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A scientific note on the partial nucleotide sequence of a US strain of Kashmir bee virus isolated from *Apis mellifera* L.

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Apis mellifera / Kashmir bee virus / nucleotide sequence / RT-PCR

Kashmir bee virus (KBV) was first isolated from a diseased adult bee of *Apis cerana* Fabr. [5]. Strains of KBV have been found in adult bees of *Apis mellifera* in Canada, Spain, India, Australia and New Zealand [1], Fiji [3] and the US [8, 9].

The immunodiffusion test has been routinely used to detect bee viruses because it is rapid, inexpensive and specific [2]. Fresh and aged virus samples of KBV, however, are known to have different capsid protein profiles ([6, 7], D. Anderson unpublished data). Therefore, the results of analyses based on serology are not necessarily reliable. The reverse transcription-polymerase chain reaction (RT-PCR) was first used by Stoltz et al. [14] for the diagnosis of KBV. However, their paper contains no sequence information other than that of the two putative 'KBV-specific' primers used for PCR. Here we report the sequence of a 393-bp PCR amplicon of KBV using the two 'KBV-specific primers' of Stoltz et al. [14].

One dead worker bee from a colony (BRL-9) [10] in our experimental apiary was tested positive for KBV in an immunodiffusion test. The homogenate of this bee was used to inoculate drone pupae with white to purple eyes collected from a *Varroa jacobsoni*-free colony. Inoculated

drone pupae were used individually in viral RNA extraction. Liquid nitrogen was used in combination with the guanidium- C_5Cl isopycnic purification protocol of Sambrook et al. [13] to obtain high quality total viral RNA. Reverse transcription of viral RNA and amplification of cDNA by PCR were performed [14]. The PCR reaction product was purified with the High Pure PCR Product Purification Kit (Boehringer Mannheim). Both DNA strands were sequenced three times on an ABI DNA sequencer (model 373a) to confirm the accuracy of the sequence data.

Figure 1 shows the edited 393-bp sequence of the amplified product. The BLASTN search of the Nucleotide Sequence Database at the National Center for Biotechnology Information showed that this KBV 393-bp nucleotide sequence had the highest alignment scores and identities with *Drosophila C* virus strain EB and hepatitis A virus.

Cricket paralysis virus (CrPV) is known to infect several species of Orthoptera and Lepidoptera [12]. Sequence data are now available for CrPV [11]. Even though CrPV has been isolated from a bee colony in Australia, it is not known to cause natural infections in bee colonies [4]. CrPV will readily replicate in bees in the laboratory, but then so do other insect viruses

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	10	20	30	40	50	60
TCGACCTATT	GAAAAAGTTA	ATCAATTGAA	AACACGAGTA	TTCTCAAATG	GACCAATGGA	
	70	80	90	100	110	120
CTTCTCAATT	GCGTTTCGAA	TGTATTATTT	GGGCTTTTATA	GCTCATTTGA	TGGAGAATCG	
	130	140	150	160	170	180
AATTGCAAAT	GAAGTGTCTA	TTGGAACGAA	TGTTTATTCA	CAAGACTGGA	GTAAACTGT	
	190	200	210	220	230	240
TAGGAAATTA	ACCAAATTTG	GAAATAAAGT	AATTGCTGGT	GATTTTTCAA	CTTTTGATGG	
	250	260	270	280	290	300
ATCACTAAAC	GTTTGCATTA	TGGAAAAATT	TGCAGATTTA	GCTAATGAGT	TTTACGATGA	
	310	320	330	340	350	360
TGGACCAGAA	AATGCTTTGA	TCCGTCATGT	ATTATTGGTG	GATGTGTACA	ATTCTGTGCA	
	370	380	390	400	410	420
TATTTGTAAT	GACTCAGTAT	ACATGATGAC	TCA.....

Figure 1. The nucleotide sequence of the 393-bp RT-PCR product (GenBank accession no. AF027125).

such as *Drosophila C* virus, many iridescent viruses and viruses from mosquitoes (B.V. Ball, unpublished data). This paper is the first report of the partial nucleotide sequence of a bona fide bee virus.

Note scientifique sur la séquence partielle de nucléotides d'une souche américaine du virus Kashmir de l'abeille isolé chez *Apis mellifera* L.

Eine wissenschaftliche Notiz zur partiellen Nukleotidsequenz einer aus *Apis mellifera* L. isolierten Nordamerikanischen Abstammungslinie des Kashmirvirus

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