

Supplementary Information

Title:

Deformed wing virus type A, a major honey bee pathogen, is vectored by the mite *Varroa destructor* in a non-propagative manner.

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Supplementary Figure S3. Single stranded RNA degradation in disrupted honey bee hemolymph cells.

Supplementary Table 1. Primers used in this study.

Primer ID	Nucleotide sequence (5' to 3')	Target: GenBank accession number, (position in the nucleotide sequence), polarity	Applications
DWV-qPCR-F	GAGATTGAAGCGCATGAACA	AY292384, (6471- 6490), sense	DWV RT-qPCR
DWV-qPCR-R	tgaattcagtgctgccata	AY292384, (6600 - 6581), complement	DWV RT-qPCR
Varr-qbACT-R	TGAAGGTAGTCTCATGGATAC	XR_002672520 , (142 - 122) complement	Varroa actin mRNA RT-qPCR
Varr-qbACT-F	GTCTCTGTTCCAGCCCTCGTTC	XR_002672520, (81 - 102), sense	Varroa actin mRNA qRT-PCR
Amel-qbACT-F	AGGAATGGAAGCTTGCGGTA	NM_001185146, (919 - 938), sense	Honey bee actin mRNA RT-qPCR
Amel-qbACT-R	AATTTTCATGGTGGATGGTGC	NM_001185146, (1099 - 1079), complement	Honey bee actin mRNA RT-qPCR
DWV900F	GTTAATGTCTCATAGCCAGACG	AY292384, (899 - 921), sense	RT-PCR, 1.2 kbp fragment, DWV strain identification
DWV2050Rev	CATATAGCATCAGAATTAGCCTC	AY292384, (2071 - 2049), complement	RT-PCR, 1.2 kbp fragment, DWV strain identification
APO3Q-F1	GCGAAATCTCTGAGCCAAC	NM_001114198, (473 - 491), sense	Honey bee Apo3 mRNA RT-qPCR
APO3Q-R1	GAGTTGCGGCAGTTTGAAG	NM_001114198 (599 - 581), complement	Honey bee Apo3 mRNA RT-qPCR
Tag1-DWV-For	CTTGTTAGCTGTGTGCAGTTGCTGTAGTCAAGCGTTACTTGAG	AY292384, (4909 - 4934), sense	Negative-strand DWV RNA detection (Assay 1), RT
DWV-1-rev	GGAGCTTCTGGAACGGCAGAT	AY292384, (5008 - 4988), complement	Negative-strand DWV RNA detection (Assay 1), qPCR
Tag1	CTTGTTAGCTGTGTGCAGTTG	not applicable	Negative-strand DWV RNA detection (Assay 1), qPCR
Tag2-DWV-2-For	AGCCTGCGCACGTGGcgaaccaactctgaggaa	AY292384, (7330 - 7349), sense	Negative-strand DWV RNA detection (Assay 2), RT
DWV-2-rev	GTGTTGATCCCTGAGGCTTA	AY292384, (7503 - 7484), complement	Negative-strand DWV RNA detection (Assay 2), qPCR
Tag2	AGCCTGCGCACGTGG	not applicable	Negative-strand DWV RNA detection (Assay 2), qPCR

Supplementary Table 2. Detection of honey bee mRNA in *Varroa* mites by RT-qPCR. Ct values for honey bee actin (BeeBase gene identifier GB44311) and Apolipoprotein III-like, Apo3 (BeeBase gene identifier GB55452) mRNAs, *Varroa destructor* actin (GenBank Accession XR_002672520) mRNA, and DWV genomic RNA in pooled *Varroa* mite and control honey bee pupae.

Pooled samples	DWV RNA	<i>Varroa</i> actin mRNA	Honey bee actin mRNA	Honey bee Apo3 mRNA
<i>Varroa</i> mites				
V-Pho ₁	25.8	19.7	26.8	36.6
V0-PBS ₁	nd	21.9	31.0	nd
V0-DWV ₁	14.7	22.8	36.4	41.2
V4-PBS ₁	25.1	24.4	34.2	nd
V4-DWV ₁	21.5	22.8	33.5	37.9
Honey bee pupae				
P0-DWV ₁	11.0	nd	17.3	23.6
P4-DWV ₁	18.0	nd	24.3	31.3
Water control	nd	nd	nd	nd

nd - not detected

Supplementary Table 3. Detection and quantification of honey bee mRNAs in RNAseq libraries from phoretic *Varroa* mites following starvation or feeding. Numbers of NGS reads corresponding a set of *Varroa* and honey bee mRNAs as well as DWV genomic RNA were determined in *Varroa destructor* RNAseq libraries (Egekwu & Cook, submitted).

Varroa RNA-seq library ID		Var_C333_VaD_WaS_12H	Var_C3_VaD_WaF_15H	Var_C3_VaD_WpF_15H
Varroa mite treatment		Phoretic mites starved for 12 hours	Phoretic mites fed on adult honey bees for 15 hours	Phoretic mites fed on honey bee pupae for 15 hours
Total number of reads in the library		59560566	49673302	65165306
DWV reads		38870677	18964968	24572362
Reads numbers ratio, honey bee beta-actin mRNA to <i>Varroa</i> beta-actin		0.017	0.039	0.034
Reads numbers ratio, total honey bee mRNAs to <i>Varroa</i> beta-actin mRNA		2.540	24.152	19.066
<i>Apis mellifera</i> mRNA	<i>A. mellifera</i> beta-actin (GB44311) NM_001185146	626	674	836
	<i>A. mellifera</i> myosin heavy chain (GB51653)	1428	4094	5231
	<i>A. mellifera</i> apolipoprotein III-like protein (GB55452)	1	240	220
Total <i>A. mellifera</i> mRNAs reads		92604	414034	470256
<i>Varroa destructor</i> mRNA	XR_002672520, <i>V. destructor</i> beta actin	36462	17143	24665
	<i>V. destructor</i> myosin heavy chain	2246	3318	4859
	<i>V. destructor</i> vitellogenin 1 JQ974976	1901	773	1289

Supplementary Table 4. Detection of honey bee mRNAs in RNAseq libraries from different *Varroa* mite developmental stages. Numbers of NGS reads corresponding a set of *Varroa* and honey bee mRNAs were determined in *Varroa destructor* RNAseq libraries²³ (NCBI BioProject PRJNA392105).

<i>Varroa destructor</i> RNA-seq library, accession number, mite description		SRX2960636 Emerging mites (collected from P8 to P9 brood cells)	SRX29606371 Young mites (collected from P8 to P9 brood cells)	SRX2960647 Young mites (collected from P8 to P9 brood cells)	SRX2960634 Laying mites (collected from sealed brood cells containing pre-pupae)	SRX2960649 Phoretic mites (collected on adult bees)	SRX2960670 Phoretic mites artificially reared in cages with adult bees	SRX2960669 Phoretic mites (collected on adult bees)	SRX2960632 Arresting mites (collected in unsealed L5 brood cells)
Total number of reads in the library		26531852	27083570	33433690	26670037	17467280	29446920	32563456	28709594
<i>Apis mellifera</i> mRNAs	NM_001185146 <i>A. mellifera</i> actin related protein 1 (Arp1)	140	473	683	174	93	206	88	87
	XM_02644216 <i>A. mellifera</i> myosin heavy chain	122	758	355	36	56	92	57	14
	NM_001114198 <i>A. mellifera</i> apolipoprotein III-like protein	3	51	77	11	1	0	0	2
<i>Varroa destructor</i> mRNAs	XR_002672520, <i>V. destructor</i> actin-like protein	>20000	>20000	>20000	8606	17838	>20000	13931	9678
	XM_022800959 <i>V. destructor</i> myosin heavy chain	2314	2523	4080	1847	1494	2920	1017	1061
	IQ974976 <i>V. destructor</i> vitellogenin 1	1649	524	1182	>20000	848	1446	756	3405

Supplementary Figure S1. Alignments of consensus DWV RNA nucleotide sequences from honey bees and mites in Experiment 2: “Varroa-mediated DWV transmission experiment”. RT-PCR fragments corresponding to the DWV LP region were amplified using RNA extracted from bees and mites pooled according to their treatment (Experiment 2, Figs. 2 and 4). **(a, b)** Complete identity between the DWV sequences amplified from honey bee pupae P0-DWV₂, P1-DWV₂, and the cloned isolate DWV-304. **(c, d)** Complete nucleotide identity between the sequenced terminal portions of the RT-PCR fragment amplified from V0-DWV₂ mites, the sequences from honey bee pupae P0-DWV₂, P1-DWV₂, and the cloned isolate DWV-304. Due to high polymorphism in the mite DWV load only the terminal portions of V0-DWV₂ were sequenced. Cloned DWV isolate DWV-304 (GenBank accession number MG831200, isolated in the USA in 2015) was used for pupae P0-DWV₂ injection. The type DWV isolate DWV-PA isolated the USA in 2006 (GenBank Accession number AY292384), was included with alignments **(b)** and **(c)** to show distribution of polymorphic sites in the LP region of DWV RNA.

(a)

DWV-304	agacatcatatntttatntttaaagctgctcttttattgctgattatnttggctgnttttattt	1020
Pupae-P0-DWV-2	-----TATTTTAATGCTGCTTTATTTGCTGATTATTTTGCCTGTTTTATTT	47
Pupae-P1-DWV-Ext-2	-----CATATTTTATTTTAATGCTGCTTTATTTGCTGATTATTTTGCCTGTTTTATTT	54

DWV-304	gctatnttatatnttggtaattttcattattgcaaatatattacattgctatntttatta	1080
Pupae-P0-DWV-2	GCTATTTTATATTTGCTAATTTTCATTATTGCGAAATATATTACATTGCTATTTTATTTA	107
Pupae-P1-DWV-Ext-2	GCTATTTTATATTTGCTAATTTTCATTATTGCGAAATATATTACATTGCTATTTTATTTA	114

DWV-304	tatacgctagattcaatntttatntttcctatatntttcaatnttatttggatnttgaaggt	1140
Pupae-P0-DWV-2	TATACGCTAGATTCAATTTTATTTTCCATATTTTCAATTTAATTTTGATTTTGAAGGT	167
Pupae-P1-DWV-Ext-2	TATACGCTAGATTCAATTTTATTTTCCATATTTTCAATTTAATTTTGATTTTGAAGGT	174

DWV-304	aaatatatataaattaattataaaaaatggccttcagttgtggaactctctcctactctgc	1200
Pupae-P0-DWV-2	AAATATATATAAATTAATTTATTTAAAAATGGCCTTCAGTTGTGGAACCTCTCCTACTCTGC	227
Pupae-P1-DWV-Ext-2	AAATATATATAAATTAATTTATTTAAAAATGGCCTTCAGTTGTGGAACCTCTCCTACTCTGC	234

DWV-304	tgtcgcccaagctccgctccgttgcccatgcacctcgatcgatgggaagttgatgaagccag	1260
Pupae-P0-DWV-2	TGTCGCCCAAGCTCCGCTCCGTTGCCATGCACCTCGTACATGGGAAGTTGATGAAGCCAG	287
Pupae-P1-DWV-Ext-2	TGTCGCCCAAGCTCCGCTCCGTTGCCATGCACCTCGTACATGGGAAGTTGATGAAGCCAG	294

DWV-304	gcgcgccgagtcacaaacgnttggcgctggagcaagaacgatttcgcaacgnttcttga	1320
Pupae-P0-DWV-2	GCGGCGCCGAGTCATCAAACGTTTGGCGCTGGAGCAAGAACGATTTCCGCAACGTTCTTGA	347
Pupae-P1-DWV-Ext-2	GCGGCGCCGAGTCATCAAACGTTTGGCGCTGGAGCAAGAACGATTTCCGCAACGTTCTTGA	354

DWV-304	cgccggcgtctatgaccaggcgacatgggaacaggaggacgcgcgcgataaatgagttcct	1380
Pupae-P0-DWV-2	CGCCGGCGTCTATGACCAGGCGACATGGGAACAGGAGGACGCGCGGATAAATGAGTTCCCT	407
Pupae-P1-DWV-Ext-2	CGCCGGCGTCTATGACCAGGCGACATGGGAACAGGAGGACGCGCGGATAAATGAGTTCCCT	414

DWV-304	aacggaacaattaacaatnttatataactatnttctgatcgccgaacgnttgcacgctcg	1440
Pupae-P0-DWV-2	AACGGAACAATTAACAATTTATATACTATTTATTCGATCGCCGAACGTTGCACGCGTCCG	467
Pupae-P1-DWV-Ext-2	AACGGAACAATTAACAATTTATATACTATTTATTCGATCGCCGAACGTTGCACGCGTCCG	474

DWV-304	acctattaagagcactctcctatatcagtttctgaataggttctgctccattggaatccct	1500
Pupae-P0-DWV-2	ACCTATTAAGAGCACTCTCCTATATCAGTTTTCGAATAGGTTTGCCTCCATTGGAATCCCT	527
Pupae-P1-DWV-Ext-2	ACCTATTAAGAGCACTCTCCTATATCAGTTTTCGAATAGGTTTGCCTCCATTGGAATCCCT	534

DWV-304	caaagtcgaggtcggtaagaagcaggcgaatgtatatntttaaagaacctaaatacacgcg	1560
Pupae-P0-DWV-2	CAAAGTCGAGGTCGGTCAAGAAGCAGGCGAATGTATATTTAAGAAACCTAAATACACGCG	587
Pupae-P1-DWV-Ext-2	CAAAGTCGAGGTCGGTCAAGAAGCAGGCGAATGTATATTTAAGAAACCTAAATACACGCG	594

DWV-304	cgtttgcaagaaagtgaagcgtgttgcaacccgcttcggtcgtaaaaagttggtcgcc	1620
Pupae-P0-DWV-2	CGTTTGCAAGAAAGTGAAGCGTGTGCAACCCGCTTCGTTTCGTGAAAAAGTTGTTTCGTCC	647
Pupae-P1-DWV-Ext-2	CGTTTGCAAGAAAGTGAAGCGTGTGCAACCCGCTTCGTTTCGTGAAAAAGTTGTTTCGTCC *****	654
DWV-304	tatgtgttctagatctcctatgctattatattaagcttaagaaaattatattatgatttgca	1680
Pupae-P0-DWV-2	TATGTGTTCTAGATCTCCTATGCTATTATTTAAGCTTAAGAAAATTTATTTATGATTTGCA	707
Pupae-P1-DWV-Ext-2	TATGTGTTCTAGATCTCCTATGCTATTATTTAAGCTTAAGAAAATTTATTTATGATTTGCA *****	714
DWV-304	cttatatagattaagaaaacagattaggatgtttgagacgtcaaaaacagcgcgagatgga	1740
Pupae-P0-DWV-2	CTTATATAGATTAAGAAAACAGATTAGGATTTTTCGACGCTCAAAAACAGCGCGAGTATGA	767
Pupae-P1-DWV-Ext-2	CTTATATAGATTAAGAAAACAGATTAGGATTTTTCGACGCTCAAAAACAGCGCGAGTATGA *****	774
DWV-304	gtagagtggtgctactaatctgtttacaattatcgaaatccgggtgcaggcaaaaaccagagat	1800
Pupae-P0-DWV-2	GTTAGAGTGTGCTACTAATCTGTTTACAATTATCGAATCCGGTGCAGGCAAAAACCAGAGAT	827
Pupae-P1-DWV-Ext-2	GTTAGAGTGTGCTACTAATCTGTTTACAATTATCGAATCCGGTGCAGGCAAAAACCAGAGAT *****	834
DWV-304	ggataaccctaataccaggacctgatggcgaggggtgaagttgaattagaaaaggatagtaa	1860
Pupae-P0-DWV-2	GGATAACCCCTAATCCAGGACCTGATGGCGAGGGTGAAGTTGAATTAGAAAAGGATAGTAA	887
Pupae-P1-DWV-Ext-2	GGATAACCCCTAATCCAGGACCTGATGGCGAGGGTGAAGTTGAATTAGAAAAGGATAGTAA *****	894
DWV-304	tgttggttttaacaactcagcgagatcctagtagatctattccagcgcgggtgagcgtaaa	1920
Pupae-P0-DWV-2	TGTTGTTTTAACAACCTCAGCGAGATCCTAGTACATCTATTCCAGCGCCGGTGAGCGTAAA	947
Pupae-P1-DWV-Ext-2	TGTTGTTTTAACAACCTCAGCGAGATCCTAGTACATCTATTCCAGCGCCGGTGAGCGTAAA *****	954
DWV-304	atggagtagatggactagtaatgatgtagtagatgattacgccacaatcacatctcgatg	1980
Pupae-P0-DWV-2	ATGGAGTAGATGGACTAGTAATGATGATGATGATTACGCCACAATCACATCTCGATG	1007
Pupae-P1-DWV-Ext-2	ATGGAGTAGATGGACTAGTAATGATGATGATGATTACGCCACAATCACATCTCGATG *****	1014
DWV-304	gtatcagattgctgaatttgggtcgaaggatgatccatttgataaggagttagcacg	2040
Pupae-P0-DWV-2	GTATCAGATTGCTGAATTTGTTGGTTCGAAGGATGATCCATTTGATAAGGAGTTAGCACG	1067
Pupae-P1-DWV-Ext-2	GTATCAGATTGCTGAATTTGTTGGTTCGAAGGATGATCCATTTGATAAGGAGTTAGCACG *****	1074

(b)

DWV-PA	AGACATCATATTTTATTTAATGCTGCTTTTATGCTGATTTATCTTGCTGTTTTTATTT	994
DWV-304	agacatcatatthttatthttaatgctgcttttattgctgatttattttgctgthtttatttt	1020
Pupae-P0-DWV-2	-----TATTTAATGCTGCTTTTATGCTGATTTATTTGCTGTTTTTATTT	47
Pupae-P1-DWV-Ext-2	-----CATATTTATTTAATGCTGCTTTTATGCTGATTTATTTGCTGTTTTTATTT *****	54
DWV-PA	GCTATTTTATATTTGCTAATTTTCATTATTTGCGAAATATATTACATTGCTATTTTATTA	1054
DWV-304	gctatthttatatttgctaattttcattatggcgaataatattacattgctatthttatta	1080
Pupae-P0-DWV-2	GCTATTTTATATTTGCTAATTTTCATTATTTGCGAAATATATTACATTGCTATTTTATTA	107
Pupae-P1-DWV-Ext-2	GCTATTTTATATTTGCTAATTTTCATTATTTGCGAAATATATTACATTGCTATTTTATTA *****	114
DWV-PA	TATACGCTAGATTCAATTTTATTTTTCATATTTTCAATTTAATTTTGATTTGGAAGGT	1114
DWV-304	tatacgtagattcaatthttatthttccctatattttcaatthttatthttgattttgaaggt	1140
Pupae-P0-DWV-2	TATACGCTAGATTCAATTTTATTTTTCATATTTTCAATTTAATTTTGATTTTGAAGGT	167
Pupae-P1-DWV-Ext-2	TATACGCTAGATTCAATTTTATTTTTCATATTTTCAATTTAATTTTGATTTTGAAGGT *****	174
DWV-PA	AAATATATATAATTAATTTAATAAAATGGCCTTTAGTTGTTGGAACCTCTTCTACTCTGC	1174
DWV-304	aaatataataaataattataaaaaatggccttcagttgtggaactctctcctactctgc	1200
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Pupae-P1-DWV-Ext-2	AAATATATATAATTAATTTAATAAAATGGCCTTCAGTTGTTGGAACCTCTCTCTACTCTGC *****	234
DWV-PA	CGTCGCCAAGCTCCGCTCGTCCATGACACCTCGTACATGGGAAGTTGATGAAGCTAG	1234
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Pupae-P0-DWV-2	TGTCGCCAAGCTCCGCTCGTCCATGACACCTCGTACATGGGAAGTTGATGAAGCCAG	287
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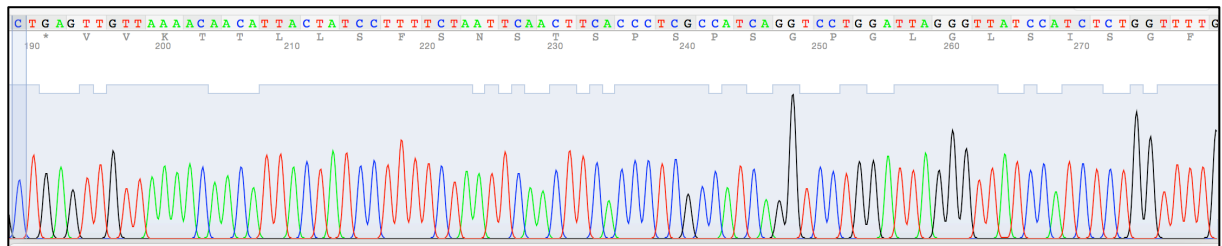
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Pupae-P0-DWV-2	GCGGCGCCGAGTCATCAACGTTTGGCGCTGGAGCAAGAACGTATTCGCAACGTTCTTGA	347
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DWV-PA	CGTFGGCGTCTATGACCAGGCGACATGGGAACAGGAGGACGCGCGGATAATGAGTTCCT	1354
DWV-304	cgccggcgctctatgaccagggcgacatgggaacaggaggacgcgcgataatgagttcct	1380
Pupae-P0-DWV-2	CGCCGGCGTCTATGACCAGGCGACATGGGAACAGGAGGACGCGCGGATAATGAGTTCCT	407
Pupae-P1-DWV-Ext-2	CGCCGGCGTCTATGACCAGGCGACATGGGAACAGGAGGACGCGCGGATAATGAGTTCCT **	414
DWV-PA	AACGGAACAATTAACAATTTATATACTATTTATTCGATCGCTGAACGTTGTACGCGTGC	1414
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Pupae-P0-DWV-2	AACGGAACAATTAACAATTTATATACTATTTATTCGATCGCGGAACGTTGCACGCGTGC	467
Pupae-P1-DWV-Ext-2	AACGGAACAATTAACAATTTATATACTATTTATTCGATCGCGGAACGTTGCACGCGTGC *****	474
DWV-PA	GCCTATCAAAGAGTACTCTCCTATATCAGTTTCGAATAGGTTTGTCTCCACTGGAATCCCT	1474
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DWV-PA	CAAGGTCGAGGTCGGTCAAGAAGCAGGCGAATGTATATTTAAGAAACCTAAATATACGCG	1534
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Pupae-P0-DWV-2	CAAAGTCGAGGTCGGTCAAGAAGCAGGCGAATGTATATTTAAGAAACCTAAATACACGCG	587
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DWV-PA	CGTTTGCAAGAAAGTGAAGCGTGTGCAACTCGCTTCGTTTCGTGAAAAAGTTGTTTCGTCC	1594
DWV-304	cgtttgcaagaaagtgaagcgtgtgcaacctcgttcggtgaaaaagttgttcgtcc	1620
Pupae-P0-DWV-2	CGTTTGCAAGAAAGTGAAGCGTGTGCAACTCGCTTCGTTTCGTGAAAAAGTTGTTTCGTCC	647
Pupae-P1-DWV-Ext-2	CGTTTGCAAGAAAGTGAAGCGTGTGCAACTCGCTTCGTTTCGTGAAAAAGTTGTTTCGTCC *****	654
DWV-PA	TATGTGTTCTAGATCCCTATGCTATTATTTAAGCTTAAGAAAATTTATTTATGATTTGCA	1654
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Pupae-P1-DWV-Ext-2	GTTAGAGTGTGTCACTAATCTGTACAATTATCGAATCCGGTGCAGGCAAAACCAGAGAT *****	834
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DWV-304	ggataaccctaataccaggacctgatggcgaggggtgaagttgaattagaaaaggatagtaa	1860
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Pupae-P1-DWV-Ext-2	GGATAACCCCTAATCCAGGACCTGATGGCGAGGGTGAAGTTGAATTAGAAAAGGATAGTAA *****	894
DWV-PA	TGTTGTTTTAACAACTCAGCGAGATCCTAGTACATCTATTCAGCGCCGGTGAGCGTAAA	1894
DWV-304	tgttgttttaacaaactcagcgagatcctagtacatctatccagcgccggtgagcgtaaa	1920
Pupae-P0-DWV-2	TGTTGTTTTAACAACTCAGCGAGATCCTAGTACATCTATTCAGCGCCGGTGAGCGTAAA	947
Pupae-P1-DWV-Ext-2	TGTTGTTTTAACAACTCAGCGAGATCCTAGTACATCTATTCAGCGCCGGTGAGCGTAAA *****	954
DWV-PA	ATGGAGTAGATGGACTAGTAATGATGTAGTAGATGATTACGCCACAATCACATCTCGATG	1954
DWV-304	atggagtagatggactagtaatgatgtagtagatgattacgccacaatcacatctcgatg	1980
Pupae-P0-DWV-2	ATGGAGTAGATGGACTAGTAATGATGTAGTAGATGATTACGCCACAATCACATCTCGATG	1007
Pupae-P1-DWV-Ext-2	ATGGAGTAGATGGACTAGTAATGATGTAGTAGATGATTACGCCACAATCACATCTCGATG *****	1014
DWV-PA	GTATCAGATTGCTGAATTTGTTTGGTCAAGGATGATCCATTTGATAAGGAGTTAGCACC	2014
DWV-304	gtatcagattgctgaatttgtttgggtcaaggatgatccatTTGATAAGGAGTTAGCACC	2040
Pupae-P0-DWV-2	GTATCAGATTGCTGAATTTGTTTGGTCAAGGATGATCCATTTGATAAGGAGTTAGCACC	1067
Pupae-P1-DWV-Ext-2	GTATCAGATTGCTGAATTTGTTTGGTCAAGGATGATCCATTTGATAAGGAGTTAGCACC *****	1074

(c)

V0-DWV-2	-----TCATATTTGCTGCTTTATTGCTGATTTATTTGCTGTTTTTATTT	55
DWV-304	agacatcatatattttattttaatgctgtctttattgctgatttattttgctgtttttattt	1020
Pupae-P0-DWV-2	-----TATTTTAATGCTGCTTTATTGCTGATTTATTTGCTGTTTTTATTT	47
Pupae-P1-DWV-Ext-2	-----CATATTTTATTTTAAATGCTGCTTTATTGCTGATTTATTTGCTGTTTTTATTT *****	54
V0-DWV-2	GCTATTTTATATTTGCTAATTTTCATTATTGCGAAATATATTACATTGCTATTTTATTA	115
DWV-304	gctattttataatttgctaattttcattattgCGAAATATATTACATTGCTATTTTATTA	1080
Pupae-P0-DWV-2	GCTATTTTATATTTGCTAATTTTCATTATTGCGAAATATATTACATTGCTATTTTATTA	107
Pupae-P1-DWV-Ext-2	GCTATTTTATATTTGCTAATTTTCATTATTGCGAAATATATTACATTGCTATTTTATTA *****	114
V0-DWV-2	TATACGCTAGATTCAATTTTATTTTCCCTATATTTCAATTTAATTTTGATTTTGAAGGT	175
DWV-304	tatacgttagattcaattttatttttccctataattttcaatttaattttgattttgaaggt	1140
Pupae-P0-DWV-2	TATACGCTAGATTCAATTTTATTTTCCCTATATTTCAATTTAATTTTGATTTTGAAGGT	167
Pupae-P1-DWV-Ext-2	TATACGCTAGATTCAATTTTATTTTCCCTATATTTCAATTTAATTTTGATTTTGAAGGT *****	174
V0-DWV-2	AAATATATATAATTAATTTATTAATAATGGCCTTnnnnnnnnnnnnnnnnnnnnnnnn	235
DWV-304	aaatataataaatttaattataaaaaatggccttcagttgtggaactctctcctactctgc	1200
Pupae-P0-DWV-2	AAATATATATAATTAATTTATTAATAATGGCCTTCAGTTGTGGAACCTCTCCTACTCTGC	227
Pupae-P1-DWV-Ext-2	AAATATATATAATTAATTTATTAATAATGGCCTTCAGTTGTGGAACCTCTCCTACTCTGC *****	234
V0-DWV-2	nn	295
DWV-304	tgctgcccagctccgctcgttgcccatgcacctcgtacatgggaagttgatgaagccag	1260
Pupae-P0-DWV-2	TGTCGCCCAAGCTCCGTCGCTTGCCCATGCACCTCGTACATGGGAAGTTGATGAAGCCAG	287
Pupae-P1-DWV-Ext-2	TGTCGCCCAAGCTCCGTCGCTTGCCCATGCACCTCGTACATGGGAAGTTGATGAAGCCAG	294
V0-DWV-2	nn	355
DWV-304	gcgcgccgagtcatacaaacggtttggcgctggagcaagaacgtattcgcaacgcttctga	1320
Pupae-P0-DWV-2	GCGGCGCCGAGTCATCAAACGTTTGGCGCTGGAGCAAGAACGTATTCGCAACGTTCTTGA	347
Pupae-P1-DWV-Ext-2	GCGGCGCCGAGTCATCAAACGTTTGGCGCTGGAGCAAGAACGTATTCGCAACGTTCTTGA	354
V0-DWV-2	nn	415
DWV-304	cgccggcgtctatgaccaggcgacatgggaacaggaggacgCGCGGATAATGAGTTCCT	1380
Pupae-P0-DWV-2	CGCCGGCGTCTATGACCAGGCGACATGGGAACAGGAGGACGCGCGGATAATGAGTTCCT	407
Pupae-P1-DWV-Ext-2	CGCCGGCGTCTATGACCAGGCGACATGGGAACAGGAGGACGCGCGGATAATGAGTTCCT	414
V0-DWV-2	nn	475
DWV-304	aacggaacaattaacaatttatataactatttattcgatcgccgaacgctgcaacgctcg	1440
Pupae-P0-DWV-2	AACGGAACAATTAACAATTTATATACTATTTATTCGATCGCCGAACGTTGCACGCGTGC	467
Pupae-P1-DWV-Ext-2	AACGGAACAATTAACAATTTATATACTATTTATTCGATCGCCGAACGTTGCACGCGTGC	474
V0-DWV-2	nn	535
DWV-304	acctattaagagcactctcctataatcagtttcgaataggtttgctccattggaatccct	1500
Pupae-P0-DWV-2	ACCTATTAAGAGCACTCTCCTATATCAGTTTCGAATAGGTTTGTCTCCATGGAATCCCT	527
Pupae-P1-DWV-Ext-2	ACCTATTAAGAGCACTCTCCTATATCAGTTTCGAATAGGTTTGTCTCCATGGAATCCCT	534
V0-DWV-2	nn	594
DWV-304	caaagtcgaggtcggtaagaagcaggcgaatgtatatttaagaacctaatacacgCG	1560
Pupae-P0-DWV-2	CAAAGTCGAGGTCGGTCAAGAAGCAGGCGAATGTATATTTAAGAAACCTAAATACACGCG	587
Pupae-P1-DWV-Ext-2	CAAAGTCGAGGTCGGTCAAGAAGCAGGCGAATGTATATTTAAGAAACCTAAATACACGCG	594
V0-DWV-2	nn	654
DWV-304	cgtttgcaagaaagtgaagcgtgtgcaaccgcttcgctgtaaaaagttgctcgtcc	1620
Pupae-P0-DWV-2	CGTTTGCAAGAAAGTGAAGCGTGTGCAACCCGCTTCGTTTCGTGAAAAAGTTGTTTCGTCC	647
Pupae-P1-DWV-Ext-2	CGTTTGCAAGAAAGTGAAGCGTGTGCAACCCGCTTCGTTTCGTGAAAAAGTTGTTTCGTCC	654
V0-DWV-2	nn	714
DWV-304	tatgtgttctagatctcctatgctattatttaagccttaagaaaattatttatgatttgca	1680
Pupae-P0-DWV-2	TATGTGTTCTAGATCTCCTATGCTATTATTTAAGCTTAAGAAAATTTATTTATGATTTGCA	707
Pupae-P1-DWV-Ext-2	TATGTGTTCTAGATCTCCTATGCTATTATTTAAGCTTAAGAAAATTTATTTATGATTTGCA	714
V0-DWV-2	nn	774
DWV-304	cttatataagattaagaaaacagatttagattttgagacgtcaaaaacagcgcgagtatga	1740
Pupae-P0-DWV-2	CTTATATAGATTAAGAAAACAGATTAGGATTTTGAGACGTCAAAAACAGCGGAGTATGA	767
Pupae-P1-DWV-Ext-2	CTTATATAGATTAAGAAAACAGATTAGGATTTTGAGACGTCAAAAACAGCGGAGTATGA	774

Supplementary Figure S2. DWV polymorphisms in honey bees and mites in Experiment 2: “*Varroa*-mediated DWV transmission experiment”. Electropherograms of direct Sanger sequencing of RT-PCR fragments corresponding to the DWV LP region were amplified using RNA extracted from bees and mites pooled according to their treatment (Experiment 2, Figs. 2 and 4). **(a)** Honey bee pupae P0-DWV₂, infected with a clone-derived DWV isolate served as the “source bees”; **(b)** *Varroa* mites V0-DWV₂, which acquired DWV from the “source bees”; **(c)** Honey bee pupae P1-DWV-Ext₂ infected with DWV strains transmitted by the *Varroa* mites fed first on the “source bees”. Clonal DWV accumulated in the honey bee pupae and divergent DWV accumulated in the *Varroa* mites, as evidenced by the presence of double peaks indicated by arrows.

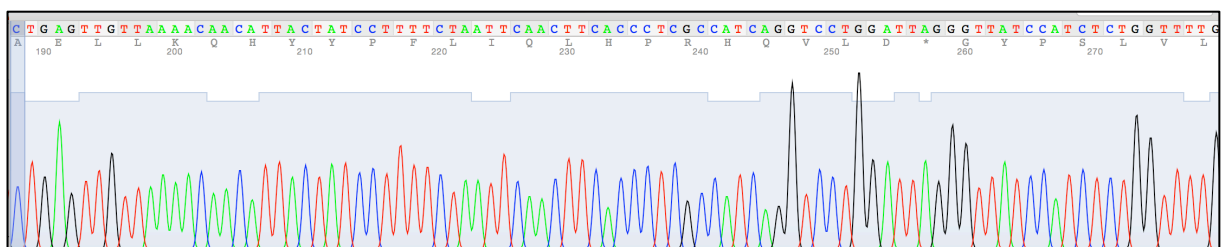
(a) Pupae P0-DWV₂ – clonal DWV



(b) *Varroa* mites V0-DWV₂ - polymorphic DWV



(c) Pupae P1-DWV-Ext₂ – clonal DWV



Supplementary Figure S3. Single stranded RNA degradation in disrupted honey bee hemolymph cells.

