Oligonucleotide Fingerprinting of the RNA Species Obtained from Six
Drosophila C Virus Isolates

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SUMMARY

The virion RNAs of six independent isolates of Drosophila C virus (DCV) have been characterized by ribonuclease T1 oligonucleotide fingerprinting. All six isolates share common large oligonucleotides. Two of the isolates, from Vigier and Charolles, are closely related while a third French isolate from Gif is more distantly related. The other three isolates, two from Morocco (Taroudant and Ouarzazate) and one from the French Antilles were mixtures of more than one variant of DCV, but were clearly related to the three French isolates.

Laboratory and wild populations of Drosophila melanogaster are susceptible hosts for the picornavirus Drosophila C virus (DCV). Little is known about the vertical or lateral transmission of this virus, which can lead to the collapse of laboratory colonies of flies, or its ability to exist as an inapparent infection or in tissue culture. Initial work with several isolates of DCV demonstrated that differences in pathogenicity and possibly in host range occurred (Plus et al., 1978).

Recent work on the polypeptides induced by several isolates of DCV in infected Drosophila cells has indicated that the proteins appear to be very similar. The similarities were confirmed by limited proteolysis of the major induced proteins (Moore et al., 1982). It was hence necessary to examine the different strains by a more sensitive method in an attempt to differentiate between them. Oligonucleotide mapping was used to investigate the differences between the RNAs of six isolates from various locations. Viruses infecting insects are not under pressure from an immune system based on immunoglobulins and this may result in less pressure to mutate or alter the structural proteins. Hence the genomes of insect viruses from different locations may not differ greatly.

The 32P-labelled RNA isolated from purified virions of DCV from Vigier, Charolles and Gif was analysed by two-dimensional polyacrylamide gel oligonucleotide fingerprinting after digestion with ribonuclease T1 (Fig. 1, V, C and G respectively). The three RNA species each gave characteristic oligonucleotide fingerprints which are obviously closely related to each other. DCVc has oligonucleotides in common with DCVV, but is missing five DCVV oligonucleotides (open circles). It has at least six large oligonucleotides not found in DCVV (arrow heads). Extensive sequence homology is therefore shared by DCVC and DCVV, suggesting that they are probably variants of the same parental variety. The fingerprint of DCVG resembles DCVV less than DCVC does. There are at least seven large DCVV oligonucleotides that are absent from DCVG (open circles). At least two large DCVC oligonucleotides are not found in either DCVV or DCVC (arrowed). There are several differences in the migration of the T1 oligonucleotides in the central region of the gel, but these changes are such that it is not possible to draw unequivocal conclusions about which are common and which are unique. It is likely that DCVV and DCVC are more closely related to each other than either is to DCVG. Thus if the three viruses are derived from the same parental DCV, DCVG has undergone more sequence divergence than DCVV or DCVC.

Oligonucleotide fingerprinting of the RNAs extracted from the Moroccan isolates (Fig. 2, O and T) and the isolate from the French Antilles (Fig. 2, A) gave a pattern of spots different in complexity from those obtained with the three French isolates. A greater number of large oligonucleotides were observed in the fingerprint of each RNA species. In addition, the Moroccan and French Antilles isolates gave fingerprints with many unique oligonucleotides obviously present in non-equimolar amounts. These observations suggest that these three
Fig. 1. Fingerprint patterns of RNase T1-resistant oligonucleotides of the RNA of three French isolates of DCV from Vigier (V), Charolles (C) and Gif (G). Confluent 81 cm² monolayers of Drosophila melanogaster cells were infected with DCV and labelled with 0.25 mCi/ml carrier-free [32P]orthophosphate in phosphate-free Schneider's Drosophila medium as previously described (Pullin et al., 1982). Virions were purified and the 32P-labelled RNA extracted as described by Reavy & Moore (1981). The DCV RNAs were digested with RNase T1 and the oligonucleotides were separated by two-dimensional polyacrylamide gel electrophoresis using the method of De Wachter & Fiers (1972) as modified by Clewley et al. (1977). The upper and lower crosses indicate the position of the bromophenol blue and xylene cyanol FF markers respectively. In the middle panel, oligonucleotides present in DCVc but not in DCVv are indicated by arrowheads, while open circles indicate DCVv oligonucleotides absent from DCVc. Similarly, in the bottom panel DCVv oligonucleotides missing from DCVg are indicated by...
viruses are not 'pure' but are mixtures of more than one virus. In the case of DCV\textsubscript{O}, all the oligonucleotides that occur with DCV\textsubscript{C} are present with one possible exception (indicated in Fig. 1). Many additional oligonucleotides are also present. Similarly, DCV\textsubscript{T} appears to contain all the oligonucleotides present in DCV\textsubscript{V} and has, in addition, other oligonucleotides. The fingerprint of DCV\textsubscript{A} is very similar, if not identical, to that of DCV\textsubscript{T} so that these two isolates may be mixtures of the same viruses.

It is likely that DCV\textsubscript{O}, DCV\textsubscript{T} and DCV\textsubscript{A} are mixtures of DCV variants and not of DCV and another virus species, since the patterns of spots are compatible with those found with the French isolates except that there are more than expected. While all of the isolates contain a single piece of RNA with mol. wt. approx. 2.8 \times 10^{6}, as we have previously demonstrated with Cricket paralysis virus (CrPV) and DCV\textsubscript{O} (Pullin \textit{et al.}, 1982), it cannot be ruled out that more than one insect picornavirus, of identical genome size, is present. While it has been possible to plaque-purify CrPV (Moore \& Pullin, 1982) it has not been possible to obtain plaques with DCV although this virus does cause a measurable cytopathic effect (Moore \textit{et al.}, 1981).

We have demonstrated previously that it is possible to distinguish DCV\textsubscript{O} and CrPV by oligonucleotide mapping (Pullin \textit{et al.}, 1982). CrPV and DCV can be distinguished on the basis of such criteria as host range, pathogenicity, structural protein content and the induction of obviously different proteins in infected tissue culture cells, but it is much more difficult to differentiate between the different DCV isolates. They are serologically very similar, having a similar protein content, although they were found to grow to different titres in different hosts (Plus \textit{et al.}, 1978). Slight differences in the induced proteins appeared to indicate that DCV\textsubscript{A} and DCV\textsubscript{T} are very similar to each other, as are DCV\textsubscript{V} and DCV\textsubscript{O} (Moore \textit{et al.}, 1982). This is supported by the information presented here, demonstrating the relationship between these viruses much more clearly.

While it is of obvious interest to determine the pressures which cause alterations in the genome of insect picornaviruses in the absence of an immune system, it will be necessary in the future to ensure that the insects are free of inapparent viruses and to plaque-purify the isolates.

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open circles, while arrowheads show two oligonucleotides not found in DCV\textsubscript{V} or DCV\textsubscript{C}. Poly(A) is visible on two panels (V and C) and is indicated by the arrowed 'A'. The directions of migration in the first and second dimensions are indicated on the lower panel. 'B' is the only nucleotide present in DCV\textsubscript{C} that is absent from DCV\textsubscript{O}.

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Fig. 2. Fingerprint patterns of RNase T\textsubscript{1}-resistant oligonucleotides of the RNA of the two Moroccan isolates of DCV from Ouarrazate (O) and Taroudant (T) and the isolate from the French Antilles (A). Poly(A) was apparent with all the DCV isolates as has been described previously for CrPV (Eaton \& Steacie, 1980; Pullin \textit{et al.}, 1982). Poly(A) is indicated on panels O and A. The two directions of migration are indicated.
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Short communications


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