A Provisional Classification of Cytoplasmic Polyhedrosis Viruses Based on the Sizes of the RNA Genome Segments

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SUMMARY

The RNA genome segments of thirty-three isolates of cytoplasmic polyhedrosis viruses (CPVs) were examined by polyacrylamide gel electrophoresis. Major differences were observed in the gel profiles of the RNA segments from many of the viruses; differences which were reinforced by polyacrylamide gel electrophoresis of the virus structural proteins. As a result of these studies, a provisional classification scheme for CPVs is proposed, where viruses with similar RNA gel profiles are included within the same ‘type’, while isolates differing in the molecular weights of most, or all of the RNA segments are assigned to different types. Using this system, eleven distinct CPV types were recognized. All eleven CPV types, like reoviruses, probably contain ten segments of RNA with a total mol. wt. of approx. $15 \times 10^6$.

INTRODUCTION

In those viruses of higher plants and animals which contain double-stranded RNA (dsRNA), the genome is segmented into 10 to 12 components of discrete sizes (Fujii-Kawata, Miura & Fuke, 1970; Verwoerd, Louw & Oellerman, 1970; Reddy & Black, 1973; Newman et al. 1975). There is good evidence, for reovirus, that the segments of RNA code for proteins which are closely similar in size to the theoretical primary gene products from each segment (Smith, Zweerink & Joklik, 1969; Both, Lavi & Shatkin, 1975). A similar relationship may also hold for cytoplasmic polyhedrosis viruses (CPVs) and other viruses containing dsRNA (Lewandowski & Traynor, 1972; Verwoerd et al. 1972; Payne & Tinsley, 1974; Payne & Kalmakoff, 1975).

Implicit in such a relationship is the understanding that viruses which differ from each other in the number and/or sizes of the RNA genome segments must also differ in other properties. For example, CPVs isolated from Inachis io (= Nymphalis io) and Spodoptera exempta have different RNA segments, different structural proteins, and are serologically unrelated, despite morphological similarity (Payne, 1976).

In recent investigations into the properties of CPVs, the RNA genome segments of many virus isolates have been examined. It appeared that the fractionation of the virus RNAs provided a good method for the provisional classification of these isolates into ‘types’ which differ markedly from each other in the sizes of the RNA segments. Such a scheme may also be applicable for other viruses containing dsRNA.
Methods

Purification of polyhedra and virus particles. Details of the 33 virus isolates used in this study are given in the results section. Many of the isolates had been collected by the former ARC Virus Research Unit, Cambridge. Polyhedra were purified as described by Payne (1976). Virus particles were isolated by dissolving the polyhedra for 3 to 30 min in 0.2 M-sodium carbonate–sodium bicarbonate buffer, pH 10.8, and were subsequently purified by sucrose gradient sedimentation (Payne, 1976).

Extraction of virus RNA. Virus dsRNA was obtained from purified polyhedra using a hot phenol-sodium dodecyl sulphate (SDS) extraction procedure (Payne & Tinsley, 1974).

Polyacrylamide gel electrophoresis of RNA and proteins. Purified RNA was electrophoresed on 3% gels as described by Payne & Tinsley (1974). The mol. wt. of the virus RNA segments were calculated by comparison with known values for the RNA segments of Bombyx mori CPV (Fujii-Kawata et al. 1970). Molar proportion measurements were made from gels scanned at 260 nm (Payne & Tinsley, 1974). The polypeptide components of polyhedra and virus particles were fractionated on 7% polyacrylamide gels (Payne, 1976), unless stated otherwise. The mol. wt. of the polypeptides were measured using standard proteins of known mol. wt. over the range 26000 to 200000 (α-chymotrypsinogen-A, 26000; carbonic anhydrase, 29000; lactic dehydrogenase, 36000; ovalbumin, 43000; bovine serum albumin, 68000; transferrin, 88000; phosphorylase, 94000; β-galactosidase, 130000; IgG, 150000; myosin, 200000).

Results

An analysis of the RNA components of several CPV isolates revealed considerable differences in the mobilities of the RNA genome segments. For example, a comparison of the RNAs from Bombyx mori, Inachis io, Spodoptera exempta, Actias selene, Trichoplusia ni, Biston betularia and Triphena pronuba CPVs is shown in Fig. 1. These RNA gel profiles were consistent for each virus isolate. A cold phenol extraction gave the same result as hot phenol, and RNA segments from virus particles had the same sizes as those obtained from polyhedra. Analysis of the structural proteins of polyhedra and virus particles further demonstrated the differences between the isolates (Fig. 2, 3, Table 2).

When the analysis was extended to a range of CPV isolates it became evident that some isolates contained RNA segments with similar mobilities. Thus, an RNA profile of the type obtained from Inachis io CPV was observed from viruses isolated from several different insect species (Table 3). It is reasonable to assume that viruses which have identical RNA gel profiles are very similar, if not identical, whereas viruses which have different RNA segments must differ in many properties. It was possible therefore to group CPVs into ‘type’ classes on the basis of similarities or major differences in the mobilities of the RNA components. Additional data on the structural polypeptides of polyhedra and virus particles were also obtained, where possible, to complement the information on the RNAs.

Eleven major ‘types’ of CPVs were recognized. The information on these viruses is presented below in the form of a brief description of some of the properties of one member of each ‘type’.

Type 1 Bombyx mori CPV

Virus isolate. Obtained from Professor K. Miura (National Institute of Genetics, Yata 1, 111 Misima, 411 Japan).

**CPV classification**

**Fig. 1.** Electrophoretic separation on 3% gels of the RNA components of six CPV isolates (a) Bombyx mori CPV; (b) Inachis io CPV; (c) Spodoptera exempta CPV; (d) Actias selene CPV; (e) Trichoplusia ni CPV; (f) Triphena pronuba CPV.

**Structural properties**

**RNA.** Nine components were separated on 3% gels (Fig. 2a). Band 2 has been resolved into 2 separate segments (Fujii-Kawata et al. 1970) and molar ratio measurements have confirmed the existence of 10 pieces of RNA with a total mol. wt. of $14.6 \times 10^6$ (Table 1, Fujii-Kawata et al. 1970).

**Proteins.** Virus particles contained five polypeptides, including three with a mol. wt. greater than 100000 (Lewandowski & Traynor, 1972; Payne & Kalmakoff, 1975). After prolonged storage, two polypeptides were resolved in the region of polypeptide 2 (Fig. 2c), probably arising as a result of proteolytic cleavage (Payne & Kalmakoff, 1975).

Polyhedra contained two polypeptides in addition to the three high mol. wt. virus proteins (Fig. 2b). The major polypeptide of the polyhedra (‘polyhedral protein’) has a mol. wt. of 27000 (Table 2). The mol. wt. of the structural polypeptides are in good agreement with values calculated by Lewandowski & Traynor (1972). It is likely that other estimates (Payne & Kalmakoff, 1975), particularly of the high mol. wt. virus proteins, are less...
Fig. 2. The structural components of CPV 'types' 1 to 5. (a to c): type 1 (Bombyx mori) CPV RNA, polypeptides of polyhedra and virus particles, respectively; (d to f): type 2 (Inachis io) CPV; (g to i): type 3 (Spodoptera exempta) CPV; (j to l): type 4 (Actias selene) CPV; (m to o): type 5 (Trichoplusia ni) CPV; (p to r): a comparison of the RNA segments of CPVs isolated from T. ni and Heliothis armigera; (p) H. armigera CPV; (q) H. armigera and T. ni CPV RNAs mixed and run on the same gel; (r) T. ni CPV.
Table 1. Molecular weights ($\times 10^{-6}$) of the RNA segments of CPV ‘types’ 1-11

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* The number of RNA segments was estimated by molar proportion measurements (‘types’ 1 to 8) or by staining intensity (‘types’ 9 to 11).
† From Fujii-Kawata et al. (1970).

Table 2. Molecular weights ($\times 10^{-3}$) of the polypeptides of CPV types 1-7

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accurate, as standard proteins of comparable size were not used. The relationship between the sizes of the structural proteins and the theoretical primary gene products (calculated as described by Smith et al. 1969) is close, with the exception of polypeptide V4.

Other isolates. A virus with the same RNA profile has been isolated from the pine caterpillar (Lymantria dispar; Miura & Shibuya, 1970).

Type 2 (Inachis io = Nymphalis io) CPV

Virus isolate. Obtained in 1962 from laboratory-reared larvae raised from field-collected adults (Agricultural Research Council Virus Research Unit, Cambridge, U.K.).


Structural properties

RNA. Nine components were separated on 3% gels (Fig. 2d). Molar proportion measurements confirmed that 10 segments of RNA are present (band 1 contains 2 segments) with a total mol. wt. of $14.36 \times 10^6$ (Table 1, Payne & Tinsley, 1974).

Proteins. Four virus particle polypeptides were resolved on 7% gels (Fig. 2f). Two of these (V1 and V2) had mol. wt. greater than 100000. Polyhedra contained one major poly-
peptide with three minor components (Fig. 2 e). The polyhedral protein (P4) had a mol. wt. of 30000 (Table 2). Using proteins of high mol. wt. as standards, the calculated mol. wt. values of P1, P2, V1 and V2, in particular, are greater than reported earlier (Payne & Tinsley, 1974). However, all the structural proteins still may be primary gene products of five of the RNA segments.

Other isolates. Viruses with the same RNA profile were also isolated from Glossiana dia, Dasychira pudibunda, Operophtera brumata, Papilio machaon and Phalera bucephala.

Type 3 Spodoptera exempta CPV


Structural properties

RNA. Seven to nine components were resolved on 3 % gels (Fig. 2g) although molar ratio measurements were consistent with there being 10 pieces of RNA with a total mol. wt. of approx. $15 \times 10^6$ (Table 1, Payne 1976).

Proteins. On 7 % polyacrylamide gels, virus particle proteins were resolved into six components, of which three had mol. wt. in excess of 100000 (Fig. 2 i, Table 2). Four proteins were consistently observed in samples of purified polyhedra (Fig. 2 h, Table 2; Payne 1976), the polyhedral protein having a mol. wt. of 28000. Minor components of intermediate size were sometimes observed in samples of polyhedra (Fig. 2 h, arrowed). These may represent proteins which are trapped non-specifically during formation of the polyhedra. All of the structural proteins could be assigned to specific RNA segments, using newly-calculated mol. wt. for the polyhedra and virus particle proteins. The greatest discrepancy occurred between the sizes of P1 (V1) and the theoretical product of the largest RNA segment.

Other isolates. Viruses with the same RNA profile were also isolated from Arctia villica, Automeris io, Danaus plexippus, Gonometa rufibrunnea, Operophtera brumata and Pieris rapae.

Type 4 Actias selene CPV

Virus isolate. From laboratory-reared larvae bred from pupae (received at ARC Virus Research Unit, Cambridge) from a site in the Himalayas.

Previous biochemical descriptions. None.

Structural properties

RNA. Seven bands were observed after fractionation of the RNA (Fig. 2 j) on 3 % gels. Molar proportion measurements were consistent with the presence of 10 components with a total mol. wt. of $15.5 \times 10^6$ (Table 1).

Proteins. Only three virus particle proteins were resolved by electrophoresis on 5 % SDS gels (Fig. 2 l). The two high mol. wt. polypeptides were also observed in samples of polyhedra (Fig. 2 k, Table 2), together with a major polypeptide of low mol. wt. (25000) and another minor component (P3). In contrast to the polyhedral protein molecules of most other CPVs, it was not possible to assign P4 to any specific RNA segment, as the smallest RNA segment could theoretically code for a protein much larger in size. P4 may be a cleavage product of a larger protein.

Other isolates. Viruses with the same RNA profile were also isolated from Antherea mylitta and Antherea pernyi.
CPV classification

Type 5 Trichoplusia ni CPV

Virus isolate. Received from Dr H. Stockdale and Dr R. Priston (Shell Research Laboratories, Sittingbourne, Kent). Virus infection was detected in laboratory cultures of T. ni in 1974.

Previous biochemical descriptions. None.

Structural properties

RNA. Eight bands were resolved on 3 % gels (Fig. 2m). Molar proportion measurements suggested that component 1 contained 3 segments of RNA of similar size, providing a genome with 10 pieces of RNA with a total mol. wt. of $14.8 \times 10^6$ (Table 1).

Proteins. Five virus particle polypeptides were observed (Fig. 2o). The two major proteins had mol. wt. in excess of 100000 (Table 2). In samples of polyhedra (Fig. 2n) the major polypeptide (polyhedral protein) had a mol. wt. of 26000. Five other (minor) polypeptides were also observed in polyhedra, including the two major virus particle proteins ($V_1 = P_1$; $V_2 = P_2$). The relationship between specific RNA segments and size of virus proteins is close, with the exception of $P_4$.

Other isolates. A virus with a similar RNA profile was observed in larvae of Heliothis armigera received from South Africa (from Miss R. Rubinstein, Virus Research Unit, Medical School, Observatory 7900, Capetown, South Africa). However, when samples of RNA from T. ni and H. armigera CPVs were mixed and run on the same gel, minor differences were observed (Fig. 2p to r). In particular, RNA segment 7 of H. armigera CPV had a fractionally greater mobility than the corresponding segment (band 5) in preparations of T. ni CPV. In addition, there was better resolution of the larger mol. wt. segments in H. armigera CPV which may reflect minor size differences between bands 1 to 3 of H. armigera CPV and the components of band 1 of T. ni CPV. However, these differences were small in comparison with the differences observed between the CPV ‘types’ described here. Differences of similar degree have been observed in isolates of wound tumour virus (Reddy & Black, 1974), and this may provide sufficient justification for placing H. armigera CPV in ‘type 5’, rather than including it as a distinct ‘type’ in its own right.

Type 6 Biston betularia CPV

Virus isolate. Dead larvae received for diagnosis at ARC Virus Research Unit, Cambridge, from Dr H. Kettlewell (Oxford).

Previous biochemical descriptions. None.

Structural properties

RNA. Ten bands were observed after fractionation of the virus RNA, and these components were present in approx. equimolar amounts (Fig. 3a, Table 1). The total mol. wt. of these segments was $15.3 \times 10^6$.

Proteins. Virus particles were not examined. The major polypeptide in samples of polyhedra had a mol. wt. of 29000 (Fig. 3b, Table 2). As with most other CPVs, the relation between the sizes of RNA segments and the mol. wt. of the virus structural proteins was close.

Other isolates. Viruses with the same RNA profile were also isolated from Agrochola lychnidis, Anchoscelis helvola, Antitype xanthomista and Phalera bucephala.
Fig. 3. RNA and polypeptide components of CPV 'types' 6, 7 and 11. (a and b): type 6 (Biston betularia) CPV RNA and polypeptides of polyhedra, respectively; (c and d): type 7 (Triphena pronuba) CPV; (e and f): the RNA segments of type 11 (Spodoptera exigua) CPV (e) compared with those of Arctia caja CPV (f), a naturally occurring mixture of types 2 and 3.

**Type 7 Triphena pronuba CPV**

**Virus isolate.** From ARC Virus Research Unit, Cambridge.

**Previous biochemical descriptions.** None.

**Structural properties**

**RNA.** Eight bands were separated when the virus RNA was fractionated on 3% gels (Fig. 3e). Like reovirus, but unlike the majority of CPVs, the RNA segments fall into three major size classes (1) 2·1 to 2·25 × 10⁶; (2) 1·34 to 1·49 × 10⁶; (3) 0·57 to 0·89 × 10⁶ (Table 1). Molar proportion measurements were consistent with there being 10 pieces of RNA, with a total mol. wt. of 15·35 × 10⁶. Band 1 probably contains 3 segments of similar mol. wt.

**Proteins.** Virus particles were not examined. The major protein of the polyhedron (P5) had a mol. wt. of 28000. This polypeptide and the four minor components (Fig. 3d, Table 2) could all be primary gene products of five of the virus RNA segments.

**Other isolates.** None.
Types 8-11

In addition to the seven 'types' described above, four other CPV isolates were obtained which also differed from each other and types 1 to 7, in the fractionation of the virus RNA segments. In these examples, we were not able to examine the structural proteins nor, in all cases, to measure the molar proportions of the viral RNA segments.

Type 8 Abraxas grossulariata CPV

Virus isolate. Dead insects from U.K. received for diagnosis at ARC Virus Research Unit, Cambridge.

Previous biochemical descriptions. None.

Structural properties

RNA. Nine bands were resolved after fractionation of the virus RNA (Fig. 4a). The staining intensity of band 1 was consistent with it containing 2 segments of RNA of similar size. This would provide a virus genome containing 10 RNA segments, with a total mol. wt. of $15\,23 \times 10^6$ (Table 1).

Other isolates. None. However, the mobilities of the RNA segments of this virus are similar to the intensely-staining RNA bands observed in the virus RNA of *Malacasoma disstria* CPV (Hayashi & Krywienczyk, 1972). The large number (16) of pieces of RNA observed in this virus probably results from a mixture of two CPVs (Payne, 1976), one of which may be similar to *A. grossulariata* CPV.

Type 9 Agrotis segetum CPV

Virus isolate. Dead larvae from U.K. received at ARC Virus Research Unit, Cambridge.

Previous biochemical descriptions. None.

Structural properties

RNA. Eight bands of RNA, falling into 3 major size classes were separated on gel (Fig. 4b). Bands 1 to 4 were in the ranges $2.04$ to $2.44 \times 10^6$; bands 5 to 6, $0.97$ to $1.32 \times 10^6$, and bands 7 to 8, $0.39$ to $0.44 \times 10^6$ (Table 1). The staining intensity with methylene blue suggested that bands 6 and 8 each contained 2 RNA segments of similar size.

Other isolates. None.

Type 10 Aporophylla lutulenta CPV

Virus isolate. Dead larvae received at ARC Virus Research Unit, Cambridge.

Previous biochemical descriptions. None.

Structural properties

RNA. Of the 7 RNA bands separated (Fig. 4c), the staining intensities of bands 1, 2 and 4 implied that each may contain two RNA segments (Table 1).

Other isolates. None.

Type 11 Spodoptera exigua CPV

Virus isolate. Virus diagnosed in larvae of *S. exigua* during cross-transmission experiments carried out at the Unit of Invertebrate Virology, Oxford.

Previous biochemical descriptions. None.
Fig. 4. The RNA segments of CPV types 8 to 10, separated by electrophoresis on 3 % gels stained with methylene blue and scanned at 600 nm. (a) Type 8 (Abraxas grossulariata) CPV, 3 mA/gel for 18 h; (b) type 9 (Agrotis segetum) CPV, 3 mA/gel for 12 h; (c) type 10 (Aporophylla lutulenta) CPV, 3 mA/gel for 18 h. Arrows (*) mark the mobility of RNA segments 1 to 9 of Bombyx mori (type 1) CPV.

Structural properties

RNA. Eight bands were separated by gel electrophoresis (Fig. 3c). Of these, bands 2 and 4 may each contain 2 segments (from their staining intensity) producing a virus genome with a total mol. wt. of 14.38 × 10^6 (Table 1). At least 2 minor components (arrowed in Fig. 3c) were also observed in the RNA samples examined. Further analysis may indicate whether these minor components imply that this virus isolate is a mixture of two or more distinct CPVs, similar to Arctia caja CPV (Fig. 3f, Payne 1976).

Other isolates. None.

Mixed CPV infections

In the CPV ‘types’ described above, it appears that each virus probably contains 10 segments of RNA, even though these segments have different size distributions. However, in four CPV isolates examined, more than 10 RNA segments were resolved on 3 % gels. In all these examples the observed RNA profile could be accounted for as a mixture of two distinct viruses replicating in the same insect species.
Arctia caja CPV. The twelve RNA components separated on 3% gels (Fig. 3f) result from a joint infection of A. caja with viruses of types 2 and 3 (Payne, 1976).

Anaitis plagiata CPV. The twelve RNA components in samples from this virus have the same mobilities as the RNA segments of CPV's types 3 and 6 (Fig. 5).

Aglais urticae CPV. Thirteen RNA components with the same mobilities as segments of types 2 and 6 were observed.

Phlogophora meticulosa CPV. Of the twelve RNA components, the major ones had the same mobilities in gel as the segments of type 3 CPV, while the minor components could be attributed to a joint infection with type 8 CPV.

**DISCUSSION**

Cytoplasmic polyhedrosis viruses have been distinguished from other insect viruses by a variety of criteria. In the replication process, polyhedral inclusion bodies (polyhedra) are formed in the cytoplasm of infected cells. These polyhedra contain icosahedral virus particles, 50 to 70 nm in diam. which can be released by treatment with alkali (Hills & Smith, 1959; Hosaka & Aizawa, 1964; Aruga & Tanada 1971; Lewandowski & Traynor, 1972). The virus nucleic acid is dsRNA, and separates into a number of segments when the RNA is fractionated by polyacrylamide gel electrophoresis (Kalmakoff, Lewandowski & Black, 1969; Fujii-Kawata et al. 1970; Payne & Tinsley, 1974; Payne, 1976). However, no
Table 3. A summary of the classification of CPV isolates from 31 insect species

<table>
<thead>
<tr>
<th>Insect host</th>
<th>CPV 'type'</th>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>Abraxas grossulariata</td>
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<tr>
<td>Actias selene</td>
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<tr>
<td>Aglais urticae</td>
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<td>Agrochola lychnidis</td>
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<td>Agrotis segetum</td>
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<td>Anaitis plagiata</td>
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<td>Anchocelis helvola</td>
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<td>Antheraea mylitta</td>
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<td>Antheraea pernyi</td>
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<tr>
<td>Antitype xanthomista</td>
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<tr>
<td>Apororphylla lutentula</td>
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<td>Arctia caja</td>
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<td>Arctia villica</td>
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<tr>
<td>Automeris io</td>
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<td>Biston betularia</td>
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<td>Bombyx mori</td>
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<td>Clossiana dia</td>
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<td>Danausplexippus</td>
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<td>Dasychira pudibunda</td>
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<td>Gonometa rufibrunnea</td>
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<tr>
<td>Heliolith armigera</td>
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<tr>
<td>Inachis io</td>
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<tr>
<td>Operophthera brumata</td>
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<tr>
<td>Papilio machaon</td>
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<tr>
<td>Phalera bucephala</td>
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<tr>
<td>Philogophora meticulosa</td>
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<td>Pieris rapae</td>
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<td>Spodoptera exempta</td>
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<td>Spodoptera exigua</td>
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<tr>
<td>Trichoplusia ni</td>
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<tr>
<td>Triphena pronuba</td>
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</tbody>
</table>

* Virus is probably a mixture of two distinct CPV types.

satisfactory classification of these viruses has been devised. As with other dsRNA viruses, an analysis of the virus RNA by gel electrophoresis provides insight into several properties of the virus. For example, it reveals the probable size of the virus genome, whether or not it is segmented, the number of segments which are involved, the theoretical coding potential of each segment, and hence the possible sizes of virus structural and non-structural proteins. Such information is important for any classification of these viruses.

The fractionation of the virus RNA also provides a method for classifying CPVs based on the properties of the virus, rather than, as in the past, by reference to the insect host from which the virus was isolated. The obvious dangers of the older method are highlighted by the observation that at least two distinct types of CPVs can replicate in the same insect species (e.g. *P. bucephala* and *O. brumata*, Table 3).

More conventional classifications of dsRNA viruses are based on serological typing. Thus mammalian reovirus types 1 to 3 were resolved first by their antigenic differences (Sabin, 1959), differences which were later confirmed by an analysis of the virus RNA segments (Shatkin, Sipe & Loh, 1968) and by RNA homology studies (Martinson & Lewandowski, 1975). Certainly, a gel analysis of the RNAs of dsRNA viruses can be performed more rapidly than many serological analyses, allowing a larger number of
CPV classification

In this study, polyhedra of 33 CPV isolates from 31 species of lepidopterous insects were examined. The virus RNA was segmented into components of discrete sizes in all examples. Not all the RNA profiles were different from each other, but the data enabled 29 of the virus isolates to be grouped into 11 virus types which were identified by major differences in the mobilities of the RNA segments. The remaining four CPV isolates were interpreted as probable mixtures of two distinct virus types. These results are summarized in Table 3. Although the scope of this survey was biased unavoidably towards viruses isolated from insects occurring in Europe, it is of interest to examine some of the factors which might influence the natural distribution of these types.

All isolates of type 4 CPV were obtained from the same locality in Asia, and therefore this virus may be restricted geographically. Insects infected with viruses of type 2, 3 and 6 shared a predominantly European or Palearctic geographical distribution. In instances where this did not occur (e.g. S. exempta and G. rufibrunnea) the virus infection could have arisen as a result of cross-contamination within laboratories which were handling similar viruses.

The sharing of a common food plant by the insect hosts, represents an ecological niche, and so this association may influence viruses. It is significant that several insect species susceptible to viruses of types 2, 3 and 6 use nettle (Urtica dioica) as their principal food. Moreover, the taxonomic status of the insect host may be significant in determining its susceptibility to a CPV of a particular type. Several members of the family ‘Nymphalidae’ are capable of infection with viruses of type 2 CPV (I. io, A. urticae and C. dia) while S. exempta and P. meticulosa (which are susceptible to infection with type 3 CPV) have no common geographical or ecological distribution, but are both members of the same sub-family (Amphipyrae).

Certain unifying features emerge from an examination of CPV components, despite the large differences in both RNAs and structural proteins. In all isolates examined, with the exception of the ‘mixtures’, the CPVs probably contained 10 pieces of RNA, although all 10 segments were only clearly resolved in CPV type 6. At least four of these RNA segments had mol. wt. in excess of \(2 \times 10^6\). The total mol. wt. of the virus genome is approx. \(15 \times 10^6\), although a rather low value of \(13.62 \times 10^6\) was found for A. segetum (Type 9) CPV. Polyhedra contain one polypeptide (polyhedral protein) in much greater amounts than any other and this has a mol. wt. within the range 25000 to 30000. Virus particles contain at least two (Types 2, 4 and 5) and sometimes 3 (Types 1 and 3) structural proteins with mol. wt. in excess of 100000. Finally, although the comparative morphology of CPV virus particles requires more study, the results which are available suggest that the virus types may be morphologically similar or identical (Cunningham & Longworth, 1968; Payne, 1976).

CPVs also possess many features in common with other dsRNA viruses (Lewandowski & Traynor, 1972; Payne & Tinsley, 1974). Reoviruses, orbiviruses and certain plant viruses also contain 10 segments of RNA with a mol. wt. of \(15 \times 10^6\) (Shatkin et al. 1968; Verwoerd...
et al. 1970; Redolfi & Boccardo, 1974). Like reovirus, the sizes of most of the CPV structural proteins are close to the sizes of the theoretical primary gene products from certain RNA segments. However, there are several exceptions to this in the CPV types described above. These exceptions may result from post-translational cleavage of primary gene products. Both et al. (1975) have recently identified six virus-specific polypeptides in reovirus-infected cells which are not primary gene products and so post-translational cleavage may be a feature of the replication of dsRNA viruses.

In all viruses which contain segmented genomes, recombination or genetic reassortment of the segments provides an explanation for the origins of some new naturally occurring virus strains (Fields, 1973; Webster, Laver & Granoff, 1973). It is possible that reassortment of RNA segments in such 'mixed' infections as A. caja CPV (Payne 1976) and other apparent CPV mixtures (Table 3) could lead to the production of new CPV strains, differing in one or more segments from the parental types. In contrast, the more subtle differences in the RNAs of H. armigera and T. ni CPVs (Type 5) may have arisen by the loss of a small piece of RNA from one of the genome segments. RNA homology studies could help both in clarifying the similarities or differences between these two viruses and in comparisons of the major CPV types described here.

Although differences in the sizes of RNA segments of CPVs imply that the viruses differ in a range of biochemical and biological properties, viruses which share the same RNA profile may not necessarily be identical. The RNA segments could be the same size, but contain base sequence differences between virus strains, which could only be resolved by detailed homology studies.

We thank the many people who, in the past, sent samples of virus-infected insects to the ARC Virus Research Unit, Cambridge, and in recent years to the NERC Unit of Invertebrate Virology, Oxford.

REFERENCES


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