

Supplementary Materials

Article title: The two prevalent genotypes of an emerging infectious disease, *Deformed wing virus*, cause equally low pupal mortality and equally high wing deformities in host honey bees

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Table S1: Contents of NGS reads of DWV inocula

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Table S3: Comparison of published DWV sequences with our NGS libraries

Figure S1. Read coverage of the experimental inocula across the DWV genome (5' → 3'). Genome-wide coverage of the DWV-A (a) and DWV-B (b) reads in the DWV-A and DWV-B inocula, respectively. The first and last 200 bp were excluded.

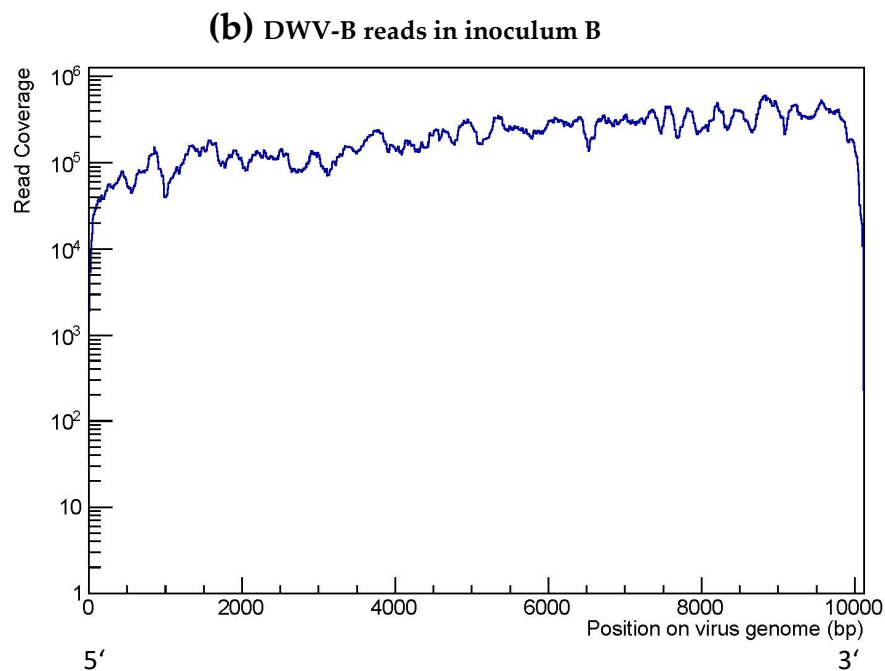
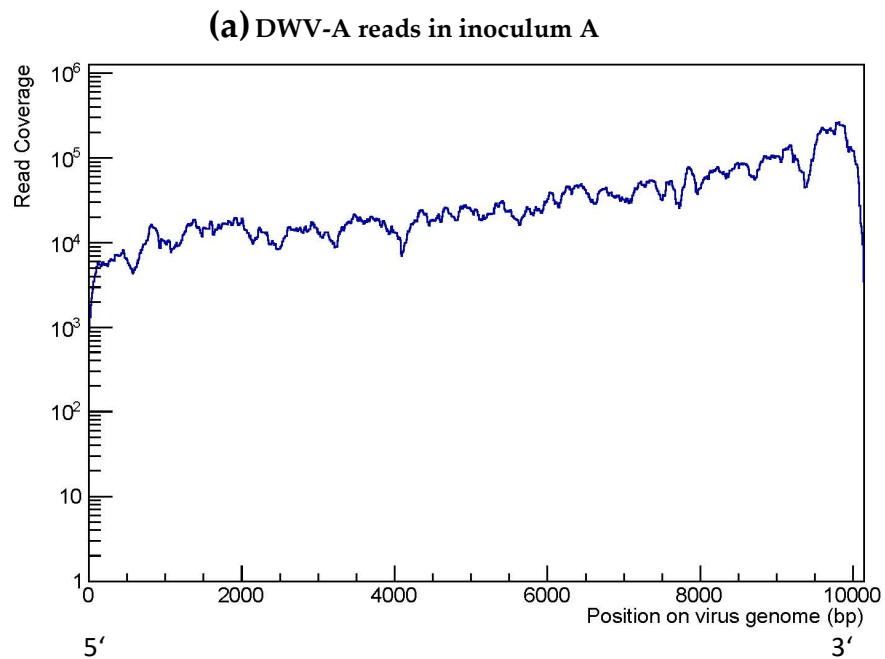
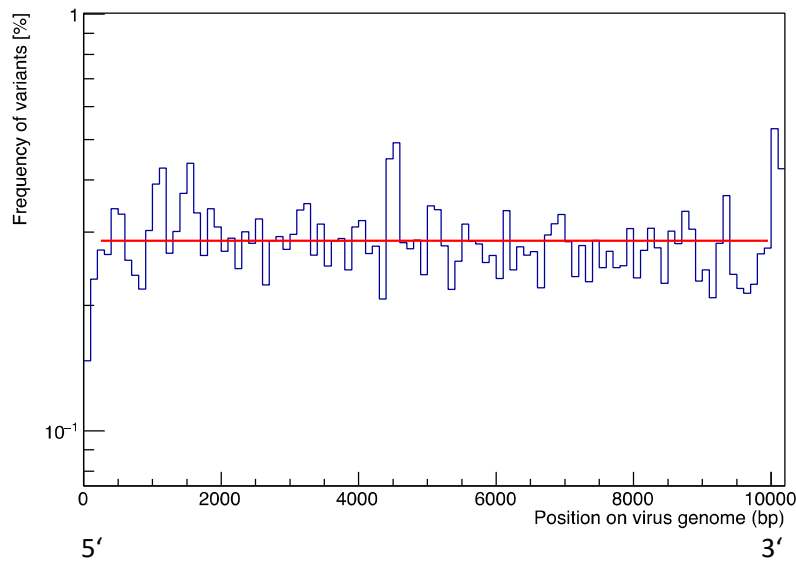


Figure S2. Genetic variability of the experimental inocula across the DWV genome (5' → 3'). (a) Average genome-wide variability of DWV-A in the DWV-A inoculum and (b) DWV-B in the DWV-B inoculum. These represent nucleotide mismatches (SNPs) between DWV-A or -B reads when matched against their respective genome sequences (Figures S3, S4). Mutational variation around each isolate (DWV-A or -B) in inocula was calculated inside of non-overlapping 100 base pair windows using a custom script and was <0.5% whereas sequence divergence between isolates was >15% (Table S3), indicating that the two viral genotypes do not form an interconnected mutant cloud. The first and last 200 bp were excluded.

(a) Inoculum DWV-A variability across the genome



(b) Inoculum DWV-B variability across the genome

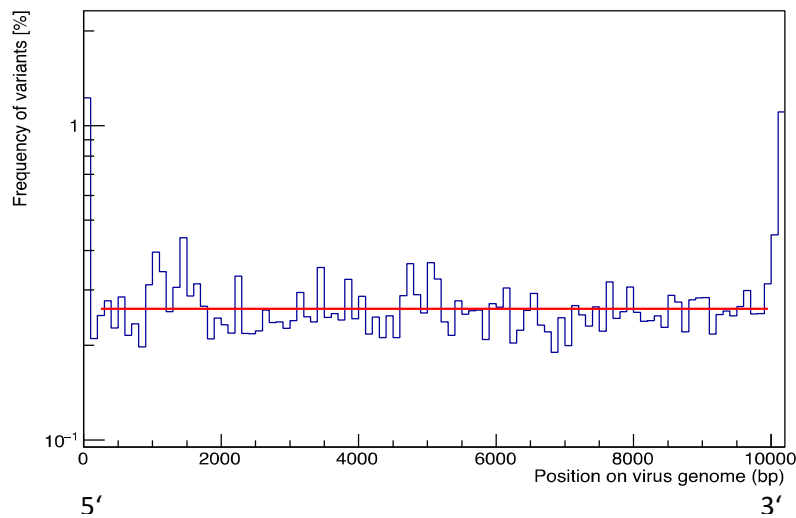


Figure S3. Full genome contig sequences of the DWV-A inoculum and DWV-A derived from a white-eyed host pupa experimentally inoculated with the DWV-A inoculum as it eclosed as an adult (individual pupal code: D4-DWV-A), as well as two partial DWV-A contigs from the DWV-B inoculum, as generated by IVA *de novo* assemblies of all DWV NGS reads.

>Inoculum-DWV-A; IVA full-genome DWV-A contig sequence
(Row 3 of Figure S5, code: DWV-A_contig.00001-Inoculum-A)

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> Code of pupa: D4-DWV-A; IVA full genome DWV-A contig sequence
(Row 4 of Figure S5, code: DWV-A_contig.00001-D4-A)

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>Inoculum-DWV-B; IVA partial genome DWV-A contig sequence
(Row 5 of Figure S5, code: DWV-A_contig.00002-Inoculum-B)

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TTTATCCGAGTTCTCTCA

>Inoculum-DWV-B; IVA partial genome DWV-A contig sequence

(Row 6 of Figure S5, code: DWV-A_contig.00003-Inoculum-B)

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Figure S4. Complete genome contig sequences of the DWV-B inoculum and DWV-B derived from a white-eyed host pupa experimentally inoculated with the DWV-B inoculum as it eclosed as an adult (individual pupal code: V4-DWV-B), as well as four partial DWV-B contigs from the DWV-A inoculated pupa D4-DWV-A, as generated by the IVA *de novo* assemblies of all DWV NGS reads.

>Inoculum-DWV-B; IVA full-genome DWV-B contig sequence

(Row 9 of Figure S5, code: DWV-B_contig.00001-Inoculum-B)

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[illegible]

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> Code of pupa: V4-DWV-B; IVA full genome DWV-B contig sequence
(Row 10 of Figure S5, code: DWV-B_contig.00001-V4-B)

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> Code of pupa: D4-DWV-A; IVA partial genome DWV-B contig sequence

(Row 11 of Figure S5, code: DWV-B_contig.00004-D4-A)

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> Code of pupa: D4-DWV-A; IVA partial genome DWV-B contig sequence

(Row 12 of Figure S5, code: DWV-B_contig.00005-D4-A)

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> Code of pupa: D4-DWV-A; IVA partial genome DWV-B contig sequence

(Row 13 of Figure S5, code: DWV-B_contig.00002-D4-A)

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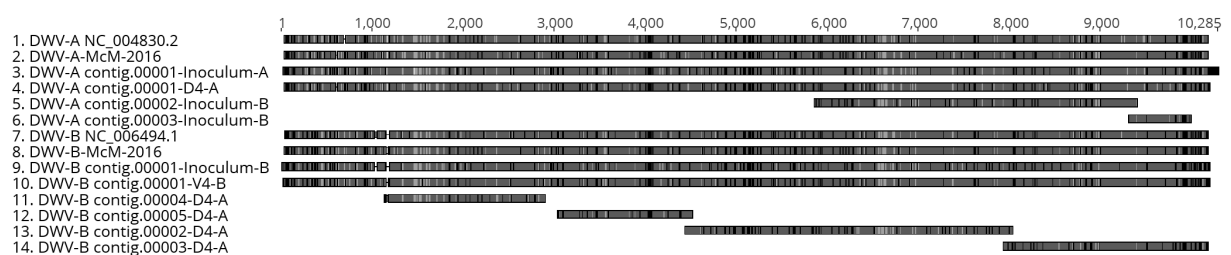
> Code of pupa: D4-DWV-A; IVA partial genome DWV-B contig sequence

(Row 14 of Figure S5, code: DWV-B_contig.00003-D4-A)

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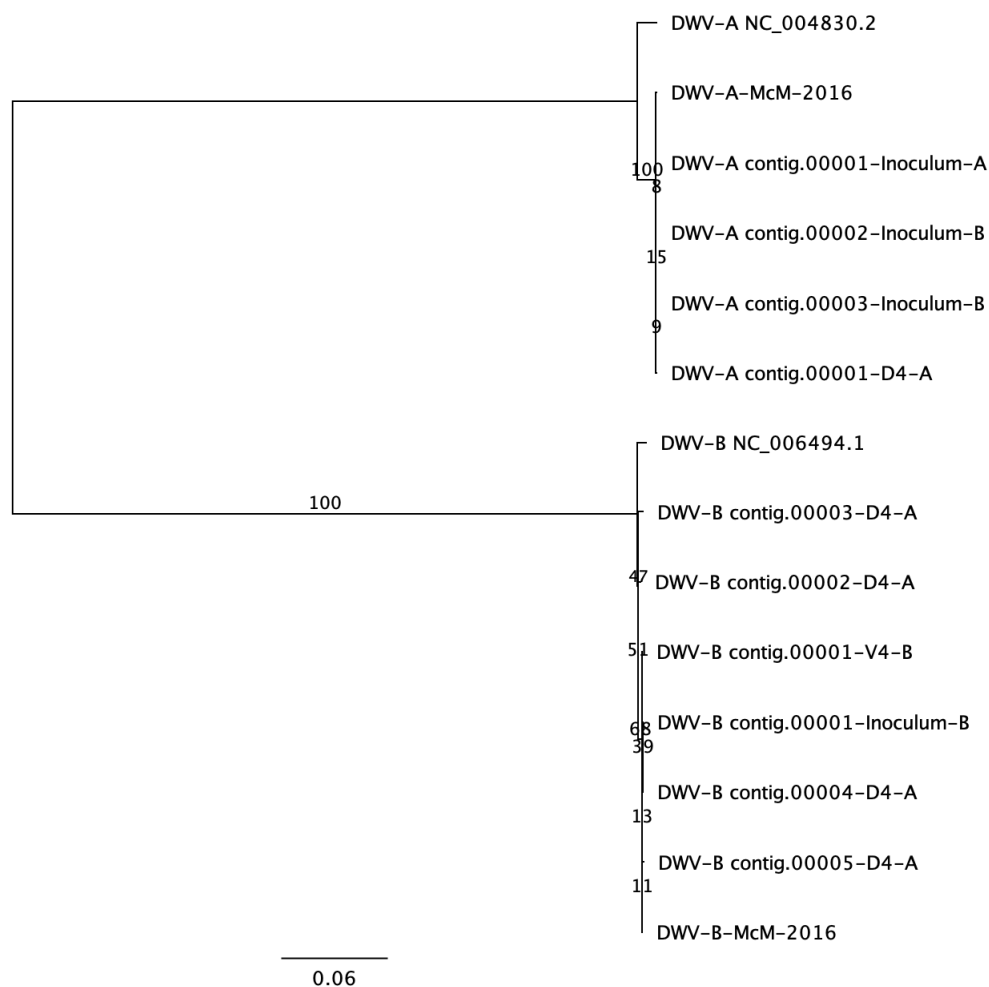
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Figure S5. Nucleotide alignment of all DWV contigs generated by IVA *de novo* assemblies of all DWV reads in our four NGS datasets. DWV-A Accession No. NC_004830.2 and DWV-B Accession No. NC_006494.1 were used as reference sequences. The McM-2016 sequences correspond to the reference sequences of DWV-A and DWV-B generated by McMahon *et al.* (2016), and from which our inocula were derived. Inoculum refers to our inocula (DWV-A or DWV-B), D4-A to a pupa inoculated with inoculum A, and V4-B to a pupa inoculated with inoculum B. The grey scale colours indicate percent identity between all simultaneously aligned sequences within either DWV-A (rows 1-6) or DWV-B (rows 7-14) (black=100% identity).



McMahon, D.P.; Natsopoulou, M.E.; Doublet, V.; Fürst, M.; Weging, S.; Brown, M.J.F.; Gogol-Döring, A.; Paxton, R.J. Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proceedings of the Royal Society of London B: Biological Sciences* **2016**, *283*, 20160811, doi: 10.1098/rspb.2016.0811

Figure S6. Maximum Likelihood phylogenetic tree of all DWV contig sequences generated by IVA *de novo* assemblies of all DWV reads in our four NGS libraries (taxa 'contigs'). DWV-A Accession No. NC_004830.2 and DWV-B Accession No. NC_006494.1 were used as reference sequences. The McM-2016 sequences correspond to the references sequences of DWV-A and DWV-B generated by McMahon *et al.* (2016), and from which our inocula were derived. Inoculum refers to our inocula (DWV-A or DWV-B), D4-A to a pupa inoculated with inoculum A, and V4-B to a pupa inoculated with inoculum B. The scale bar represents substitution rate per site. Bootstrap values were calculated after 100 replicates using the HKY85 model.



McMahon, D.P.; Natsopoulou, M.E.; Doublet, V.; Fürst, M.; Weging, S.; Brown, M.J.F.; Gogol-Döring, A.; Paxton, R.J. Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proceedings of the Royal Society of London B: Biological Sciences* **2016**, *283*, 20160811, doi: 10.1098/rspb.2016.0811

Figure S7. Titres of DWV-A and DWV-B per honey bee pupa at days 0 and 3 post inoculation (p.i.) with either DWV-A or DWV-B (n = 4 per treatment and age class). Box plots show the median (dark bar), upper and lower interquartiles (coloured box), and 95% confidence limits (whiskers). Within a viral genotype, titres did not differ between bees injected with 10^2 or 10^4 virus particles (GLMs $p > 0.05$). The DWV-A titre after 3 days was higher than the DWV-B titre (GLM $p < 0.01$).

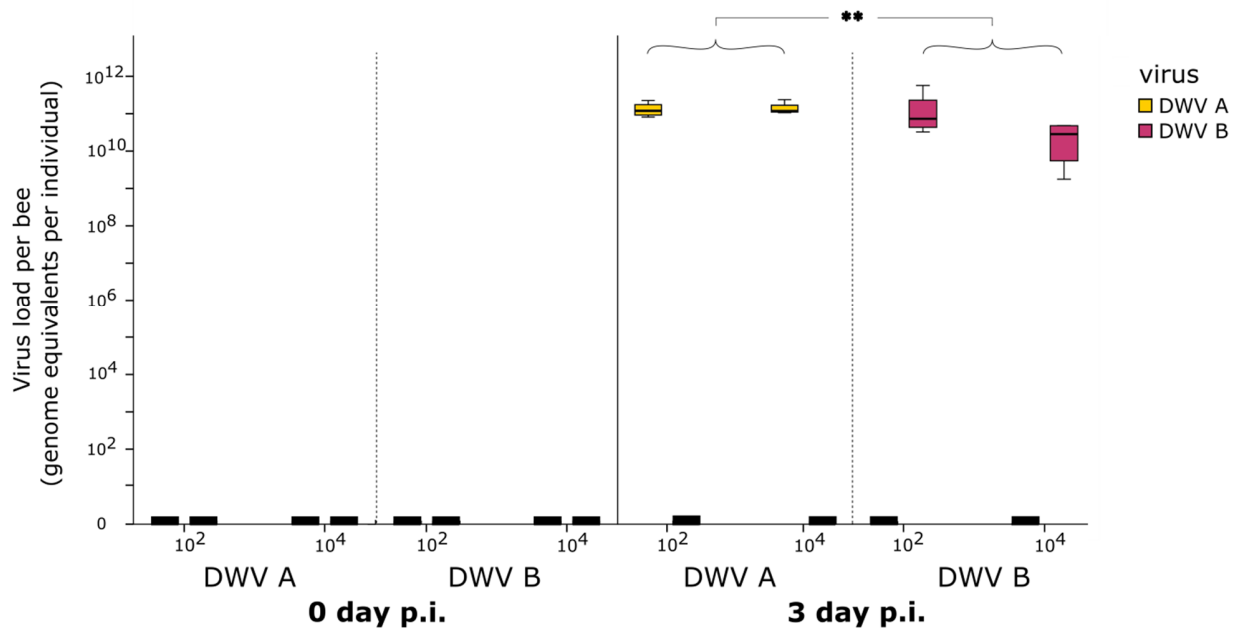
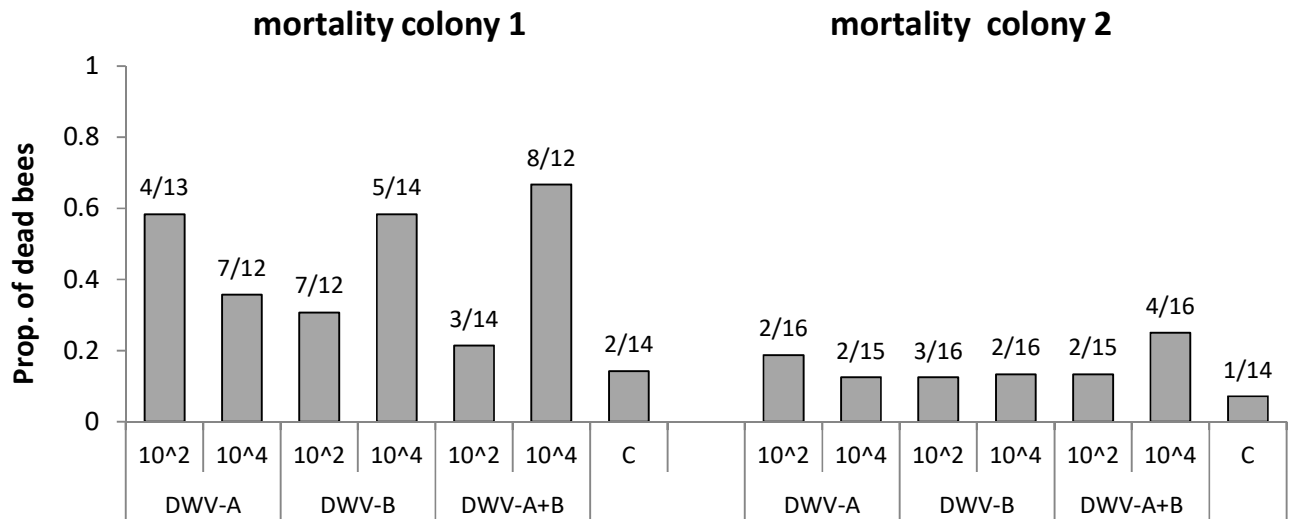


Figure S8. Survival (a) and wing deformities (b) in treatment groups split by colony of origin (colony 1, colony 2). Values above histograms give the number of individuals exhibiting the trait/total number of individuals. C represents the control inoculation devoid of virus.

(a)



(b)

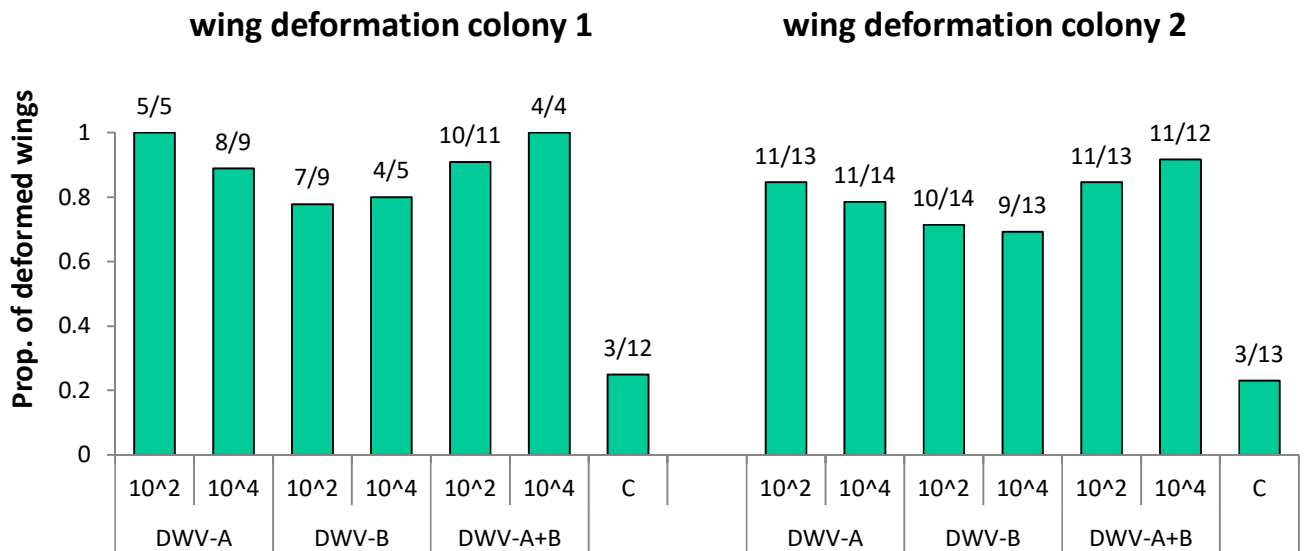


Figure S9. Viral titres per bee in eclosing honey bee adults that as white-eyed pupae had been inoculated with DWV. Data from all our viral treatments (A, B, A+B) were used to generate mean titres of DWV-A and DWV-B, and partitioned by whether the eclosing bee had normal or deformed wings. Only those bees where the experimental infection worked successfully and the bees therefore showed virus titres above 10^7 genome equivalents (Ct <33) were included (n = 4 of 44 bees were excluded, including one bee inoculated with A, one with B and two with A+B). There was no difference in titre between bees with normal *versus* deformed wings for DWV-A (GLMM, $z = -0.648$, $p = 0.517$) or for DWV-B (GLMM, $z = -0.764$, $p = 0.445$). Viral titres (DWV-A, DWV-B) separated by viral treatment are given in Figure 4.

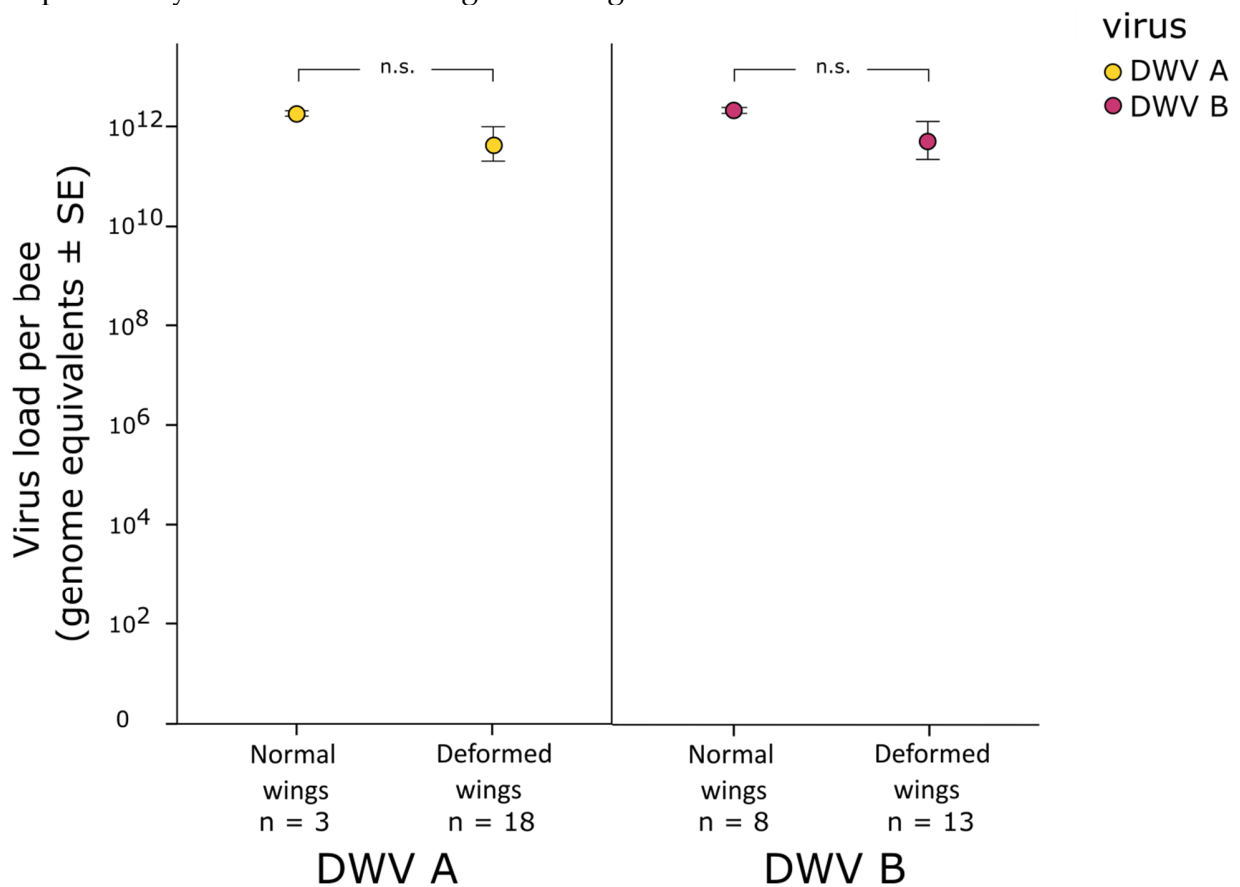


Table S1. Contents (number and % of all NGS reads that could be mapped to contigs or that remained unmapped) of the two NGS libraries generated using either the DWV-A or the DWV-B inoculum.

		DWV-A inoculum		DWV-B inoculum	
Origin		N reads	% reads	N reads	% reads
<i>Apis</i>		12,868,152	86.794	21,763,325	67.855
DWV					
	DWV-A	1,843,693	12.435	6,143	0.019
	DWV-B	65,169	0.440	10,236,879	31.917
Other					
	<i>Apis rhadbovirus-1</i>	6,113	0.041	33,735	0.105
	Other Eukaryota	4,064	0.027	6,347	0.020
	Bacteria	3,630	0.024	6,308	0.020
	Other (viral or Eukaryota)*	999	0.007	945	0.003
	Unknown reads [§]	35,240	0.238	20,628	0.064
Total		14,826,061		32,073,365	

*; short contigs that either represent fragments of DNA viruses or eukaryote genes e.g. annotated *Apis* rRNA genes

[§]; includes NGS reads that could not be assembled into contigs

Table S2. Contents (number and % of all NGS reads that could be mapped to contigs or that remained unmapped) of the two NGS libraries generated from eclosing honey bees that had been inoculated as white-eyed pupae with either the DWV-A (individual pupal code: D4-DWV-A) or the DWV-B inoculum (individual pupal code: V4-DWV-B).

		D4-DWV-A pupa (DWV-A inoculated)		V4-DWV-B pupa (DWV-B inoculated)	
Origin		N reads	% reads	N reads	% reads
<i>Apis</i>		11,856,745	51.955	7,017,221	41.870
DWV	DWV-A	9,923,721	43.484	969	0.006
	DWV-B	30,786	0.135	9,538,992	56.916
Other					
	<i>Apis rhadbovirus-1</i>	16	<0.001	0	
	Other Eukaryota	34,225	0.150	22,893	0.137
	Bacteria	18,221	0.080	15,835	0.094
	Other (viral or Eukaryota)*	0		35	<0.001
	Unknown §	957,677	4.196	163,749	0.977
Total		22,821,391		16,759,694	

*; short contigs that either represent fragments of DNA viruses or eukaryote genes e.g. annotated *Apis* rRNA genes

§; includes reads that could not be assembled into contigs

Table S3. Comparison of published consensus sequences for DWV-A (NC_004830.2) and DWV-B (NC_006494.1), inocula A and B of McMahon *et al.* (2016; McM-2016-A and McM-2016-B respectively), those used here (DWV-A inoculum /DWV-B inoculum, which were derived from those of McMahon *et al.* 2016) and virus derived from experimentally inoculated host pupae as they eclosed as adults (individual pupal codes: D4-DWV-A and V4-DWV-B for DWV-A and DWV-B inoculated pupae respectively).

		DWV-A			
		NC_004830.2	McM-2016-A	DWV-A inoculum	D4-DWV-A
DWV-A	NC_004830.2		195 + 69N	194 + 69N	195 + 69N
	McM-2016-A	97.732%		1	2
	Inoculum A	97.742%	99.990%		2
	D4-DWV-A	97.732%	99.980%	99.980%	

		DWV-B			
		NC_006494.1	McM-2016-B	DWV-B inoculum	V4-DWV-B
DWV-B	NC_006494.1		75 (1 insert)	77 (1 insert)	75 (1 insert)
	McM-2016-B	99.258%		3	1
	Inoculum B	99.239%	99.970%		5
	V4-DWV-B	99.258%	99.990%	99.951%	

Upper right diagonal: number of nucleotide base differences

Lower left diagonal: % nucleotide identity

N: bases whose identity could not be unambiguously determined within the sequences

McMahon, D.P.; Natsopoulou, M.E.; Doublet, V.; Fürst, M.; Weging, S.; Brown, M.J.F.; Gogol-Döring, A.; Paxton, R.J. Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proceedings of the Royal Society of London B: Biological Sciences* **2016**, *283*, 20160811, doi: 10.1098/rspb.2016.0811