaI fragments that were submolar in ArGV5 (see Fig. 2 and legend). ArGV5b contained equimolar proportions of KpnI-L (Fig. 4) and BamHI-J fragments of the same size as those in PbA5/11; these two fragments overlap on the physical map of ArGV5 (Fig. 3). ArGV5b thus contains an insertion of approximately 100 bp, situated between map positions 38.45 and 38.85 kbp (Fig. 3), relative to ArGV5a. Apart from this insertion, ArGV5b is identical to ArGV5 (cf. ArGV5a, the predominant component of ArGV5; Fig. 4) and quite distinct from PbA5/11. The ArGV5 component of PbA5/11 is therefore likely to be derived from an ArGV5b genome present in wild-type ArGV5.

Analysis of progeny from infections with ArGV3a

ArGV3a, the predominant genotype of wild-type ArGV3, was isolated by low-dose infection in A. rapae. Two infections of P. brassicae with ArGV3a (infections 6 and 9; Table 1) yielded 12 and 13% mortality, respectively, whereas no deaths ensued from two other infections (7 and 8; Table 1) with this virus at doses considerably higher than that used in infection 6. EcoRI profiles of progeny from infections 6 and 9 had characteristics similar to those of most progeny from ArGV5 infections; many possessed fragments unique to ArGV3a, but in no case had ArGV3a replicated and three EcoRI profiles of progeny from infection 9 were identical to the ArGV1 profile (not shown).

Infections with AsGV

Although AsGV was found to be non-pathogenic for P. brassicae (Allaway & Payne, 1984), occasional mortalities in larvae fed high doses of AsGV OBs have been observed (C. C. Payne, personal communication). In one infection with AsGV at approximately 10⁹ OBs per larva, 43% mortality occurred (infection 10; Table 1). EcoRI profiles of 14 DNAs from progeny virus populations are shown in Fig. 5. A striking feature of many of these profiles was the presence of certain EcoRI fragments, characteristic of ArGV3, ArGV4 and ArGV6 (Crook, 1986; Smith, 1987), which were not observed among progeny from ArGV5 infections (see above). Very few of these profiles were identical to each other and all but two (PbAs/2 and PbAs/4), both of which contained submolar bands, were distinct from ArGV1. Two subsequent infections (11 and 12; Table 1) with AsGV, at the same or threefold higher doses and using more than 700 larvae, gave no mortalities.

Seventy-five fourth instar A. rapae, which are approximately 600-fold more susceptible to ArGV infection than are third instar P. brassicae (Payne et al., 1981; Crook, 1986), were each infected with approximately 10⁹ AsGV...
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(a) XhoI EcoRI HindIII BamHI KpnI Others

(b) Fig. 3. Physical maps of ArGV5 and PbA5/11. (a) Complete restriction map of the 107.9 kbp ArGV5 genome, for eight enzymes. The circular genome is depicted linearized at the start codon of the granulin gene (0 kbp), which is transcribed rightwards. The distance in kbp of each restriction site from this point is indicated. Restriction fragment nomenclature conforms to a scheme described by Smith & Crook (1988 a), in which fragments of other ArGV isolates are labelled with reference to collinear regions of ArGV1. (b) Genotypic variation between ArGV5 and ArGV1. Positions of restriction sites specific to ArGV5 are shown above the line and those of sites specific to ArGV1 below. Shaded areas depict regions, delineated by restriction sites, in which deletions of the indicated sizes (in bp) occur in ArGV5 relative to ArGV1. B, BamHI; E, EcoRI; H, HindIII; K, KpnI; P, PstI. (c) Genome composition of the ArGV5 × ArGV1 recombinant PbA5/11. Boxes represent regions in which PbA5/11 possesses either ArGV1-specific (1) or ArGV5-specific (5) restriction markers, and the vertical line indicates the position of a single ArGV1-specific HindIII site marker. As seen in (b), there are extensive tracts of ArGV5 and ArGV1 DNA in which no distinguishing markers occur; consequently, the sizes of parent-specific regions shown are likely to be underestimated and it is possible that additional ‘invisible’ crossover sites exist.

(b) OBs as a test for ArGV contamination of the AsGV preparation. All of these larvae survived.

Infections of wild P. brassicae

The P. brassicae stock used for all inoculations described above has been maintained in the laboratory since 1960. Early generations suffered several outbreaks of GV disease (David & Gardiner, 1966), but since 1963 there have been no signs of virus in the stock. Although a previous comparison of this stock with wild P. brassicae showed no significant difference in susceptibility to ArGV1 infection (Payne et al., 1981), it is possible that the results obtained with other isolates were due to some peculiarity of the laboratory stock. A similar experiment was therefore conducted with larvae reared from field-collected P. brassicae eggs. Eighty larvae were reared to fourth instar and then divided into three treatments: 25 larvae were fed $4 \times 10^9$ ArGV8 OBs, a further 25 were fed $6 \times 10^9$ AsGV OBs, and 30 were used as controls. Of the
Fig. 4. *KpnI* profiles of four distinct genotypes isolated from *ArGV5*. Five genotypes, designated *ArGV5a* to *ArGV5e*, were isolated, four of which could be distinguished by their *KpnI* profiles as shown here. *ArGV5a* (lane a) is the predominant genotype of *ArGV5*; *ArGV5b* and *ArGV5c* (lanes b and c, respectively) have insertions of different sizes within *KpnI*-L relative to *ArGV5a*, and *ArGV5c* (lane c) has an additional *KpnI* site within *KpnI*-GH which results in the appearance of two new fragments, the smaller of which comigrates with *KpnI*-I. [The map location of this additional site has been determined by Dwyer & Granados (1987), who observed the two fragments as submolar bands in *ArGV5*.] The fifth genotype, *ArGV5d* (not shown) possessed a *KpnI* profile identical to that of *ArGV5c* but an altered *ClaI* profile (see Results and legend to Fig. 2). Letter assignments for *KpnI* fragments of *ArGV5*, which are the same for *ArGV5a*, *ArGV5b* and *ArGV5c*, are shown to the right. λ, *HindIII* digest of lambda DNA, with fragment sizes indicated to the left.

latter inoculated with *ArGV8*, 18 died but only 11 yielded sufficient virus for analysis. Six of the larvae fed *AsGV* died, but very little virus was detected in the cadavers. None of the control larvae died and all pupated normally.

*HindIII* profiles of viral DNA from those *ArGV8*-infected larvae which yielded sufficient OBs (PbA8/1 to PbA8/11) are compared with profiles from *ArGV1* and *ArGV8* in Fig. 6. Two of these profiles (PbA8/1 and PbA8/3) were predominantly *ArGV1*-like but contained submolar amounts of at least three *ArGV8* bands. Two others (PbA8/8 and PbA8/11) were indistinguishable from *ArGV8* and a further one (PbA8/9) was similar but contained a submolar *ArGV1* band. Two (PbA8/6 and PbA8/10) contained bands from both *ArGV1* and *ArGV8* in apparently equimolar amounts, and the
remaining four were indistinguishable from ArGV1. *EcoRI* profiles (not shown) of the 11 DNAs gave essentially similar results. PbA8/8 and PbA8/11 were also analysed with *BamHI* and *KpnI* (not shown): *BamHI* profiles of both viruses and the *KpnI* profile of PbA8/11 were identical to those of ArGV8, but the *KpnI* profile of PbA8/8 revealed a small reduction in the size of *KpnI*-K showing that even in this case the progeny virus was not identical to the inoculum.

PbA8/10 was examined in more detail by identifying ArGV1- and ArGV8-specific fragments in comparative restriction profiles of the three isolates for *HindIII*, *EcoRI*, *BamHI* and *KpnI* (not shown). As with PbA5/11, it was found that all the fragments could be accounted for if PbA8/10 was a recombinant between ArGV1 and the inoculum. Fig. 7 shows the physical map of ArGV8, and that of a recombinant ArGV8 x ArGV1 genome which would give the profiles observed for PbA8/10.

**Discussion**

The infections described above involved exposing *P. brassicae* to ‘non-infectious’ baculoviruses at doses which, although very high, were not unrealistic in terms
of naturally occurring horizontal transmission. Cabbage is a favoured diet of several lepidopterous species including *P. brassicae* and *A. rapae*, and it is quite conceivable that, for example, a *P. brassicae* larva could fortuitously ingest $10^8$ OBs (or approximately 1% of the yield) of ArGVs released from a late-instar larva that had recently died in its proximity. Our simulations of such an event suggest that mortalities which occur in *P. brassicae* are unlikely to be the result of heterologous viral replication. Most or all of the progeny from productive infections with ArGV3a, ArGV8, AsGV and three of four infections with ArGV5 possessed genomes consisting partly or wholly of ArGV1-like DNA. Many of these genomes, including PbA5/11 and PbA8/10, appear to be recombinants which contain sequences of both the inoculum genotype and ArGV1.

There are three possible sources of ArGV1 DNA emerging from *P. brassicae* in these experiments. First, ArGV1 OBs might have been present as contaminants in the laboratory at the time of infection, and been ingested along with heterologous inocula in numbers sufficient to cause low levels of mortality. Third instar larvae would, however, have had to ingest a large number of ArGV1 OBs ($LD_5$ is approximately $10^8$ OBs) for significant numbers of larvae to die, and no uninfected larvae died of viral infection during the course of these experiments. Furthermore, levels of ambient ArGV1 contamination sufficient to cause any deaths in *P. brassicae* would have resulted in very high levels of mortality in *A. rapae* larvae which were being handled in the same area; no such mortalities occurred.

Second, inoculum OB preparations might have been contaminated with trace amounts of ArGV1 and/or pathogenic recombinants. The high relative susceptibility of *A. rapae* effectively rules out the possibility that the AsGV preparation, which led to deaths in *P. brassicae* in infection 10, was contaminated with ArGV genotypes, because no mortality occurred in *A. rapae* larvae fed a dose of AsGV identical to that used in infection 10. We cannot, however, state unequivocally that recombinants recovered from *P. brassicae* following infection with wild-type ArGV genotypes were not pre-existent in the inoculum. *A. rapae* is used to propagate stocks of most ArGV isolates and, in view of its greater sensitivity to contamination than *P. brassicae* (reflected by much lower $LD_{90}$ values for all ArGVs), trace concentrations of diverse recombinants might be present at levels low enough to escape detection in ethidium bromide-stained gels of inoculum DNAs but high enough to produce significant mortality when large doses were fed to *P. brassicae*. In contrast, no such recombinants have been encountered in the course of screening large numbers of progeny populations for genotypic isolations from ArGV3 (Smith & Crook, 1988b) or ArGV5 (this study). Furthermore, such recombinants are extremely unlikely to have been present as contaminants in the single-genotype ArGV3a preparation used in infections 6 and 9.

Third, a latent GV infection may exist in *P. brassicae*. The possibility that a GV from *A. rapae* might ‘trigger’ a latent GV in *P. brassicae* was considered as early as 1956 (Smith & Rivers, 1956). Our infections with heterologous GV isolates suggested that a latent genotype might be ArGV1, although restriction fragments in progeny from AsGV infection indicated that other ArGV genotypes might also be present in *P. brassicae*. Most studies in which latent baculovirus infection has been proposed to occur have, like the present one, been ‘indirect’: what is detected is not the putative latent genome itself but an amplified population, presumed to represent the result of its activation and replication. There are, however, four reports describing direct detection of latent NPV DNA in host insects (Skuratovskaya et al., 1982; Miryuta et al., 1985; Wood et al., 1986; Burand et al., 1990). In the course of this work we failed to detect ArGV1 DNA in uninfected *P. brassicae* genomic DNA preparations by Southern hybridization (Smith, 1987). This experiment is not considered to be definitive because the limit of detection was approximately two ArGV genome copies per cell [cf. latent infection of mice with herpes simplex virus type 1, in which viral genomes are maintained episomally at 0·01 to 0·2 copies per cell (Rock & Fraser, 1987; Mellerick & Fraser, 1987)]. In summary, although we cannot unequivocally exclude contamination with ArGV OBs as an explanation of the results presented in this work, the possibility that some proportion of *P. brassicae* larvae harbour latent ArGV infections should not be dismissed.

Irrespective of the source of non-inoculum ArGV DNA, it is clear from these results that extensive homologous recombination among ArGVs can take place in vivo. This observation concurs with several reports of recombination between closely related NPV genotypes (Croizier et al., 1980, 1988; Miller et al., 1980; Summers et al., 1980; Croizier & Quiot, 1981; Kondo & Maeda, 1991), and we have suggested that recombination may have contributed to the genesis of eight distinct genotypes isolated from wild-type ArGV3 (Smith & Crook, 1988b). In contrast, more distantly related baculovirus genotypes may not be capable of homologous recombination (Miller, 1981). With regard to ArGV pathogenicity for *P. brassicae*, it may be feasible to obtain recombinants between, for example, ArGV1 and ArGV5 in *A. rapae* possessing either (i) minimal ArGV5 sequence and avirulence or (ii) minimal ArGV1 sequence and virulence towards *P. brassicae*. The generation and characterization of such recombinants would constitute an important step towards identifying a gene(s) that specifies the ability or inability of particular
ArGV isolates to replicate productively in *P. brassicaceae*. Kondo & Maeda (1991) have recently described a similar strategy for generating recombinants between *Autographa californica* NPV and *Bombyx mori* NPV which possess a host range wider than that of either parental genotype.

It was anticipated that, since genotypes such as PbA5/1 to PbA5/13 had caused lethal infection in the larvae from which they emerged, OBs recovered from infections with heterologous ArGVs would be pathogenic for *P. brassicaceae*. Preliminary infections (unpublished observations) of *P. brassicaceae* with a number of these genotypes have indicated that generally this is indeed the case. PbA5/11, however, gave low levels of mortality and the majority of restriction profiles among 18 third-passage progeny populations were indistinguishable from ArGV1; only one was identical to PbA5/11. We cannot draw any firm conclusions from these findings, but they suggest that productive replication of low-pathogenicity ArGVs may be blocked at an early stage of infection, perhaps in the midgut epithelium, the first host tissue to be affected during baculovirus infection (reviewed by Granados, 1980). Fraser & Stairs (1982) and Ignoffo et al. (1985) have shown that the midgut may be a decisive barrier in two NPV–host systems by comparing the pathogenicities of OBs fed *per os* with those of non-occluded virus particles, the latter bypassing the midgut by being injected directly into the haemocoele. In this context it would be of considerable interest to determine to what extent non-occluded virus particles of genotypes such as ArGV3 and PbA5/11, which could be obtained from the haemolymph of infected *A. rapae* larvae, are pathogenic when injected into the haemocoele of *P. brassicaceae*.

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Recombination of *Autographa californica* and *Rachiplusia* or nuclear polyhedrosis viruses in *Galleria mellonella* L. *Journal of General Virology* 69, 177–185.


Fraser, M. J. & Stairs, G. R. (1982). Susceptibility of *Trichoplusia ni*, *Heliothis zea* (Noctuidae), and *Manduca sexta* (Sphingidae) to a nuclear polyhedrosis virus from *Galleria mellonella* (Pyralidae). *Journal of Invertebrate Pathology* 40, 255–259.


Jukkovičová, M. (1979). Activation of latent virus infections in larvae of *Adoxophyes orana* (Lepidoptera: Tortricidae) and *Barathra brassicae* (Lepidoptera: Noctuidae) by foreign polyhedra. *Journal of Invertebrate Pathology* 34, 213–223.


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