Restriction Map of *Rachiplusia ou* and *Rachiplusia ou*-*Autographa californica* Baculovirus Recombinants

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The restriction sites of *Rachiplusia ou* nuclear polyhedrosis virus (RoMNPV) DNA were mapped for the endonucleases *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, and *Eco*RI. Of the 60 DNA restriction sites of RoMNPV, 35 mapped in similar positions as compared to the restriction sites of *Autographa californica* nuclear polyhedrosis virus (AcMNPV) DNA. Two plaque-purified viruses, obtained from randomly picked plaques of a wild-type isolate of RoMNPV, were recombinants of RoMNPV and AcMNPV. The recombinants were shown to have RoMNPV and AcMNPV restriction fragments as well as structural polypeptides from each parental virus. Both recombinant viruses had a major RoMNPV capsid protein but were occluded in the AcMNPV polyhedrin protein.

The nuclear polyhedrosis virus of *Rachiplusia ou* (RoMNPV) is an insect baculovirus with a double-stranded, circular, supercoiled DNA genome of about 82×10^6 daltons (7). Previous reports (7, 9-11, 13) demonstrated that RoMNPV is distinct from, but closely related to, *Autographa californica* nuclear polyhedrosis virus (AcMNPV). Restriction endonuclease analysis of RoMNPV and AcMNPV DNAs (7) has shown that the genomes are of similar size and that at least 40% of RoMNPV DNA *Eco*RI restriction fragments representing about 50% of the genome have the same mobilities in agarose gel electrophoresis as AcMNPV *Eco*RI fragments. AcMNPV and RoMNPV structural polypeptides when compared by polyacrylamide gel electrophoresis were also very similar (10). Yet the mobilities of several structural proteins are distinct between these two viruses; for example, a major RoMNPV capsid polypeptide of 36,000 daltons is about 1,000 daltons smaller than a major AcMNPV capsid polypeptide (10). Polyhedrin, the protein that makes up the crystalline matrix in which these viruses are often found occluded, is composed of a single polypeptide of about 30,000 daltons for both AcMNPV and RoMNPV. The amino acid sequences of AcMNPV and RoMNPV polyhedrins are similar but unique as determined by tryptic peptide analysis (9).

An elegant technique involving an analysis of viral recombinants has been used to map adenovirus *ts* mutations and the location of templates specifying polypeptides on the physical map of the DNA (3, 14). More recently, many polypeptides and several viral functions of herpes simplex virus types 1 and 2 have been mapped by similar methods (5, 6). This report

will initiate studies to use viral recombinants for the mapping of AcMNPV and RoMNPV gene products. The restriction endonuclease cleavage map of RoMNPV DNA will be shown and compared to the cleavage sites mapped for AcMNPV DNA (8). In addition, two recombinants of AcMNPV and RoMNPV were isolated from randomly picked plaques of a wild isolate of RoMNPV. The DNA structure and viral structural proteins of the two recombinants will be presented. In a subsequent report, the physical location of crossover points between AcMNPV and RoMNPV in recombinant genomes will be determined and related to the segregation of those viral proteins that are different between the two parental viruses.

MATERIALS AND METHODS

Cells and virus. A continuous cell line of *Trichoplusia ni*, TN-368, was maintained as described by Volkman and Summers (12) and used for the preparation of viral isolates.

The E2 variant of AcMNPV for which we have prepared a physical map (8) was used in this study. Two wild isolates of RoMNPV were obtained from C. Y. Kawanishi (Environmental Protection Agency, Research Triangle Park, N.C.). Originally, one isolate, designated as RoMNPV wt-1, had been obtained from James Harper (Auburn University). A second isolate, designated as RoMNPV wt-2, was from the Cornell Experiment Station (Geneva, N.Y.). The RoMNPV isolates were plaque purified as described by Summers and Smith (10). RoMNPV R1 was isolated from RoMNPV wt-2. The AcMNPV and RoMNPV recombinants AR7 and AR8 and the AcMNPV variant R9 were isolated from RoMNPV wt-1. Isolation of polyhedra and purification of virus were done as described previously (10).

Polyacrylamide gel electrophoreses. To analyze the viral structural polypeptides, purified virus was

disrupted in 1.0% sodium dodecyl sulfate–5.0% 2-mercaptoethanol at 100°C for 3 min. Samples of 30 to 40 µg of viral protein were electrophoresed in 11% polyacrylamide vertical gel slab in the presence of 0.1% sodium dodecyl sulfate as described by Laemmli (1) as modified for baculovirus preparations (10).

Preparation and purification of ³²P-labeled DNA. Viral DNA was labeled with radioactive isotope by adding phosphate-free medium (9) containing 50 µCi of carrier-free ³²Pi per ml (50 Ci/mmol; New England Nuclear Corp.) to infected cell monolayers as described previously (8). Viral DNA was isolated by disrupting purified virus with sodium dodecyl sulfate and protease K followed by phenol extraction (8). About 5 × 10⁶ cpm of viral [³²P]DNA with a specific activity of 0.5 × 10⁶ to 1.0 × 10⁶ cpm/µg was recovered per 10⁷ infected cells.

Restriction endonucleases and agarose gel electrophoresis. *Bam*HI, *Eco*RI, *Kpn*I, *Sac*I, *Sma*I, and *Xho*I restriction endonucleases were purchased from New England Biolabs (Beverly, Mass.). All restriction enzyme digests were done as recommended by the supplier with demonstrated excess enzyme to achieve a limit digestion. λ DNA (0.5 µg) was added to all [³²P]DNA digests as an internal standard to confirm that a limit digest had occurred.

Horizontal agarose gel electrophoresis and autoradiography were done as described previously (8).

Isolation and digestion of DNA restriction fragments from Sea Plaque agarose. Isolation of DNA restriction endonuclease fragments was carried out by the method of Parker and Seed (R. Parker and B. Seed, Methods Enzymol., in press). Low-melting-temperature agarose gels were made from Sea Plaque agarose (Marine Colloids, Rockland, Maine). Horizontal gels of 0.6% were made by dissolving the agarose at 100°C in E buffer (40 mM Tris, 5 mM NaOAc, 1 mM EDTA, 0.5 µg of ethidium bromide per ml adjusted to pH 7.4 with glacial acetic acid). About 1 µg of [³²P]DNA restriction fragments were electrophoresed in each slot at 75 V for 36 to 48 h. The UV visible bands were excised from the gel with a razor blade and stored at –70°C.

DNA restriction fragments recovered from Sea Plaque agarose gels were incubated at 70°C to melt the agarose without denaturing the DNA. The samples were allowed to equilibrate to 37°C, at which the low-melting-temperature agarose remained in solution. Ten microliters of [³²P]DNA restriction fragments was added to 20 µl of an enzyme digestion solution to give a final concentration of 1.0× restriction endonuclease buffer, 0.1 mg of bovine serum albumin per ml, 0.5 µg of λ DNA, and 2 U of enzyme (1 U of enzyme digest and 1 µg of λ DNA to completion in 15 min). The samples were incubated for 3 h at 37°C, and the reactions were terminated by adding 3 µl of a solution containing 0.1 M EDTA, 1.0% bromophenol blue, 1.0% sodium dodecyl sulfate, and 40% glycerol. The DNA samples, still in a solution of Sea Plaque agarose, were layered directly into the wells of the second gel and electrophoresed as described above.

Tryptic peptide mapping of polyhedrin. Virus polyhedra labeled with ³²P were purified from 2 × 10⁷ infected TN-368 cells at 72 h postinfection as described previously (7). The ³²P-labeled polyhedrin protein was

released by disrupting the polyhedra in 0.1 M Na₂CO₃–0.17 M NaCl–0.001 M EDTA (pH 10.8) and separated from the virus by centrifugation at 100,000 × *g* for 30 min. The supernatant containing the phenol-soluble polyhedrin protein (9) was extracted twice with redistilled phenol and then precipitated from the phenol phase by adding 3 volumes of cold acetone and incubating at –20°C overnight. The precipitated polyhedrin was pelleted at 10,000 × *g* for 30 min and washed twice with distilled water, and the pellet was resuspended in 0.05 M NH₄HCO₃. TR tolylsulfonyl phenylalanyl chloromethyl ketone-treated trypsin (Worthington Biochemicals Corp., Freehold, N.J.) was added at an enzyme-to-substrate ratio of 50:1. Polyhedrin was digested at 37°C for 18 h, lyophilized, and resuspended in 10 to 20 µl of 0.05 M NH₄OH. About 100 µg of digest protein was spotted onto cellulose thin-layer sheets (20 by 20 cm) and electrophoresed in the first dimension at 600 V for 45 min in 10% pyridine–0.03% acetic acid, pH 4.5. The tryptic peptides were further separated by ascending chromatography at room temperature as described in Fig. 7. Positions of the hydrolysates were detected with 1% ninhydrin–0.1% cadmium acetate. To detect ³²P-phosphopeptides the sheets were exposed to Kodak NS-54T X-ray film with a Dupont Lightning-Plus intensifying screen at –70°C for 2 days.

RESULTS

Twenty two plaque-purified viral isolates were obtained from RoMNPV wt-2. The viral DNAs from each were purified, restricted with *Eco*RI, and then electrophoresed in agarose gels. The restriction patterns of the 22 viral isolates, as well as the RoMNPV wt-2 DNA, were the same as those shown for RoMNPV R1 in Fig. 1. Three additional plaque-purified isolates, R9, AR7, and AR8, were obtained from RoMNPV wt-1. The DNA restriction sites of one of these isolates (R9) have been physically mapped (8) and were the same as those of AcMNPV E2 DNA with the exception of an additional *Eco*RI site between physical coordinants 59.2 and 70.2%. The other two RoMNPV plaque-purified isolates, AR7 and AR8, will be shown to be recombinants of AcMNPV and RoMNPV. The restriction patterns of RoMNPV wt-1 were indistinguishable from those of RoMNPV R1 (data not shown).

RoMNPV R1 restriction endonuclease fragments. RoMNPV R1 DNA was cleaved with the restriction endonucleases *Eco*RI, *Xho*I, *Kpn*I, *Sac*I, *Bam*HI, and *Sma*I and electrophoresed in agarose gels. The fragment patterns are shown in Fig. 1 with individual fragments lettered sequentially by increased mobility. DNA bands which comigrate have been given a double-letter designation, e.g., *Eco*RI fragments IJ and MN. The molecular weights of RoMNPV R1 restriction fragments (Table 1) were estimated by comparing their mobility in agarose gels with restriction fragments of AcMNPV E2

FIG. 1. Autoradiogram of electrophoretically separated restriction fragments of RoMNPV R1 [32 P]DNA. The DNA was restricted with EcoRI, XhoI, KpnI, SacI, BamHI, and SmaI and then electrophoresed in 0.75% agarose. The EcoRI and SmaI fragments of AcMNPV E2 DNA were run in the first and last slots, respectively, as molecular weight standards. The size of each AcMNPV E2 fragment is labeled in megadaltons. Minor fragments in the EcoRI pattern were partially digested fragments and were not present in subsequent digests of RoMNPV R1 DNA.

[32 P]DNA and λ [32 P]DNA (9). RoMNPV R1 DNA has 22 EcoRI, 14 XhoI, 7 KpnI, 7 SacI, 8 BamHI, and 4 SmaI restriction enzyme fragments. DNA bands of a molecular weight less than 0.3×10^6 would not have been detected. The sum of the molecular weights of the restriction bands for each enzyme was approximately 82.0×10^6 .

Restriction map of RoMNPV R1 DNA.

The methods used to establish the order of restriction fragments have been presented in detail previously (8) in which the DNA restriction fragments of our AcMNPV variants were mapped. As RoMNPV and AcMNPV DNA restriction maps are quite similar, only an example of the reciprocal double-digest data used to map RoMNPV R1 DNA will be shown.

The KpnI fragments A through G of Ro-

TABLE 1. *Molecular weights of RoMNPV R1 restriction fragments*

Frag- ment	Mol wt ^a (× 10 ⁶)					
	<i>EcoRI</i>	<i>XhoI</i>	<i>SacI</i>	<i>BamHI</i>	<i>KpnI</i>	<i>SmaI</i>
A	8.7	19.5	20.0	32.5	26.6	45.4
B	8.0	14.8	20.0	18.7	18.5	16.1
C	7.8	10.4	14.5	16.2	12.6	12.2
D	7.0	8.7	14.5	5.4	8.4	8.2
E	6.9	6.8	7.0	5.0	6.5	
F	5.6	4.8	5.6	2.10	5.1	
G	5.0	4.1	0.68	1.20	4.0	
H	4.8	3.9		0.60		
I	4.1	3.6				
J	4.1	1.80				
K	4.0	1.52				
L	3.4	1.40				
M	2.50	0.74				
N	2.50	0.70				
O	1.90					
P	1.55					
Q	1.49					
R	1.20					
S	0.90					
T	0.78					
U	0.30					
V	0.30					

^a Molecular weights of restriction fragments were the mean of three independent determinations. Molecular weights of fragments greater than 10.0×10^6 were calculated from the sizes of restriction fragments after digestion of the isolated fragments with *EcoRI*.

MNPV [³²P]DNA were isolated, digested with *XhoI*, and then electrophoresed in agarose (Fig. 2). The following results were observed. (i) *KpnI* fragments F and G contained no *XhoI* cleavage sites. (ii) *KpnI* fragments D and E were each cleaved once by *XhoI* resulting in two end fragments. (iii) *KpnI* fragments A, B, and C each had two or more *XhoI* restriction cleavage sites to give at least one internal *XhoI* fragment and two end fragments, e.g., *KpnI* fragment A is cleaved by *XhoI* into *XhoI* fragments D, G, H, J, K, MN, and two end fragments of 4.2 and 3.0 megadaltons (Fig. 2).

A similar analysis was made with RoMNPV R1 [³²P]DNA *XhoI* fragments A through I that had been digested with *KpnI* (Fig. 2). By connecting the overlapping end fragments a partial order of *KpnI* and *XhoI* fragments could be determined as A-(F,G)-D-B-E-C and B-A(L,MN)-E-I,F-C(D,K,G,J,H,MN), respectively. The following experiments established a restriction map for the RoMNPV R1 genome. *SmaI*-A through D was cleaved with *KpnI*, *BamHI*, *SacI*, *XhoI*, and *EcoRI*; *KpnI*-A through G was cleaved with *SmaI*, *XhoI*, *SacI*, and *EcoRI*; *BamHI*-A through G was cleaved with *SmaI*, *KpnI*, and *EcoRI*; *SacI*-A through F

was cleaved with *SmaI*, *KpnI*, *XhoI*, and *EcoRI*; *XhoI*-A through I was cleaved with *SmaI*, *KpnI*, *SacI*, and *EcoRI*; and *EcoRI*-A through Q was cleaved with the other five enzymes. The order of *SmaI*, *KpnI*, *BamHI*, *SacI*, *XhoI*, and *EcoRI* fragments of RoMNPV R1 DNA that is consistent with the above data is presented in Fig. 3. The *XhoI* fragments K, G, and J were difficult to order by reciprocal digestion techniques, and the order of these fragments should be confirmed by other methods. To facilitate the comparison of restriction sites, the circular genome of RoMNPV R1 has been illustrated as a linear form in a manner similar to that presented for AcMNPV (8). The cleavage site between *BamHI* fragments A and E was chosen as 0.0% of the genome and used to orient the other restriction enzyme cleavage sites. In addition, each restriction site was labeled as a percent of the genome with respect to its position and the *BamHI*-A, 0.0% cleavage site.

AcMNPV and RoMNPV DNA linkage map. The restriction maps of RoMNPV R1 and AcMNPV E2, when aligned relative to the cleavage site between *BamHI* fragments A and F and *BamHI* fragments A and E, respectively (Fig. 4), show that these two genomes have many restriction sites in common. Of the 60 RoMNPV R1 restriction sites that have been mapped, 35 of these have the same or very similar positions as those in AcMNPV E2 DNA. Many of the RoMNPV cleavage sites that differ from AcMNPV are a result of either (i) the loss of a RoMNPV restriction site as compared to AcMNPV, e.g., RoMNPV *KpnI* fragment F maps in the same position as two AcMNPV *KpnI* fragments D and E or (ii) additional RoMNPV restriction sites as compared to AcMNPV, e.g., RoMNPV *BamHI* fragments A, E, and BC map in the same position as AcMNPV *BamHI* fragment A. The linkage map (Fig. 4) also suggests that the RoMNPV genome does not have any major deletions, insertions, or inversions in its nucleotide sequence when compared to AcMNPV.

Recombinants of AcMNPV and RoMNPV. The plaque-purified viruses AR7 and AR8 were isolated from RoMNPV wt-1. The *EcoRI* restriction patterns of AR7 and AR8 DNAs (Fig. 5) when compared to AcMNPV E2 and RoMNPV R1, suggest that AR7 and AR8 are recombinants of AcMNPV and RoMNPV. Both AR7 and AR8 have *EcoRI* restriction fragments with mobilities in agarose gels which are either (i) the same as RoMNPV R1 *EcoRI* fragments (ii) the same as AcMNPV E2 *EcoRI* fragments or (iii) different from both R1 and E2 *EcoRI* fragments. Analysis of AR7 and AR8

FIG. 2. *KpnI* fragments A through G of RoMNPV R1 [32 P]DNA were purified from 0.6% Sea Plaque agarose gels, and isolated fragments were restricted with *XhoI* and electrophoresed in 0.75% agarose (*KpnI* + *XhoI*). *XhoI* fragments A through I of RoMNPV R1 [32 P]DNA were purified from 0.6% Sea Plaque agarose, restricted with *KpnI*, and electrophoresed in 0.75% agarose (*XhoI* + *KpnI*). The resulting DNA fragments were labeled in megadaltons. *KpnI*, *XhoI*, and *EcoRI* digests of RoMNPV R1 DNA were electrophoresed in adjacent slots to provide molecular weight standards.

DNAs with *Bam*HI and *XhoI* (Fig. 5) gave similar results.

Structural polypeptides of recombinants AR7 and AR8. The viral structural polypeptides of the AcMNPV and RoMNPV recombinants, AR7 and AR8, were compared to the structural polypeptides of AcMNPV variants S1, E2, and R9 and RoMNPV R1 by polyacrylamide

gel electrophoresis. Four AcMNPV polypeptides with molecular weights of 90,000, 56,000, 40,000, and 37,000 are reproducibly distinct from four corresponding RoMNPV polypeptides with molecular weights of 91,000, 55,000, 39,500, and 36,000 (Fig. 6). The recombinant, AR7, has the RoMNPV major capsid polypeptides (molecular weight, 36,000) (10), the RoMNPV polypeptides

FIG. 3. Physical maps of RoMNPV R1 DNA derived in this study for the restriction enzymes *SmaI*, *KpnI*, *BamHI*, *SacI*, *XhoI*, and *EcoRI*. The circular DNA of RoMNPV is presented in a linear form for convenience of comparing the cleavage maps. The horizontal lines refer to the DNA and the vertical lines indicate cleavage sites. The *BamHI* cleavage site between fragments A and F at 0.0% was used to orientate all other cleavage sites. One hundred percent of the genome is equal to a molecular weight of 82.0×10^6 . Each cleavage site is labeled as to a percent of the genome and its position relative to the 0.0% cleavage site.

FIG. 4. Physical linkage maps of AcMNPV E2 and RoMNPV R1 DNAs. The cleavage sites of RoMNPV R1 are illustrated as described in Fig. 3, and the AcMNPV E2 cleavage sites are as presented by Smith and Summers (8). The vertical lines above and below each horizontal are the cleavage sites of AcMNPV E2 and RoMNPV R1 DNAs, respectively.

with molecular weights of 55,000 and 91,000, and the AcMNPV structural polypeptides of 40,000 and 56,000 molecular weights. The AR8 recombinant also has the RoMNPV capsid polypeptide of 36,000 molecular weight and the AcMNPV polypeptides of 40,000, 56,000, and 90,000 molecular weights.

The tryptic peptides of ^{32}P -labeled polyhedrin proteins of AcMNPV E2, RoMNPV R1, AR7, and AR8 were compared by two-dimensional

analysis on thin-layer cellulose plates as described above. The tryptic peptide patterns of AcMNPV E2 and RoMNPV R1 are similar but have several distinct differences (Fig. 7). The tryptic peptide patterns of AR7 and AR8 polyhedrins are indistinguishable from that of AcMNPV E2. An autoradiogram of AcMNPV E2 and RoMNPV R1 ^{32}P -labeled polyhedrin tryptic peptides (Fig. 7) shows that both polyhedrins contain two major phosphopeptides and

FIG. 5. The *EcoRI*, *BamHI*, and *XhoI* restriction fragments of RoMNPV R1, AR7, AR8, and AcMNPV E2 DNAs were electrophoresed in 0.75% agarose. The RoMNPV R1 and AcMNPV E2 DNA restriction fragments are lettered as described in the text. The *EcoRI* digest is an autoradiogram of ^{32}P -labeled viral DNAs and the *BamHI* and *XhoI* digests are the UV fluorescent bands of ethidium bromide-stained DNA.

that one of the phosphopeptides of AcMNPV E2 polyhedrin migrates differently when compared to the phosphopeptides of RoMNPV R1 polyhedrin (Fig. 7). The phosphopeptides of AR7 and AR8 polyhedrin were the same as AcMNPV E2 (data not shown). Thus, the AR7 and AR8 isolates have the AcMNPV polyhedrin protein.

DISCUSSION

In previous reports (2, 8, 9) wild isolates of AcMNPV derived from infected insects have been shown to be a mixture of several genomic variants. In addition, the genomes of *Trichoplusia ni* (TnMNPV) and *Galleria mellonella* (GmMNPV) NPVs are also very similar to that of AcMNPV (4, 9). Specifically, of the 60 restriction fragments mapped for AcMNPV (9), TnMNPV has 59 and GmMNPV has 56 endonuclease fragments with similar mobilities in agarose as AcMNPV fragments. That is, TnMNPV and GmMNPV both have greater than 90% of their restriction fragments in common

with AcMNPV. This degree of relatedness of the viral genomes is similar to the differences between AcMNPV variants mapped in our laboratory (9). We propose that GmMNPV and TnMNPV should also be considered as variants of AcMNPV. In contrast, only about 50% of the RoMNPV restriction fragments had the same mobility in agarose gels as AcMNPV. Therefore, RoMNPV may not be as closely related to AcMNPV as the AcMNPV variants. Consistent with this observation are differences in several major structural polypeptides of RoMNPV and AcMNPV, including the polyhedrin gene. In comparison, differences in the major structural polypeptides among AcMNPV variants or TnMNPV were not observed (9). The major structural polypeptides of GmMNPV are also very similar to those of AcMNPV with the exception of the AcMNPV protein of 40,000 molecular weight which is about 40,500 molecular weight in GmMNPV (9).

In this study we obtained plaque-purified isolates of RoMNPV from two sources of wild-type

FIG. 6. Polyacrylamide gel analysis of the structural polypeptides of AcMNPV variants S1, E2, and R9; RoMNPV R1; and AcMNPV and RoMNPV recombinants AR7 and AR8. About 40 μ g of protein of each purified virus was disrupted, electrophoresed,

virus, RoMNPV wt-1 and wt-2. Before plaque purification, both wild-type preparations had DNA restriction patterns that were indistinguishable from that of a plaque-purified isolate, RoMNPV R1. An analysis of the DNA restriction patterns of plaqued isolates from RoMNPV wt-2 did not reveal the presence of RoMNPV genetic variants. However, of three randomly picked viral plaques from RoMNPV wt-1, two were recombinants of AcMNPV and RoMNPV (AR7 and AR8) and one was a variant of AcMNPV (R9). As the restriction bands unique to the recombinants or the AcMNPV variant R9 cannot be detected in the DNA restriction patterns of RoMNPV wt-1, we would estimate that these genomes were present at no more than 10% of the total. In the absence of any selection, and assuming all three types of genomes were present at the 10% level, the probability of picking the two recombinants and the one AcMNPV

and stained as described in the text. Those virus proteins characteristically associated with AcMNPV (AMN VPs) and RoMNPV (RMN VPs) are labeled in kilodaltons.

FIG. 7. Two-dimensional analysis of the tryptic peptides of 32 P-labeled polyhedrin protein from AcMNPV E2, RoMNPV R1, AR7, and AR8. The digests were spotted in the lower right, electrophoresed for 30 min at 750 V, and then chromatographed in butanol/pyridine/acetic acid/water (50:33:1:40). The ninhydrin-stained tryptic peptides are shown in A, B, D, and E, and the autoradiograms of AcMNPV E2 and RoMNPV R1 32 P-phosphopeptides are shown in C and F, respectively. A mixture of four amino acids was spotted on the left of each plate before being chromatographed.

variant out of three randomly picked plaque would have been 1 in 1,000. One explanation for the occurrence of such an improbable event would be that genomes with certain AcMNPV sequences are more efficient at replication as detected by plaque formation. Information in a subsequent report, where isolates from mixed infection of RoMNPV and AcMNPV will be analyzed, supports this hypothesis.

The loss of or acquisition of restriction sites as a result of mutation is the simplest interpretation for many of the differences observed between the restriction maps of RoMNPV and AcMNPV DNA. There is presently no evidence that these viruses have evolved through major deletions or by insertions of other viral or host genetic information. However, minor deletions or insertions in the genome of RoMNPV relative to AcMNPV could have gone undetected. Recombination may also have played a role in the evolution of RoMNPV as the recombination frequency of RoMNPV and AcMNPV during mixed infection will be shown in a subsequent report to be high.

The segregation of AcMNPV and RoMNPV DNA restriction endonuclease cleavage sites as well as several viral structural proteins between the plaque-purified isolates AR7 and AR8 are convincing evidence that these are genetic recombinants. The recombination of baculovirus genes in nature and under controlled laboratory conditions may be important in the evolution of this group of viruses and in the development of our knowledge of the physical location and function of baculovirus genes.

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