A Virus Made from Parts of the Genomes of Brome Mosaic and Cowpea Chlorotic Mottle Viruses

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Brome mosaic virus (BMV) and cowpea chlorotic mottle virus (CCMV) are spherical plant viruses which have recently been shown to possess divided genomes (Lane & Kaesberg, 1971; Bancroft, 1971) even though the virus particles sediment as single species. BMV contains four species of RNA with molecular weights of $1.09 \times 10^6$, $0.99 \times 10^6$, $0.75 \times 10^6$ and $0.28 \times 10^6$ and CCMV contains four species with molecular weights of $1.15$, $1.0$, $0.85$ and $0.32 \times 10^6$ (Fowlks & Young, 1970). The species are named 1, 2, 3 and 4, respectively. Species 1 + 2 + 3 are required to initiate BMV infections (Lane & Kaesberg, 1971) but CCMV seems only to require species 1 + 2, although the addition of species 3 enhances lesion production (Bancroft, 1971). Species 4 is needed by neither virus for infection. I wished to determine if a virus possessing some properties of both viruses could be obtained by mixing BMV species 1 + 2 with CCMV species 3 prior to biological assay. This report describes the construction of such a virus.

BMV and CCMV, grown in barley (Hordeum vulgare L.) and cowpea (Vigna unguiculata (L.) Walp), respectively, were purified by two cycles of differential ultracentrifugation after an initial clarification step at pH 4.7 to 5 (Hiebert, Bancroft & Bracker, 1968). RNA was obtained by phenol extraction and the RNA species were separated by electrophoresis in 2.6% polyacrylamide gels (Loening, 1967). The RNA bands were lightly stained with toluidine blue and the BMV species 1 + 2 and 3 and CCMV species 1 + 2 and 3 were recovered by phenol extraction as reported (Bancroft, 1971). These were assayed separately and together on young, horticulturally ‘soft’ Chenopodium hybridum L. The assay plants were kept for 1½ to 2 weeks and suitable single lesions were picked and subcultured on C. hybridum before transfer to Chenopodium quinoa Willd. The progeny, purified as for the normal viruses, was examined by various biological and physical tests, to be described, to ascertain its nature.

The preparations of BMV species 1 + 2 had only about 4% of the infectivity of homologous mixtures of species 1 + 2 + 3, and, like the complete genome, produced large necrotic lesions, probably as a result of imperfect separations. CCMV species 3 produced no lesions, but augmented the infectivity of species 1 and 2 by about fourfold to produce moderately sized necrotic lesions. As previously reported (Bancroft, 1971), addition of BMV species 3 to a combination of CCMV species 1 and 2 did not increase lesion numbers significantly or produce atypical lesions. However, a combination of BMV species 1 + 2 with CCMV species 3 produced small lesions, not normally present, at about 10% of the efficiency of the complete homologous BMV mixture. These small lesions are easily distinguishable from those of the parental viruses (Fig. 1). CCMV and BMV have distinct host ranges, the former being largely limited to the Leguminosae and the latter to the Gramineae; CCMV infects cowpea but not barley, whereas BMV infects barley but not cowpea. The ‘hybrid’ virus infects neither, but seems to be restricted to hosts common to both viruses. In Chenopodium quinoa, BMV multiplies systemically, whereas CCMV is confined to local lesions, at least at temperatures of about 25°C. The ‘hybrid’ virus is also confined to local lesions. Thus, the biological properties of the ‘hybrid’ virus were restricted by the limiting virus; there was no combination of biological properties. The ‘hybrid’ virus reached a concentration of about
Fig. 1. Chenopodium hybridum leaves with local lesions caused by ‘hybrid’ virus (upper left and lower right), CCMV (upper right) and BMV (lower left) 11 days after inoculation.

4 mg./100 g. wet weight tissue in C. quinoa leaves which was about three times the concentration found in C. hybridum leaves. About 100 mg. of BMV and about 4 mg. of CCMV were obtained from 100 g. samples of C. quinoa infected with the parental viruses. About 70 mg. of virus was obtained from doubly-infected plants.

The ‘hybrid’ virus did not react against BMV antiserum in either ring interface or gel-diffusion tests in which the homologous reactions were positive, but did against CCMV antiserum. The precipitin lines between CCMV and the ‘hybrid’ virus in the gel diffusion tests fused (Fig. 2). This means that CCMV species 3 carries, as does BMV species 3 (Lane & Kaesberg, 1971), the coat protein cistron. It was reported (Bancroft, 1971) that CCMV species 1 + 2 were sufficient to cause infection and produce mature virus. However, since
CCMV species 3 carries the coat cistron, it would seem that it should, under the simplest assumptions, be required for the production of mature virus in the homologous system even though repeated efforts to establish this were unsuccessful.

BMV and CCMV cannot be clearly distinguished from one another or from the ‘hybrid’ virus by appearance in the electron microscope or by sedimentation rate. They can, however, be distinguished from one another or from the ‘hybrid’ virus by their behaviour in CsCl. BMV and CCMV have different densities, largely due to the different compositions of their coat proteins, and density patterns. The latter are probably a result of slightly different molecular weights of the sums of the jointly encapsidated 3 + 4 RNA species relative to the molecular weights of the separately encapsidated 1 and 2 species which comprise the heavy and light bands in CsCl (details of density-distribution considerations, which obviate the necessity of the monomer-tetramer theory (Bancroft et al. 1968), will be published separately). This difference in relative densities results in CCMV having a clear central component whereas BMV seems to lack one (Fig. 3). The ‘hybrid’ virus is similar to BMV in its apparent density distribution (Fig. 3), but similar in density to CCMV. The densities of the particles which form the heavy and light BMV bands are 1.355 and 1.348 g./cm$^3$, respectively, whereas those of the ‘hybrid’ virus are 1.366 and 1.358 g./cm$^3$, which are similar to the 1.364 and 1.356 g./cm$^3$ densities found for the extreme bands of CCMV. The density data are consistent with the expected properties of a virus composed of CCMV protein and RNA species 1 and 2 from either virus, since the true molecular weight of CCMV species 2 RNA is complicated by conformational factors which can result in the formation of doublets of this RNA species on acrylamide gels (Bancroft, 1971).
When CCMV or BMV are grown in *Chenopodium quinoa* separately or together, the four principal RNA species associated with these viruses are obtained. In contrast, the 'hybrid' virus yields only two major RNA species after extraction with phenol and polyacrylamide gel electrophoresis (Fig. 4). These RNAs have the molecular weights of BMV RNA species 1 and 2 and the RNA species 2 does not form conformational doublets if not treated in 1 M-urea as does CCMV RNA (Bancroft, 1971). The minor RNA peak, identified by an arrow in Fig. 4, may be a degradation product since its molecular weight (0.9 × 10^6) is distinct from species 3 from either CCMV or BMV; there appears to be no CCMV RNA in the 'hybrid' virus. However, since purified 'hybrid' virus incites small lesions containing virus with CCMV coat protein, CCMV RNA must be encapsidated. Thus, the easiest explanation of the acrylamide patterns is that CCMV RNA species 3 – and perhaps 4 as well – is actually present, but in amounts below the limits of the detection system. Such a result provides a sobering illustration of the difficulty that may be encountered in assessing the extent of cross-contamination in separations of RNA species.

The identification of the two RNA species from the 'hybrid' virus, which are not infective together, was verified biologically by adding separately prepared BMV species 3 to them. Such a mixture incited normal BMV lesions containing BMV on *Chenopodium*...
Fig. 4. Distribution of CCMV (top), 'hybrid' virus (middle) and BMV (bottom) RNAs after 3 hr electrophoresis in 2.6% polyacrylamide gels. The arrow designates the minor species mentioned in the text. The inset shows 'hybrid' virus and BMV RNAs after 4½ hr electrophoresis.

hybridum, whereas the addition of CCMV species 3 resulted in small lesions. Therefore, BMV species 1 and 2 were not changed by their association with CCMV species 3. No biological function could be associated with the $0.9 \times 10^6$ RNA found in the 'hybrid' virus.

The production of 'hybrid' viruses from admixtures of suitable complementary parts of divided genomes have so far been confined to strains of the classical multi-component viruses. New strains of cowpea mosaic virus (De Jager & van Kammen, 1970), alfalfa mosaic virus (van Vloten-Doting, Kruseman & Jaspars, 1968) and tobacco rattle virus (Sanger, 1968; Lister, 1969) have been produced only between members with easily demonstrable serological affinities. Although BMV and CCMV share many physical properties and are considered to belong to the same virus group because of those properties (Harrison et al. 1971), they have been regarded as probably being distinct serologically (Bancroft et al. 1968) and they have almost totally dissimilar host ranges. Yet, these viruses have genomes which are able to complement each other. This evidence, which strongly indicates albeit by novel means that the viruses are related, would not have been obtained if very susceptible assay plants had not been used and it is conceivable that partially aborted infections have also
occurred, but have been overlooked, in complementation tests between dissimilar isolates of other viruses.

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REFERENCES


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