

Bee Virus Y

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SUMMARY

Bee virus Y (BVY), isolated from dead adult honey bees (*Apis mellifera*) collected in the field, is a commonly occurring serotype of bee virus X (BVX) in Great Britain. The viruses are very similar physically, although BVY aggregates in low salt concentrations and its single protein has a slightly lower mol. wt. than that of BVX. The viruses form separate bands when centrifuged to equilibrium in CsCl.

During recent surveys of European honey bees (*Apis mellifera*) in England and Wales we identified a common but previously undescribed virus which we called bee virus Y (BVY). We have also detected the virus in bees from Australia and Canada.

BVY occurred frequently in adult bees found moribund or dead beneath live bee colonies in England, especially in early summer. Electron microscopy of crude extracts showed many isometric particles about 35 nm in diam. (Fig. 1*a*) that failed to react with the usually employed dilutions of antiserum to BVX, the only other bee virus known to have particles of the same size and shape (Bailey & Woods, 1974).

The best method of extracting and purifying BVY was to grind 100 bees in 50 ml 0.01 M-potassium phosphate, pH 7, containing 0.02% sodium diethyldithiocarbamate (DIECA) and 5 ml diethyl ether, to emulsify the extract in 5 ml CCl₄ and separate the phases by centrifuging at 8000 *g* for 10 min and then to centrifuge the aqueous layer at 75000 *g* for 3 h, a method used for several other bee viruses (Bailey *et al.* 1979). The pellet was suspended in 4 ml of the phosphate buffer, and 4 ml 0.2 M-ammonium acetate, pH 5, added. The mixture was clarified by centrifuging at 8000 *g* for 10 min and the supernatant fluid was centrifuged at 100000 *g* for 1 h. The pellet was resuspended in 1.5 ml 0.1 M-ammonium acetate, pH 7. This procedure gave a clear solution of BVY which was readily further purified by sucrose density-gradient centrifugation (Bailey & Woods, 1974). Although suitable early in purification, dilute buffers caused increasing aggregation and almost total loss of virus as purification progressed, and omission of the phase at pH 5 left much material in suspension that sedimented at about the same rate as the virus. BVX can be purified similarly, but has given no indication of aggregation when extracted and purified in dilute buffers.

BVY particles contain RNA, as indicated by positive orcinol (Ceriotti, 1955) and negative diphenylamine (Burton, 1956) reactions. They have a sedimentation coefficient ($s_{20,w}$) of 187, a buoyant density in CsCl of 1.347 g/ml (standard error 0.001, four determinations) and an $A_{260} : A_{280}$ ratio of 1.24. Polyacrylamide gel electrophoresis (Carpenter *et al.* 1977) showed one protein component of estimated mol. wt. 50000 ± 250 (six determinations) in 5% gels, as measured on densitometer traces. BVY multiplied when fed to adult bees kept at 30 °C but not at 35 °C, nor when injected into their haemolymph or into the haemolymph of bee pupae. In all these respects BVY closely resembles BVX, but mixtures of the two viruses separated clearly into two bands after centrifuging at 44000 rev/min for 17 h at 25 °C in CsCl in an MSE Centriscan centrifuge (Fig. 1*b*). BVX has the slightly higher density of about 1.355 g/ml and its protein has a mol. wt. larger by more than 2000 (52300 ± 350 , four determinations, $P < 0.001$) when measured on 5% SDS-acrylamide gels (Fig. 1*e, f*).

BVY and BVX were purified by two cycles of centrifugation in sucrose gradients for the

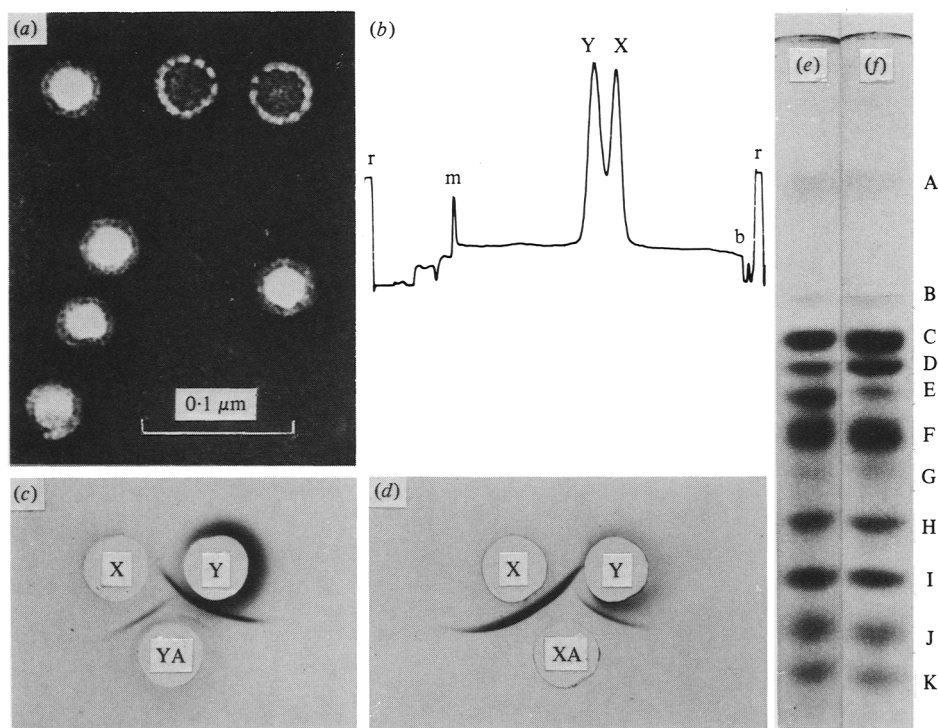


Fig. 1. (a) Sodium phosphotungstate-stained preparation of BVY. (b) U.v. scan of a mixture of BVY and BVX after centrifugation for 17 h at 44000 rev/min in CsCl buffered with 0.01 M-phosphate, pH 7, at 25°C (original density 1.350 g/ml): m, meniscus; Y, BVY; X, BVX; b, bottom of cell; r, reference mark. (c, d) Immunodiffusion tests using BVY (Y) and BVX (X) and antisera prepared against BVY (YA) and BVX (XA) respectively. (e, f) BVY and BVX proteins analysed on 5% SDS-acrylamide gels together with markers. Bands are: A, bovine serum albumin dimer; B, ovalbumin dimer; C, bovine serum albumin; D, catalase; E, bee virus Y or X; F, ovalbumin; G, pepsin; H, carbonic anhydrase; I, tobacco mosaic virus coat protein; J, chymotrypsin B chain; K, chymotrypsin C chain.

preparation of antisera. There was no evidence that either preparation contained any of the other virus. An antiserum to BVY with an homologous titre in immunodiffusion tests of 1/32 to 1/64 had a titre of about 1/2 against BVX, and an antiserum to BVX with an homologous titre of 1/128 to 1/256 had a titre of 1/4 to 1/8 against BVY. Neither antiserum reacted with preparations of uninfected bees. When tested against preparations of the two viruses (0.1 mg/ml) in adjacent wells, each antiserum gave a single strong line against the homologous virus and a single much weaker one against the heterologous virus (Fig. 1 c, d). Because of the wide difference between homologous and heterologous titres it was difficult to demonstrate fusion of the lines and so show unequivocally that the two viruses were related. However, the heterologous reactions did not occur after the antisera had been absorbed with purified preparations of the homologous virus that contained no detectable amounts of the other virus, giving only single bands when centrifuged to equilibrium in CsCl. It seems unlikely, therefore, that the antisera were contaminated with antibodies to the other virus and more probable that the heterologous reactions were evidence of a serological relationship between the two viruses.

The relationship between BVY and BVX was unexpectedly distant in view of their similar physical and biological properties and their occurrence in the same host species, sometimes in the same sample, although current investigations show that the two viruses have very

different ecological characteristics. BVY is common, with a striking peak of incidence in early summer, whereas BVX is uncommon and has been found only during winter and early spring.

The physical properties of BVY and BVX appear similar to those of *Nudaurelia* β virus and its relatives (Reinganum *et al.* 1978), and to the mammalian caliciviruses (Matthews, 1979), but they do not correspond sufficiently to suggest any relationship to either group.

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