Supporting Information

Elevated virulence of an emerging viral genotype as a driver of honeybee loss

Dino P. McMahon, Myrsini E. Natsopoulou, Vincent Doublet, Matthias Fürst, Silvio Weging, Mark J. F. Brown, Andreas Gogol-Döring, Robert J. Paxton

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Supplementary Methods: Characterization of experimental inocula via ultradeep sequencing.

After mapping reads to contigs using Bowtie2 [1], we used NCBI Blast (megablast) [2] to search for similarities between the contigs and the NCBI nucleotide collection (nt, version from November 20th 2014, downloaded from

ftp://ftp.ncbi.nlm.nih.gov/blast/db). Most contigs matched nucleotide sequences originating from Apinae spp., so we assigned the corresponding sequencing reads to A. mellifera. Other contigs represented the bee viruses DWV-A or -B. Reads that mapped equally well to both viruses were counted as either DWV-A or -B in the same proportion as the reads that could be uniquely assigned to either DWV-A or -B; this is an unbiased approach because, though sequence similarity of DWV-A and -B varies across the length of the genome (Fig. S1), reads that could be mapped to both variants were spread evenly across the consensus genome in proportion to sequence similarity of the DWV genotypes A and B (Fig. S1). Some contigs were identified as the bacteriophage phiX, which is widely used for Illumina sequencing control libraries, so we assume that these reads are technical artefacts. A manual inspection of the Blast results revealed that the remaining contigs contained either very unspecific sequences or were assigned to database entries clearly unrelated to A. mellifera. All reads either corresponding to these unspecific or unrelated contigs, or to contigs which could not be found in the NCBI nucleotide collection, together with all reads which could not be mapped to any contig, were further processed as follows. After filtering out lowcomplexity sequences with PRINSEQ [3] (threshold 15 for 'dust' and 85 for 'entropy'), reads were mapped with Bowtie2 to the NCBI virus database [4]. No beerelated virus other than the viruses mentioned above could be found this way. Then

we mapped all remaining reads one by one to the NCBI nucleotide collection using blastn [5]. In this way, we sorted these reads into the classes described before, but we could not identify any further bee-related or bee-associated organism. At the end, only 47,587 (0.23%) reads from the B inoculum and 12,434 (0.064%) reads from the A inoculum remained unassigned. Both inoculum libraries were sequenced on the same multiplexed Illumina flow cell lane, so a small fraction of sequencing reads is expected to be accidently assigned to the wrong data set, e.g. because of errors during barcode sequencing or due to chimeric reads. This could explain the very low occurrence of DWV-B in the A inoculum data set and also a part of the few DWV-A reads in the B inoculum data set. We also compared the average variability of each inoculum by calculating the proportion of nucleotide mismatches, insertions and deletions between the reads and the respective consensus genome sequences. Average mutation frequencies were calculated inside of non-overlapping 100 base pair windows (Fig. S1). Sequencing errors were avoided by conducting a restricted analysis using only identical overlapping paired-end read portions. The detected levels of variability were very low and similar for both the A and B inocula (~0.04%).

Finally, we analyzed reads from a third library prepared from RNA extracted from cage experiment-derived honeybees (N=5 pooled 9d p.i. M-treated honeybees, in total 23,546,472 reads) in order to search for the presence of a recently characterized genotype of DWV: DWV-C [6]. Reads were concordantly mapped using Bowtie2 to a single index containing the genomes of DWV-A/Kakugo virus (accession numbers NC_004830.2 and NC_005876.1 respectively), DWV-B (NC_006494.1) and DWV-C (gi|873406561|emb|CEND01000001.1) allowing for multiple hits. All reads that mapped to DWV-C also mapped to one of the other reference genomes with the same

or better alignment score, so we concluded that DWV-C was not present in the

experimental inocula.

SI References

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Fig. S1. Genetic variability and read coverage of the experimental inocula. (**A**) Average genome-wide variability of DWV-A in the A inoculum, and DWV-B in the B inoculum respectively. These represent nucleotide mismatches, insertions and deletions between DWV-A or -B reads when matched against their respective consensus genome sequences. Mutational variation around each isolate (DWV-A or -B) derived from field-infected bees was <1% whereas sequence divergence between isolates was >15% (Fig. S2), indicating that the two viral genotypes do not form an interconnected mutant cloud. (**B**) Genome-wide coverage of DWV-A and DWV-B reads in A and B inocula, respectively.



Fig. S2. Genome-wide sequence similarity and extent of recombination between DWV-A and -B. **(A)** Genome-wide sequence similarity of DWV-A and -B (derived from consensus sequences of experimental inocula) in a 100bp sliding window. **(B)** Genome-wide rate of recombination between DWV-A and -B genomes as inferred by number of DWV-A and -B discordant read-pairs from pooled M-treated individuals 9d p.i. (N=5). The length of each bar in the histogram corresponds to the number of discordant read pairs whose centers fall into a 100bp window of the virus genome. The green bars give the number of recombinants with a DWV-A to -B fusion; the blue bars give the number of recombinants with a DWV-B to -A fusion.



Fig. S3. Location of sampling sites used in the GB survey [20,41].



Fig. S4. Fitted Cox proportional hazard survival curves (solid coloured lines) of bees from 13 days p.i. onwards. C=Control (black); A=DWV-A (blue); B=DWV-B (green); M=Mix (orange) and 95% CIs for each fitted curve (dashed coloured lines). Star/lines show significant differences between treatments (P<0.05) based on post-hoc pairwise comparisons of the final model in Table S6.



Fig. S5. Population dynamics over time of colonies infected with DWV-B, with modifications only to the relative individual mortality of adult bees: the individual daily mortality rate of pupae was kept at the default setting (Table S5). The model was run in BEEHAVE [48]

Table S1. (A) Virus loads (as genome equivalents) of A and B inocula used to calculate infectious dose. Virus loads (qRT-PCR) from cage experiment individuals sampled at 9d p.i. (B) and 13d p.i. (B). Ct values transformed into virus genome equivalents per honeybee are shown.

Α.

<u>INOCULA</u>			
	DWV-B	C	DWV-A
Ct value	Q / genomes / ul	Ct value	Q / genomes / u
15.1	6.97 x 10 ⁸	13.46	1.18 x 10 ^{9 §}

[§] The A inoculum was diluted x1.69 relative to the B inoculum to equalize doses. This is consistent with the proportion of DWV-A reads compared to DWV-B reads in the illumina-sequenced A and B inocula, respectively (Table S2.)

В.

<u>9 DAYS P.I.</u>						
Treatment	Sample		DWV-B		DWV-A	RP49
		Ct value	Absolute quantity	Ct value	Absolute quantity	Ct value
	C1	na	na	37.69		24.99
	C2	na	na	37.30		24.43
С	C3	na	na	na	na	24.05
	C4	na	na	37.62		24.27
	C5	na	na	37.74		24.81
	D1	36.05		11.96	176569565921.80	25.09
	D2	na	na	11.10	414617806383.81	24.37
А	D3	36.39		11.14	212221814165.47	24.03
	D4	35.16	18916.76	11.56	302548758497.88	24.33
	D5	na	na	12.08	99653859170.76	24.68
	V1	10.08	2920480607485.87	34.50	120113.93	26.43
	V2	9.17	2901401640057.17	35.51		26.04
В	V3	9.35	2820652080550.68	31.60	496756.50	25.37
	V4	9.88	1280784021269.72	33.81	73550.91	25.38
	V5	9.27	6424227863871.44	32.75	498041.52	25.43
	M1	10.10	1645366097189.03	10.81	504504218346.07	25.82
	M2	9.95	4607122304117.58	10.38	1699703416392.77	25.48
Μ	M3	10.40	2949987704299.03	11.51	692417826596.36	26.27
	M4	10.16	2897697469151.58	10.81	925610286072.58	25.42
	M5	9.80	3892980228102.32	10.41	1285922177392.84	25.55

Bold italic Ct values > acceptance threshold (Ct = 35). na = not detected

Table S1. Cont.

C.

<u>13 DAYS P.I.</u>

Treatment	Sample		DWV-B		DWV-A	RP49
		Ct value	Absolute quantity	Ct value	Absolute quantity	Ct value
	C6	na	na	na	na	24.48
С	C7	na	na	na	na	24.32
	C8	na	na	na	na	23.99
	D6	na	na	10.26	195511930427.82	25.37
А	D7	na	na	9.96	683595893151.01	25.19
	D8	na	na	9.87	391146603892.63	24.48
	V6	10.86	396656612110.95	na	na	25.30
В	V7	10.99	566967760401.87	na	na	25.49
	V8	11.01	1116755448574.56	na	na	25.75
	M6	12.04	375004409986.09	10.85	406282317906.32	25.54
М	M7	11.58	722585275438.26	10.09	962219268280.72	24.90
	M8	12.37	355406511228.20	10.68	533357594158.83	25.50

na = not detected

Origin	B inoculum reads (M2)	A inoculum reads (M1)
Apis	15,277,458 (74.5%)	8,545,289 (44.2%)
Viruses		
DWV-B	4,547,491 (22.2%)	623 (0.0032%)
DWV-A	6,157 <i>(0.03%)</i>	10,334,080 (53.5%)
Overhead		
phiX	26,355 (0.13%)	28,010 (0.14%)
sequencing adapters	3,555 (0.017%)	4,327 (0.022%)
low complexity	602,276 (2.9%)	405,663 (2.1%)
Unknown	47,587 <i>(0.23%)</i>	12,434 (0.064%)
Total	20,510,879	19,330,426

Table S2. Origin of sequenced reads in the DWV-A and -B inocula (see Supplementary Methods for detailed description of methods).

Table S3. Sequence alignment of cloned viral RdRp gene PCR products, amplified using specific (qRT-PCR) DWV-A and -B primers from a mixed-template. Sequences are compared against DWV-A (GenBank Accession No. NC004830) and DWV-B (GenBank Accession No. NC006494) reference genes.

DWV_B_NC_006494	CTTCTGGTAA	G <mark>C</mark> GA	TGGTTG	TTTGATATTG	AA <mark>TT</mark> ACAAGA	TTCAGGATGT	TATC TTTTGA
DWV B clone1	CTTCTGGTAA	GCGA	T <mark>GGTT</mark> G	TTTGATATTG	AATTACAAGA	TT <mark>CAGGATG</mark> T	TATCTTTGA
DWV B glopo2							
DWV_B_CIONEZ	CTTCTGGTAA	GOGA			AATTACAAGA		
Dwv_B_clone3	CTTCTGGTAA	- G <mark>C</mark> GA	'T <mark>GG</mark> TT <mark>G</mark>	TTTGATATTG	AATTACAAGA	. TT <mark>CAGGATG</mark> T	TATCTTTTGA
DWV_B_clone4	CTTCTGGTAA	G <mark>C</mark> GA	T <mark>GGTT</mark> G	TTT <mark>GA</mark> TATTG	AA <mark>TTA</mark> CAAGA	TT <mark>C</mark> AGGATGT	TATCTTTTGA
DWV B clone5	CTTCTGGTAA	GCGA	T <mark>GGTT</mark> G	TTT <mark>GA</mark> TATTG	AA <mark>TT</mark> ACAAGA	TTCAGGATGT	TATCTTTTGA
DWV B clone6	CTTCTCCTCCTA A	GCGA	т <mark>сс</mark> ттс	ͲͲͲ <mark>Ⴚ</mark> ϼͲϼ <mark>ϲ</mark>	AATTACAAGA	TTCAGGATCT	Ͳ <mark>ϪͲϹ</mark> ͲͲͲͲϹϪ
DWV P glopo7		CCCA					
Dwv_b_cione/	CTTCTGGTAA	GOGA			AATTACAAGA		
DWV_B_clone8	CTTCTGGTAA	- G <mark>C</mark> GA	'T <mark>GG</mark> T"T <mark>G</mark>	'T'T'T <mark>GATA</mark> TTG	AATTA <mark>C</mark> AAGA	. TT <mark>CAGGATG</mark> T	TATCTTTTGA
DWV_B_clone9	CTTCTGGTAA	G<mark>C</mark>GA	T <mark>GG</mark> TT <mark>G</mark>	TTTGATATTG	AA <mark>TTA</mark> CAAGA	TT <mark>CAGGAT</mark> GT	TATCTTTTGA
DWV B clone10	CTTCTGGTAA	GCGA	TGGTTG	TTTGATATTG	AATTACAAGA	TTCAGGATGT	TATCTTTTGA
DWV B clone11	CTTCTCTCTTA N	CCCA	TCCTTC				
DWV_D_clonell							
Dwv_B_clonel2	CTTCTGGTAA	G <mark>C</mark> GA	.T. <mark>G.G</mark> .T.T.G	TTTGATATTG	AATTACAAGA	TTCAGGATGT	TATOTTTTGA
DWV_B_clone13	CTTCTGGTAA	G <mark>C</mark> GA	T <mark>GGTT</mark> G	TTT <mark>GA</mark> T <mark>A</mark> TTG	AA <mark>TTAC</mark> AAGA	TT <mark>CA</mark> GGATGT	TATCTTTTGA
DWV B clone14	CTTCTGGTAA	GCGA	TGGTTG	TTTGATATTG	AA <mark>TTAC</mark> AAGA	TTCAGGATGT	TATCTTTTGA
DWV B clone15	CTTCTGGTAA	GCGA	т <mark>GG</mark> TTG	ͲͲͲ <mark>Ⴚ</mark> ϷͲϷ <mark>Ⴚ</mark>	AATTACAAGA	TTCAGGATGT	TATCTTTCA
DWV P glopol6		CCCA					
Dwv_b_cloneto	CTTCTGGTAA	GOGA			AATTACAAGA		
DWV_B_clone17	CTTCTGGTAA	- <mark>G</mark> CGA	T <mark>GGTTG</mark>	TTT <mark>GATA</mark> TT <mark>G</mark>	AA <mark>TTAC</mark> AAGA	. TT <mark>CAGGATG</mark> T	TATCTTTTGA
DWV_B_clone18	CTTCTGGTAA	G <mark>C</mark> GA	T <mark>GGTT</mark> G	TTT <mark>GATA</mark> TT <mark>G</mark>	AA <mark>TT</mark> A <mark>C</mark> AAGA	TT <mark>C</mark> AGGATGT	TATCTTTTGA
DWV B clone19	CTTCTGGTAA	GCGA	TGGTTG	TTTGATATTG	AATTACAAGA	TTCAGGATGT	TATCTTTTGA
DWV B clone20	CTTCTCTCTTA N	CCCA	TCCTTC				
DWV_D_CIONE20							
Dwv_B_clone21	CTTCTGGTAA	G <mark>C</mark> GA	TGGTTG	TTTGATATTG	AATTACAAGA	TTCAGGATGT	TATCTTTTGA
DWV_B_clone22	CTTCTGGTAA	G <mark>C</mark> GA	T <mark>GGTT</mark> G	TTT <mark>GATA</mark> TTG	AA <mark>TTA</mark> CAAGA	TT <mark>C</mark> AGGATGT	TATCTTTTGA
DWV B clone23	CTTCTGGTAA	GCGA	TGGTTG	TTTGATATTG	AA <mark>TTAC</mark> AAGA	TTCAGGATGT	TATCTTTTGA
DWV B clone24	CTTCTCCTCCTA A	GCGA	T <mark>GG</mark> TTG	ͲͲͲ <mark>Ⴚ</mark> ϪͲϪͲͲϹ		TTCACCATC	Ͳ <mark>ϪͲϹ</mark> ͲͲͲͲϹϪ
DWV B alona 25		CCC					
DWV_B_CIONE25	CTTCTGGTAA	GOGA			AATTACAAGA		
DWV_B_clone26	CTTCTGGCAA	- <mark>G</mark> CGA	T <mark>GGTTG</mark>	TTT <mark>GATA</mark> TT <mark>G</mark>	AA <mark>TTAC</mark> AAGA	. TT <mark>CAGGATG</mark> T	TATCTTTTGA
DWV_B_clone27	CTTCTGGTAA	GCGA	TGGTTG	TTTGATATTG	AATTACAAGA	TT <mark>C</mark> AGGATGT	TATCTTTTGA
DWV B clone28	CTTCTGGTAA	GCGA	TGGTTG	TTTGATATTG	AATTACAAGA	TTCAGGATGT	TATCTTTTGA
DWV B clone29	CTTCTCTCTTA N	CCCA	TCCTTC				
DWV_D_CIONE25							
Dwv_B_clone30	CTTCTGGTAA	G <mark>C</mark> GA	TGGTTG	TTTGATATTG	AATTACAAGA	TTCAGGATGT	TATOTTTTGA
DWV_B_clone31	CTTCTGGTAA	G <mark>C</mark> GA	T <mark>GGTT</mark> G	TTT <mark>GATA</mark> TT <mark>G</mark>	AATTACAAGA	TT <mark>CAGGATG</mark> T	TATCTTTTGA
DWV B clone32	CTTCTGGTAA	GCGA	TGGTTG	TTTGATATTG	AA <mark>TTAC</mark> AAGA	TTCAGGATGT	TATCTTTTGA
DWV B clone33	CTTCTCCTCCTA A	GCGA	T <mark>GG</mark> TTG	ͲͲͲ <mark>Ⴚ</mark> ϷͲϷͲϹ		TTCACCATC	TATCTTTCA
DWV_B_clone34							
DWV_B_CIONE34	CTTCTGGTAA	GOGA			AATTACAAGA		
DWV_B_clone35	CTTCTGGTAA	- G <mark>C</mark> GA	'T <mark>GG</mark> T"T <mark>G</mark>	TTTGATATTG	AA <mark>TTAC</mark> AAGA	. TT <mark>CAGGATG</mark> T	TATCTTTTGA
DWV_B_clone36	CTTCTGGTAA	G<mark>C</mark>GA	T <mark>GGTT</mark> G	TTT <mark>GATA</mark> TTG	AA <mark>TT</mark> A <mark>C</mark> AAGA	TT <mark>CAGGAT</mark> GT	TATCTTTTGA
DWV B clone37	CTTCTGGTAA	GCGA	TGGTTG	TTTGATATTG	AA <mark>TTAC</mark> AAGA	TTCAGGATGT	TATCTTTTGA
DWV B clone38	CTTCTCCTCCTA A	GCGA	T <mark>GG</mark> TTG	ͲͲͲ <mark>Ⴚ</mark> ϷͲϷͲϹ		TTCACCATC	TATCTTTCA
$MV_D_croneso$							
KV_NC_005876	CATCAGGTAA	GCGA	T.GG.T.T.G	TTTG <mark>AC</mark> ATTG	AGOTACAAGA	CTCGGGATGT	TATCTCTTGC
DWV_A_NC_004830	CATCAGGYAA	- <mark>G</mark> CGA	T <mark>GGTTG</mark>	TTTGAYATTG	AG <mark>CTAC</mark> AAGA	YT <mark>C</mark> GGGATGT	TATCTCYTGC
DWV_A_clone1	CATCAGGTAA	GCGA	TGGTTG	TTTGACATTG	AGCTACAAGA	CTCGGGATGT	TATCTCTTGC
DWV A clone?	CATCAGGTAA	GCGA	т <mark>GG</mark> TTG	TTTGACATTG	AGCTACAAGA	CTCGGGATGT	TATCTCTTGC
DWV A clone3							
DWV_A_CIONES	CATCAGGIAA	GOGA			AGOTACAAGA		
DWV_A_Clone4	CATCAGGTAA	G <mark>C</mark> GA	'T <mark>GG</mark> T"T <mark>G</mark>	TTTG <mark>ACA</mark> TTG	AG <mark>CTAC</mark> AAGA	CTCGGGATGT	TATCTCTTGC
DWV_A_clone5	CATCAGGTAA	G <mark>C</mark> GA	T <mark>GGTT</mark> G	TTT <mark>GACA</mark> TT <mark>G</mark>	AGCTACAAGA	CTCGGGATGT	TATCTCTTGC
DWV A clone6	CATCAGGTAA	GCGA	TGGTTG	TTTG <mark>ACA</mark> TTG	AGCTACAAGA		TATCTCTTGC
DWV A clone7		GCGA	T <mark>GG</mark> TTG			CTCCCCATC	TATCTCTTCCTTCC
	CALCAGGTAA	GCGA		TTTGACATTG	AGCIACAAGA	CTCGGGGATGT	TATOTOTTGC
DWV_A_CIONE9	CATCAGGTAA	G <mark>C</mark> GA	T <mark>GGTTG</mark>	TTTG <mark>ACA</mark> TTG	AG <mark>CTAC</mark> AAGA	CTCGGGATGT	TATCTCTTGC
DWV_A_clone10	CATCAGGTAA	G <mark>C</mark> GA	T <mark>GGTT</mark> G	TTT <mark>GACA</mark> TTG	AGCTACAAGA	CTCGGGATGT	T<mark>A</mark>TCTCTTGC
DWV A clone11	CATCAGGTAA	GCGA	TGGTTG	TTTGACATTG	AGCTACAAGA		TATCTCTTGC
DWV A clonel?		CCCA	TCCTTC			CTCCCCATCT	TATCTCTTCCT
DWV_1 _clone12							
Dwv_A_clonel3	CATCAGGTAA	G <mark>C</mark> GA	.T. <mark>G.G.T.T.</mark> G	TTTGACATTG	AGOTACAAGA	CTCGGGATGT	TATCTCTTGC
DWV_A_clone14	CATCAGG TAA	G <mark>C</mark> GA	T <mark>GGTTG</mark>	TTT <mark>GACATT</mark> G	AG <mark>CTAC</mark> AAGA	CTCGGGATGT	T <mark>A</mark> TCTCTTGC
DWV_A_clone15	CATCAGGTAA	GCGA	TGGTTG	TTTGACATTG	AGCTACAAGA	CTCGGGATGT	TATCTCTTGC
DWV A clone16	CATCAGGTAA	GCGA	т <mark>GG</mark> TTG	TTTG <mark>AC</mark> ATTG	AGCTACAAGA	CTCGGGATGT	TATCTCTTGC
DWU λ glopol7							
DWV_A_CIONEL/	CALCAGGIAA	GOGA			AGOTACAAGA	CTCGGGGHIGI	
DMA ⁻ V_CTOUET8	CATCAGGTAA	GCGA	TGGTTG	TTTGACATTG	AGCTACAAGA	CTCGGGATGT	TATCTCTTGC
DWV_A_clone19	CATCAGGTAA	G <mark>C</mark> GA	T <mark>GGTT</mark> G	TTT <mark>GACATT</mark> G	AGCTACAAGA	CTCGGGATGT	T <mark>A</mark> TCTCTTGC
DWV A clone20	CATCAGGTAA	GCGA	TGGTTG	TTTGACATTG	AGCTACAAGA		TATCTCTTGC
DWV A clone 21					ACCTACAACA	CTCCCCATC	
	CATCACCTAA	GCCA					
	CATCAGGTAA	GCGA					
DWV_A_clone22	CATCAGGTAA CATCAGGTAA	G <mark>C</mark> GA G <mark>C</mark> GA	TGGTTG TGG <mark>TTG</mark>	TTT <mark>GAC</mark> ATT <mark>G</mark>	A <mark>GCT</mark> ACAAGA	CTCGGGATGT	TATCTCTTGC
DWV_A_clone22 DWV_A_clone23	CATCAGGTAA CATCAGGTAA C <mark>ATCAGG</mark> TAA	G <mark>C</mark> GA GCGA GCGA	TGGTTG TGGTTG T <mark>GGTT</mark> G	TTT <mark>GACA</mark> TTG TTT <mark>GAC</mark> ATTG	A <mark>GCTAC</mark> AAGA A <mark>GCTAC</mark> AAGA	CTCGGGATGT CTCGGGATGT	TATCTCTTGC TATCTCTTGC
DWV_A_clone22 DWV_A_clone23 DWV_A_clone24	CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA	GCGA GCGA GCGA GCGA	TGGTTG TGGTTG TGGTTG TGGTTG	TTTGACATTG TTTGACATTG TTTGACATTG	A <mark>GCTAC</mark> AAGA AGCTACAAGA AGCTACAAGA	CTCGGGATGT CTCGGGATGT CTCGGGATGT	TATCTCTTGC TATCTCTTGC TATCTCTTGC
DWV_A_clone22 DWV_A_clone23 DWV_A_clone24 DWV_A_clone25	CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA	GCGA GCGA GCGA GCGA GCGA	TGGTTG TGGTTG TGGTTG TGGTTG TGGTTC	TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG	AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA	CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATCT	TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC
DWV_A_clone22 DWV_A_clone23 DWV_A_clone23 DWV_A_clone24 DWV_A_clone25	CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA	GCGA GCGA GCGA GCGA GCGA	TGGTTG TGGTTG TGGTTG TGGTTG TGGTTG	TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG	AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA	CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT	TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC
DWV_A_clone22 DWV_A_clone23 DWV_A_clone24 DWV_A_clone25 DWV_A_clone26	CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA	GCGA GCGA GCGA GCGA GCGA GCGA	TGGTTG TGGTTG TGGTTG TGGTTG TGGTTG TGGTTG	TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG	AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA	CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT	TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC
DWV_A_clone22 DWV_A_clone23 DWV_A_clone23 DWV_A_clone24 DWV_A_clone25 DWV_A_clone26 DWV_A_clone27	CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA	GCGA GCGA GCGA GCGA GCGA GCGA	T GGTTG TGGTTG TGGTTG TGGTTG TGGTTG TGGTTG	TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG	AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA	CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT	TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC
DWV_A_clone22 DWV_A_clone23 DWV_A_clone24 DWV_A_clone25 DWV_A_clone26 DWV_A_clone27 DWV_A_clone28	CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA CATCAGGTAA	GCGA GCGA GCGA GCGA GCGA GCGA GCGA G <mark>C</mark> GA	TGGTTG TGGTTG TGGTTG TGGTTG TGGTTG TGGTTG TGGTTG	TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG TTTGACATTG	AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA AGCTACAAGA	CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT CTCGGGATGT	TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC TATCTCTTGC

Table S3. Cont.

6	51				
DWV B NC 006494	G	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone1	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone?	G	GGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone3					T.
DWV B clone4					T
DWV B clone5				CACATACACT	—
DWV_B_clone6				CACAMACAC	
DWV_B_CIONe6	G		ACCTGAACTT	GAGATACAGT	
DWV_B_CIONE7	G		ACCTGAACTT	GAGATACAGT	
DWV_B_Clone8	G	AGGGATGAG	ACCTGAACTT	GAGATACAGT	T
DWV_B_clone9	G	AGGGATGAG	ACCTGAACTT	GAGATACAGT	T
DWV_B_clone10	G	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone11	G.	AGGGAT GAG	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone12	G.	AGGGAT <mark>GAG</mark>	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone13	G/	AGGGAT <mark>GAG</mark>	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone14	G.	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone15	G.	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone16	G/	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone17	G/	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone18	<mark>G</mark>	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAGATACAG T	Т
DWV_B_clone19	G <mark>/</mark>	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone20	G/	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAGATACAGT	Т
DWV_B_clone21	G <mark>7</mark>	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAG<mark>ATAC</mark>AGT	Т
DWV_B_clone22	G <mark>7</mark>	AGGG <mark>AT</mark> GAG	ACCTGAACTT	GAGATACAG T	Т
DWV_B_clone23	G/	AGGG <mark>AT</mark> GAG	ACC <mark>TGAACTT</mark>	GAGATACAGT	Т
DWV B clone24	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAG T	Т
DWV_B_clone25	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone26	G <mark>7</mark>	AGGGA <mark>T</mark> GAG	ACCTGAACTC	GAGATACAG T	Т
DWV B clone27	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone28	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone29	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone30	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAG T	Т
DWV B clone31	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone32	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone33	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone34	G	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone35	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone36	G <mark>7</mark>	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone37	G	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
DWV B clone38	G	AGGGATGAG	ACCTGAACTT	GAGATACAGT	Т
KV NC 005876	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	Т
DWV A NC 004830	G		TCCCGAACTT	GAGATTCAAT	Т
DWV A clone1	G		TCCCGAACTT	GAGATTCAAT	Т
DWV A clone2	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	Т
DWV A clone3	G		TCCCGAACTT	GAGATTCAAT	Т
DWV A clone4	G		TCCCGAACTT	GAGATTCAAT	Т
DWV A clone5	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	Т
DWV A clone6	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	Т
DWV A clone7	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	T
DWV A clone8	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	T
DWV A clone9	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	T
DWV A clone10	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	T
DWV A clone11	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	T
DWV A clone12	G	GGAATGCG	TCCCGAACTT	GAGATTCAAT	T
DWV A clone13	G		TCCCGAACTT	GAGATTCAAT	Ť
DWV A clone14	G	GGA ATTGCC	TCCCGAACTT	GAGATTCAAT	Ť
DWV A clone15	G	GGA ATGCG	TCCCGAACTT	GAGATTCAAT	Ť
DWV A clone16	C			GAGATTCAAT	Ť
DWV A clone17	C				–
DWV A clone18	C				T.
DWV A clone19	C	CGA ATCCC	TCCCCAACTT	GAGATTCAAT	Ţ
DWV A clone20	C	GGAATCCC	TCCCGAACTT	GAGATTCAAT	Ţ
DWV A clone21	C	GGAATCCC	TCCCGAACTT	GAGATTCAAT	Ţ
DWV A clone??	C	GGAATCCC	TCCCGAACTT	GAGATTCAAT	Ţ
DWV A clone23	C		TCCCGAACTT	GAGATTCAAT	T
DWV A clone24	C	GGAATCCC	TCCCGAACTT	GAGATTCAAT	Ţ
DWV A clone25	C	CGAATCCC	TCCCGAACTT	GAGATTCAAT	Ţ
DWV A clone26	C		TCCCGAACTT	GAGATTCAAT	T
DWV A clone?7	C	CGA ATCCC	TCCCCAACTT	GAGATTCAAT	т Т
DWV A clone28	C	CGAATCCC	TCCCCAACTT	GAGATTCAAT	Ţ
DWV A clone20	C	GGAATCCC	TCCCGAACTT	GAGATTCAAT	Ţ
	<u> </u>				

Table S4. List of primers used in this study.

Primer				
Target	Name	Sequence	Application	Reference
DWV-A (RdRp)	DWV-F2	TGTCTTCATTAAAGCCACCTGGAA	qPCR	[1]
DWV-A (RdRp)	DWV-R2a	TTTCCTCATTAACTGTGTCGTTGAT	qPCR	"
DWV-B (RdRp)	VDV-F2	TATCTTCATTAAAACCGCCAGGCT	qPCR	"
DWV-B (RdRp)	VDV-R2a	CTTCCTCATTAACTGAGTTGTTGTC	qPCR	"
DWV-A (RdRp)	F8668	TTCATTAAAGCCACCTGGAACATC	qPCR	[2]
DWV-A (RdRp)	B8757	TTTCCTCATTAACTGTGTCGTTGA	qPCR	"
DWV-A (RdRp)	DWV-F1a	GGAAACATCTGGAATTAGCGACAAA	Stand' curve	[1]
DWV-B (RdRp)	VDV-F1a	GAAAACATTTGGAATTAGCAACGAC	Stand' curve	
DWV-A / -B (RdRp)	DWVDV-7A-R	AATCCGTGAATATAGTGTGAGG	Stand' curve	"
SBPV	SPV-F3177	GCGCTTTAGTTCAATTGCC	qPCR	[3]
SBPV	SPV-B3363	ATTATAGGACGTGAAAATATAC	qPCR	"
SBPV	SBPV-var-F2	GTGCTTTAGTTCAATTACCATTG	qPCR	This study
SBPV	SBPV-var-R	ATTATGGGACGTGAGAAT ATAC	qPCR	This study
ABPV	ABPV-F6548	TCATACCTGCCGATCAAG	qPCR	[4]
IAPV	IAPV-F6627	CCATGCCTGGCGATTCAC	qPCR	"
ABPV/IAPV	KIABPV-B6707	CTGAATAATACTGTGCGTATC	qPCR	"
BQCV	BQCV-F7893	AGTGGCGGAGATGTATGC	qPCR	[1]
BQCV	BQCV-B8150	GGAGGTGAAGTGGCTATATC	qPCR	"
SBV	SBV-F3164	TTGGAACTACGCATTCTCTG	qPCR	"
SBV	SBV-B3461	GCTCTAACCTCGCATCAAC	qPCR	"
RP49	RP49-qF	AAGTTCATTCGTCACCAGAG	qPCR	[2]
RP49	RP49-qB	CTTCCAGTTCCTTGACATTATG	qPCR	"

Table S4. References

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- 3 de Miranda, J.R. et al. Genetic characterization of slow bee paralysis virus of the honeybee (Apis mellifera L.). J Gen Virol 91, 2524-2530 (2010).
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Table S5. Description of adjusted BEEHAVE model parameters. Modified values are emphasized in underlined italics.

Model scenario (1) DWV-A.

Parameter	Parameter	Default	Description
	value	value	
MORTALITY_INHIVE	0.004	0.004	Daily mortality rate of healthy in-hive bees and foragers
MORTALITY_INHIVE_INFECTED_AS_ADULT	0.012	0.012	Daily mortality rate of in-hive bees and foragers, infected as
			adults
MORTALITY_INHIVE_INFECTED_AS_PUPAE	0.012	0.012	Daily mortality rate of in-hive bees and foragers, infected as
			pupae

Model scenario (2) DWV-B

Parameter	Parameter	Default	Description
	value	value	
MORTALITY_INHIVE	0.004	0.004	Daily mortality rate of healthy in-hive bees and foragers
MORTALITY_INHIVE_INFECTED_AS_ADULT	<u>0.016</u>	0.012	Daily mortality rate of in-hive bees and foragers, infected as
			adults
MORTALITY_INHIVE_INFECTED_AS_PUPAE	<u>0.016</u>	0.012	Daily mortality rate of in-hive bees and foragers, infected as
			pupae

Conservative model scenario (2) DWV-B

Parameter	Parameter	Default	Description
	value	value	
MORTALITY_INHIVE	0.004	0.004	Daily mortality rate of healthy in-hive bees and foragers
MORTALITY_INHIVE_INFECTED_AS_ADULT	<u>0.016</u>	0.012	Daily mortality rate of in-hive bees and foragers, infected as
			adults
MORTALITY_INHIVE_INFECTED_AS_PUPAE	0.012	0.012	Daily mortality rate of in-hive bees and foragers, infected as
			pupae

Table S6. Final Cox proportional hazard model of cage mortality following experimental inoculation, from day 13 p.i. onwards. C, control; A, DWV-A; B, DWV-B; M, mixed DWV-A and -B. SE, Standard error; SD, Standard deviation. a Equivalent to the hazard ratio, the instantaneous risk of death for bees in each treatment compared with the baseline treatment level (in this case C). Higher levels of β indicate higher risk of death.

Parameters	Coeffi	cients		Model testing		
	β	SE (β)	Exp $(\beta)^{a}$	χ^2 (LRT)	df	P-value
Fixed variable						
Treatment				43.102	3	<0.00001*
С	0	-	1			
А	4.742	0.671	114.642			
В	6.464	0.708	641.819			
М	6.061	0.698	428.914			
Random variable	SD	Variance				
Cage	0.542	0.293				