Serological Relations Between Twelve Small RNA Viruses of Insects

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SUMMARY

Serological tests were done to examine the relationships between twelve picorna-like viruses of insects. The results of the tests indicated that the majority of the viruses are unrelated. However, cricket paralysis virus, isolated from Australian wild field crickets, appeared to be identical to Drosophila C virus, independently isolated in France. Cricket paralysis virus was infective for adults of Drosophila melanogaster and its infectivity towards Galleria melonella was neutralised by Drosophila C virus antiserum. It is therefore concluded that cricket paralysis virus and Drosophila C virus are very closely related if not identical.

An increasing number of insect viruses is being isolated which have some features in common with the mammalian picornaviruses. However, very few of these viruses have been sufficiently studied to place them in the general scheme of virus classification. Apart from the report of Juckes, Longworth & Reinganum (1973), no systematic study has been made of the serological relationships which may exist between these picorna-like viruses.

This paper reports the results of tests of the serological relationships between twelve insect viruses. All the viruses have non-enveloped isometric particles 25 to 35 nm in diam., and contain, or are suspected of containing, RNA. Tests were done by the agar gel double diffusion technique or by immuno-osmophoresis (Ragetli & Weintraub, 1964). The results of the tests are presented in Table 1.

Judging from the results of the tests, the majority of the viruses appear to be serologically unrelated. However, a distinct band of precipitate was obtained between Drosophila C virus (DCV) antiserum and cricket paralysis virus (CrPV) antigen (Fig. 1 a). Tests done by the Ouchterlony method showed a reaction of identity when CrPV antigen reacted with CrPV antiserum and DCV antiserum in adjacent wells (Fig. 1 b). The relationship between the two viruses was investigated further by injecting CrPV into adult Drosophila melanogaster. Fifty flies were injected with a purified suspension of CrPV containing about 10¹⁰ particles/ml. A control group of 50 flies received virus which had been inactivated by treatment with 2 % formaldehyde and then dialysed. The injected flies were incubated at 26°C and flies killed as a result of the injection technique were culled the following day. No further deaths were noted until the third day after injection when a few of the CrPV-injected flies had died. By day six, 83 % of the flies injected with CrPV had died whereas all the control flies remained healthy. A series of neutralisation tests was also conducted, in which Galleria melonella was used as the test species; this species is one of several Lepidoptera which is susceptible to CrPV (Reinganum, 1975). Each group of 25 larvae was injected with one of the following: a purified suspension of CrPV, the same suspension treated with DCV antiserum, and a solution of physiological saline. All the larvae which had been injected with CrPV died, whereas all those which had received antiserum-treated virus or saline survived.

Taken together, the results of the serological and the infectivity tests indicate that CrPV and DCV are closely related, if not identical viruses. Extracts of Drosophila melanogaster
Table I. The serological reactions between twelve small RNA viruses of insects*

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<tr>
<th>Antiserum</th>
<th>CrPV</th>
<th>TPV</th>
<th>KFV</th>
<th>ABPV</th>
<th>AV†</th>
<th>DPV</th>
<th>DCV</th>
<th>DiV</th>
<th>GV</th>
<th>SBV</th>
<th>SFV‡</th>
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* A positive reaction is indicated by a +, and a negative one by a -. Homologous reactions are omitted. Abbreviations are as follows: CrPV, cricket paralysis virus (Reinganum, O'Loughlin & Hogan, 1970); TPV, termite paralysis virus (Gibbs, Gay & Wetherly, 1970); KFV, kelpfly virus (Scotti, Gibbs & Wrigley, 1975); ABPV, acute bee paralysis virus (Bailey, Gibbs & Woods, 1963); AV, Antheraea virus (Grace & Mercer, 1965); DPV, Drosophila P virus (Plus & Duthoit, 1969); DCV, Drosophila C virus (Jousset et al., 1972); DiV, Drosophila iota virus (Jousset, 1970); GV, Gonometra virus (Harrap et al. 1966); SBV, sacbrood virus (Bailey et al. 1964); SFV, silkworm flaccherie virus (Aizawa et al. 1964); NV, Nodamura virus (Scherer & Hurlbut, 1967).

All tests were done in our laboratories. However, where additional data was available in the literature, this information is referenced by superscript notation.

5. Jousset et al. (1972).

† Serologically related to Nudaurelia capensis β virus (Juckes et al. 1973).
‡ Silkworms affected by flaccherie may contain two types of particles which have been designated SFV I and SFV II (Himeno, Onodera & Tanami, 1974). The antiserum used in our tests was to a mixture of the two.

that died as a result of the CrPV injection gave no precipitin line when tested with CrPV antiserum; however, only a small sample of flies was used, so the negative result was not unexpected.

Certain predictions can be made regarding some of the serological relationships which remain untested. Since CrPV and DCV are serologically indistinguishable, it is unlikely that DCV shares a common antigen with Nodamura virus (NV), Gonometra virus (GV) or silkworm flaccherie virus (SFV), all of which are serologically unrelated to CrPV. Furthermore, NV and GV are unlikely to be related as they possess radically different capsid proteins (Brown & Hull, 1973).

There has been a tendency to regard insect viruses as being highly specific in host range. Although this may be true for the occluded viruses, there is increasing evidence that this may not be the case for several picorna-like insect viruses. This view is supported by the results reported here of a close relationship between DCV and CrPV which were independently isolated from Drosophila and field crickets (Teleogryllus commodus and T. oceanicus) occurring in widely separated geographical areas. Furthermore, CrPV has been experimentally transmitted to several species of Lepidoptera and has been detected as an
inapparent infection in populations of the emperor gum moth, *Antheraea eucalypti* (Reinganum, 1975). Similarly, the lepidopteran *Persectania ewingii* has recently been found to carry inapparent infections of both CrPV and KFV, which was originally isolated from the dipteran *Caetocoelopa sydneyensis* (Scotti, Gibbs & Wrigley, 1975). We would therefore stress the importance of serologically testing as many of the picorna-like insect viruses as possible, particularly newly isolated viruses, to avoid further confusion.

We are grateful to the following for gifts of antiserum: L. Bailey (ABPV, SBV, NV and TPV); H. Inoue (SFV); J. F. Longworth (GV); N. Plus (DiV, DCV, DPV); We would also like to thank L. Bailey for SBV antigen.

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