Absence of deformed wing virus and *Varroa destructor* in Australia provides unique perspectives on honeybee viral landscapes and colony losses

John M K Roberts[1*], Denis L Anderson[1,2] and Peter A Durr[3]

[1]Commonwealth Scientific and Industrial Research Organisation, Canberra, ACT, 2601, Australia

[2] ADFCA, Research and Development Division, Al Ain, UAE

[3] CSIRO-Australian Animal Health Laboratory, Geelong, Victoria, 3219, Australia

*Author for correspondence

Supplementary material 1. Primers used for RT-PCR virus detection. T_a is the PCR annealing temperature.

Virus	Primers	T_a	Reference
IAPV	F: AGACACCAATCACGGACCTCAC	58 °C	Maori et al 2007[1]
	R: AGATTTGTCTGTCTCCCAGTGCACAT		
KBV	F: GATGAACGTCGACTATTGA	58 °C	Stoltz et al 1995[2]
	R: TGTGGGTTGGCTATGAGTCA		
BQCV	F: TGG TCA GCT CCC ACT ACC TAA AAC	58 °C	Benjeddou et al 2001[3]
	R: GCA ACA AGA AAC GTA AAC CAC		
LSV1	F: TTA TCT CGC GCC GCC ACC TC	52 °C	Runckel et al 2011[4]
	R: ATC GCC GCT GCA ACG TGA CC		
LSV2	F: CGG CCG GTC TAG CGT GGT TG	52 °C	Runckel et al 2011[4]
	R: TGG CAA GCT GTG ACG AAT CCC T		
DWV-A/	F: ATA TTC ACG GAT TGT TTG AAA GA	58 °C	Meeus et al 2010[5]
DWV-B	R: CRC TAA CAT TCA TGA TAA GAT CGT C		
SBPV	F: GAT TTG CGG AAT CGT AAT ATT GTT TG	58 °C	de Miranda 2010[6]
	R: ACC AGT TAG TAC ACT CCT GGT AAC TTC G		
SBV	F: AAT GGT GCG GTG GAC TAT GG	58 °C	Grabensteiner et al 2001[7]
	R: TGA TAC AGA GCG GCT CGA CA		
ABPV	F: TGA GAA CAC CTG TAA TGT GG	58 °C	Tentcheva et al 2004[8]
	R: ACC AGA GGG TTG ACT GTG TG		
CBPV	F: AGT TGT CAT GGT TAA CAG GAT ACG AG	58 °C	Ribiere et al 2000[9]
	R: TCT AAT CTT AGC ACG AAA GCC GAG		

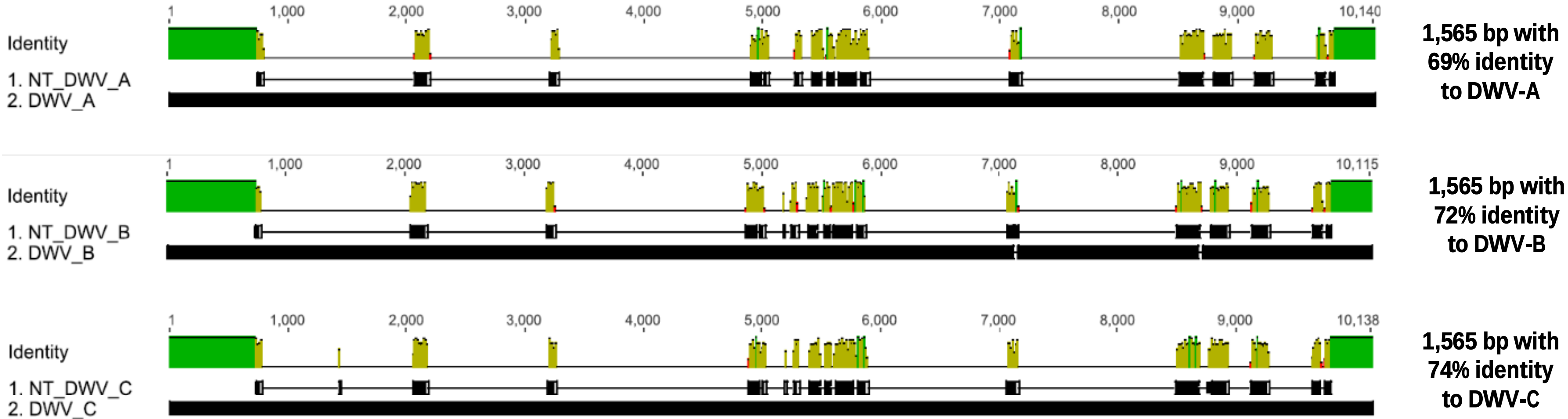
Supplementary material 2. Summary of BLAST results for sequence fragments from NT, QLD and WA-2 samples that mapped to DWV reference genomes.

Sequence fragment and length	BLAST aligned sequence	Location on aligned sequence	Highest nucleotide similarity to DWV isolates
NT DWV-like 1 (54bp)	GTAGGGACCCCTCTATCCTCTAGGTACTGTATGTCGCGAAAGTATGAAAGTAAT	734-787	91% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 2 (127bp)	ATATGCGATGTACCTAATACAATACCTTTCAAGGTGCATGCATATTGGCGTGGAGAAATGCAAGTTCGAATACAAGTTAACTCGAATAAGTTCCAGGTTGGTCAATTGC AAGCCACTTGGTACTATT	2045-2171	84% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 3 (66bp)	TGTTTATGTATGTGCAAGTACCATTGATACCGATGGAAGCTGTAGCTGATACTATCGATATTAATG	3189-3254	92% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 4 (148bp)	TGAAATGATGAATACTGTCATCACCATCGTAAAACGATTGTTAGGCAAATATAGATTGGCGACGCAACCCCAGGACCACGCCGATTCACGTACTGTTAACGCTACCCCA GAGGGACCTAGTGCTGAGGCTGAGGAAACAAGTGCGTGG	4867-5017	75% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 5 (51bp)	GTGAAGATGAACCTGAAATTGTAAAGCGATGGGTTAAAGAATGTTTGTATT	5240-5291	89% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 6 (421bp)	ATTTTGTTGCATGACCTTACAGCTGAGATGAATCAGTCGAGAAATTTGACTGTGTTTACTCGGGTGTATGATCAAATATCGAAATTGAAGACCGACCTGAATGTTTTAC GATTTGTATGACTGGTGCTCCTGGTATTGGTAAGTCATTCTTAACTGATGCAAGTAGTACTCCCGTAACTACGGGCATCAAGTGTGTCGTTAATCCTTTATCAGATTATT GGGATCAGTGTGACTTTCAACCAGTCTTGTGCGTAGACGACATGTGGAGTGTTGAGACTGGAGCAACTTTGGACAAACAACTAAATTTGCTCTTTCAGGTTCATTCTCC AATTGTCCTAAAGCTGATTTAGATGGTAAGAAGATGAGGTATAATCCTGAAATCTTCATTTATAATACCAATAAACCTTTTCCGGTATTTGAT	5375-5863	72% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 7 (95bp)	TTTGATAATCCTACGTGGCGATTAATATATAATGCATCAAGAAAGGGTTTGCCAGAATATTATACACTACCAAGTGATGAAATTTCATTAGATTC	7049-7143	78% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 8 (203bp)	CACTTGAAAGAGAAGTTTATAACCGTTATTAAACCTATAAACGGTTGTAAAATTCGTAGTCTGCAGGATGCTATTTTTGGCGCACCTGGAGTAGAAGGATATGATTCGA TTTCGTGGAATACGAGTGCCGGTTTTCCGTTGTCTACAATGAAGCCGGCTGGAACATCAGGAAAGCGCTGGTTGTTTGATATTGAACTTACAAG	8480-8681	77% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 9 (153bp)	GGGCATAAAGCCACATACGATATTTACTGACTGCCTCAAAGATACATGTTTGCCCATAGAGAAGTGTATGATACCGGGTAAAACGAGGATATTTAGTATTGCGCCTGTT CAATATACAATACCTTTTCGACAATATTTTCTCGATTTTATGGC	8761-8913	77% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 10	ATTATACAGAGGAAAAAGATAAGACTGAGATGAGTCGAGTCATGTGGACTATGGCTCAGGAGATTCTTGCACCAAGCCATCTTTGTAGAGATTTGGTGTATAGAGTAC	9111-9253	75% similar to DWV isolate leuven-dwv1 (KX783225)

(143bp)	CTTGTGGCATACCTTCGGGCTCACCCATCACGGAC		
NT DWV-like 11 (72bp)	CGTGTAGCCACTATAGAAAATGCTAAACAATCTCTAGAGTTGGCATTCGGTTGGGGTCCGCAATACTTCAAT	9626-9697	83% similar to DWV isolate leuven-dwv1 (KX783225)
NT DWV-like 12 (44bp)	AAGCTTGGTATTTATGAGGATCTCATTACATGGGAGGAGATGGA	9731-9774	89% similar to DWV isolate leuven-dwv1 (KX783225)
QLD DWV-like 1 (105bp)	TAGACCTCTACGGCATCGAGTAGAGATTAAAAAGAGTAGTGCAATAGTATAATCACTGTCTACCACCACATATGGTAATGATATGGTTTGTGGTAAACCGGTTAT	488-592	80% similar to VDV-1 genome (AY251269)
QLD DWV-like 2 (99bp)	ATGTCAGGTTATATTATGAATGCTCGAGTATGATTTTCTGCGTTGGAGTCGGGACCCCTCGGTCTCCCAGGTGTCGCATGTGACGAAAGTGCGAAAATA	687-785	80% similar to VDV-1 genome (AY251269)
QLD DWV-like 3 (135bp)	ATATGCGATGTACCTAATACAATACCATTCAAGGTACATGCATATTGGCGTGGAGAGATGCAAGTTCGAATACAAGTTAATTCGAATAAATTCCAAGTAGGTCAATTGC AAGCCACTTGGTATTATATGGATTAT	2045-2179	84% similar to VDV-1 genome (AY251269)
QLD DWV-like 4 (232bp)	ATGGATTATATGACACTTAAAGCATCAAGTTATGTGGTATTTGATTTGCAAGAAACAAATAGCTTTACGTTTGAAGTGCCTTATGTGTCATATAGACCATGGTGGTGTTT ATGTATGTACAAGTACCGTTGATACCTATGGAAGCTGTTGCAGATACCATAGATATCAATGTGTATTTAAGAGGAGGCTCTTCATTTGAAGTATGTATTCCTGTACAACC TAGTGTAGGATT	3029-3315	69% similar to VDV-1 genome (AY251269)
QLD DWV-like 5 (326bp)	TTTCCGCAACACCAGAAGGACCTAGTGCTGAGGCTGAGGAAACAAGTGCGTGGGTATCCGTTATTTATAACGGTGTTTGTAATATGTTGAATGTGGTGGCACAAAAGC CAAAACAATTTAAAGACTGGGTAAAAATTGTTTCCCAAGATTTTGGAAACAATTGTAGAAGTAGTAATCAGGTATTCATATTCTTTAAGAACACCTTTGAGGTTTTGAA GAATTTGTAGGACTAATCCTGCCGCTCGCCTATTGAAGGCTGTGAGCGATGAGCCCGAGATACTCAAAGCGTGGGTTAAAGAATGTTTGTACTTAAATGATCCCAAATT	4965-5307	77% similar to VDV-1 genome (AY251269)
QLD DWV-like 6 (111bp)	TACACCTGTCACCACTGGAATTAAGTGTGTTGTTAATCCCCTCTCTGACTATTGGGATCAGTGTGATTTCCAACCGGTTTTGTGCGTTGATGATATGTGGAGTGTGGAAA C	5599-5709	82% similar to VDV-1 genome (AY251269)
QLD DWV-like 7 (98bp)	AACTATTTTAGTTATTTATTCCATAAATCATGGTTATACGCAAATCCCACGTGGAGACTGATTTTTAATGGTTCAAAGAAAGGTATGCCAGAGTATTT	7013-7110	80% similar to VDV-1 genome (AY251269)
QLD DWV-like 8 (90bp)	TGGAATACGAGTGCAGGTTTTCCATTGTCAACAATGAAACCTGCTGGAACATCTGGAAAGAGATGGTTGTTTGATATTGAACTAAAAGAT	8594-8683	80% similar to VDV-1 genome (AY251269)
QLD DWV-like 9 (88bp)	CAACGACTCAAGAAATGAGAAAGAGAGGAATCAAGCCTCACACCATCTTCACAGATTGCCTCAAAGATACATGTTTGCCAGTAGAAAA	8736-8823	82% similar to VDV-1 genome (AY251269)
QLD DWV-like 10	AGGGAAGTACCACCCCCCCACTCGTTCGTTTTTAAATCTGCTAATAGGAATGAACTCTATTATAAGAGTCCAAAAGCAGTGTGGATTAGACCATCACTTTTGGCTTATAC	9854-10050	85% similar to VDV-1 genome (AY251269)

(198bp)	TAGAGAAAGGATGAGCTGCCTCTAAAGGCTCAATTCCGTAGTAGAGTAGGTTTAATTAGGATTAAAGTGGTACTCTAGGTTAGGTATT		
WA-2 DWV-like 1 (127bp)	ATAGGTAATGATCCCAATACGGTACCATTTAGAGTACATGCATATTGGCGTGGAGATATTGAAGTTAAAATTCAGATAAATTCTAATAAATTTCAAGTTGGGCAATTGC AAGCTACTTGGTATTATT	2072-2198	84% similar to DWV isolate Chilensis A1 (JQ413340)
WA-2 DWV-like 2 (91bp)	TGGCGTGGATCTTTGGAGTATCGATTTGATATCGTTGCTTCTCAATTTCATACTGGTAGGTTGATTGTAGGCTATATCCCCGGATATGACA	2948-3037	80% similar to DWV isolate Chilensis A1 (JQ413340)
WA-2 DWV-like 3 (98bp)	AATTGTCAAAGTAGTAATCAAGTATTTATTTTCTTTAAGAATACATTTGAAGTATTAAAGAAGACTTGGGCTTATTTATTTTGTCGAAGTAATCCTGC	5150-5247	81% similar to DWV isolate Chilensis A1 (JQ413340)

Supplementary material 3. Alignment of mapped reads from the NT sample to DWV-A (NC_004830), DWV-B (NC_006494) and DWV-C (ERS657949) using Geneious (Version 9.1.5, created by Biomatters, Auckland, New Zealand)



Supplementary material 4. Australian apiary survey results within the context of comparable molecular-based surveys. In relation to Figure 4, populations with profiles 1-3 are on the lower end of the stress spectrum, populations with profile 4 range across the middle of the stress spectrum and populations with profile 5 are on the high end of the stress spectrum.

Survey reference	**Country: Region**	**Increased colony losses recorded**	**Colony stressors**						**Stress profile**	**Commercial-scale beekeepers**
			Varroa destructor	**DWV complex**	**Other viruses detected**	**Viruses tested for but not detected**	**Other pathogens**	**Abiotic stressors**		
This survey	Australia	No	No	No	BQCV SBV LSV1 LSV2 IAPV ALPV	DWV SBPV KBV CBPV ABPV	Most	Most	1	Yes
Malfroy et al. [10]	Australia: Norfolk Island	No	No	No	LSV	DWV ABPV IAPV KBV BQCV SBV CBPV SBPV	Few	Few	2	No

Kajobe et al. [11]	Uganda	No	No	No	BQCV	DWV SBV CBPV ABPV IAPV	Few	Few	2	No
Shutler et al. [12]	Canada : Newfoundland	No	No	DWV-A	BQCV	IAPV KBV SBV	Few	Few	3	No
Sanpa and Chantawannakul [13]	Thailand : Northern provinces	No	Yes	DWV-A	ABPV KBV SBV	BQCV CBPV	Most	Some	4	Yes
Ai et al. [14]	China	No	Yes	DWV-A	ABPV IAPV BQCV SBV CBPV	KBV	Most	Most	4	Yes
Kojima et al. [15]	Japan	No	Yes	DWV-A	BQCV SBV IAPV	CBPV KBV ABPV	Most	Most	4	Yes
Strauss et al. [16]	South Africa	No	Yes	DWV-B	BQCV IAPV	DWV-A SBV CBPV KBV VdMLV	Most	Some	4	Yes
Muli et al. [17]	Kenya	No	Yes	DWV-A	BQCV ABPV	SBV KBV IAPV	Some	Some	4	No

						CBPV				
Yang et al. [18]	China	No	Yes	DWV-A	BQCV IAPV SBV CBPV	ABPV KBV	Most	Most	4	Yes
Mondet et al. [19]	New Zealand	No	Yes	DWV-A	BQCV KBV SBV CBPV		Some	Most	4	Yes
Adjlane et al. [20]	Algeria	No	Yes	DWV-A	BQCV SBV ABPV IAPV CBPV	DWV-B	Most	Some	4	No
Tsevegmid et al. [21]	Mongolia: northern regions	No	Yes	DWV-A	SBV BQCV CBPV	ABPV KBV IAPV LSV2	Some	Some	4	No
Giacobino et al. [22]	Argentina	No	Yes	DWV-A	BQCV ABPV CBPV	KBV IAPV	Most	Some	4	Yes
Anido et al. [23]	Uruguay	No	Yes	DWV-A	BQCV SBV ABPV	IAPV KBV	Most	Some	4	Yes
Teixeira et al. [24]	Brazil: southeast	No	Yes	DWV-A	BQCV ABPV	KBV CBPV	Most	Some	4	Yes

	region					SBV				
Soroker et al. [25]	Israel	No	Yes	DWV-A DWV-B	BQCV SBV ABPV IAPV	KBV CBPV	Most	Most	4	Yes
Rodriguez et al. [26]	Chile: Biobio region	No	Yes	DWV-A	BQCV SBV ABPV		Most	Some	4	Yes
Desai et al. [27]	Canada	Yes	Yes	DWV-A	BQCV SBV ABPV KBV IAPV CBPV		Most	Most	5	Yes
Forgach et al. [28]	Hungary	Yes	Yes	DWV-A	BQCV SBV ABPV	KBV CBPV	Most	Most	5	Yes
Tentcheva et al. [8]	France	Yes	Yes	DWV-A	BQCV SBV ABPV KBV CBPV		Most	Most	5	Yes
Berenyi et al. [29]	Austria	Yes	Yes	DWV-A	BQCV SBV ABPV CBPV	KBV	Most	Most	5	Yes

Genersch et al. [30]	Germany	Yes	Yes	DWV-A	SBV ABPV KBV	IAPV	Most	Most	5	Yes
Berthoud et al. [31]	Switzerland	Yes	Yes	DWV-A	ABPV	KBV CBPV	Most	Most	5	Yes
Nguyen et al. [32]	Belgium	Yes	Yes	DWV-A	BQCV SBV ABPV CBPV		Most	Most	5	Yes
Baker and Schroeder [33]	England: Devon	Yes	Yes	DWV-A	SBV BQCV ABPV	KBV CBPV	Most	Most	5	Yes
Antunez et al. [34]	Spain	Yes	Yes	DWV-A	BQCV SBV IAPV KBV		Most	Most	5	Yes
Cornman et al. [35]	USA	Yes	Yes	DWV-A	BQCV SBV LSV1 LSV2 ABPV IAPV KBV		Most	Most	5	Yes
Cox-Foster et al. [36]	USA	Yes	Yes	DWV-A	CBPV SBV BQCV ABPV		Most	Most	5	Yes

					KBV IAPV					
Dainat et al. [37]	Switzerland	Yes	Yes	DWV-A	BQCV SBV SBPV ABPV	IAPV KBV CBPV	Most	Most	5	Yes
Budge et al. [38]	England and Wales	Yes	Yes	DWV-A	BQCV CBPV SBV ABPV KBV		Most	Most	5	Yes
Welch et al. [39]	USA: Massachusetts	Yes	Yes	DWV-A	BQCV SBV	ABPV KBV IAPV CBPV	Most	Most	5	Yes
Bacandritsos et al. [40]	Greece	Yes	Yes	DWV-A	CBPV BQCV SBV ABPV		Most	Most	5	Yes
Runckel et al. [4]	USA	Yes	Yes	DWV-A	BQCV CBPV SBV ABPV KBV IAPV		Most	Most	5	Yes
Traynor et al. [41]	USA	Yes	Yes	DWV-A	BQCV SBV CBPV	SBPV	Most	Most	5	Yes

					LSV2 ABPV IAPV KBV					
Tozkar et al. [42]	Turkey	Yes	Yes	DWV-A DWV-B	BQCV LSV CBPV ABPV KBV IAPV	SBV SBPV	Most	Some	5	Yes
Ravoet et al. [43]	Belgium	Yes	Yes	DWV-A	BQCV SBV CBPV LSV ABPV VdMLV ALPV	IAPV SBPV	Most	Most	5	Yes
Amiri et al. [44]	Denmark	Yes	Yes	DWV-A	SBV BQCV CBPV ABPV KBV		Most	Most	5	Yes
Gajger et al. [45]	Croatia	Yes	Yes	DWV-A	BQCV SBV ABPV CBPV	KBV IAPV	Most	Most	5	Yes
Granberg et al. [46]	Spain: Navarre	Yes	Yes	No	IAPV ALPV	BQCV SBV ABPV	Most	Most	5	Yes

					LSV	KBV DWV				
Nielsen et al. [47]	Denmark	Yes	Yes	DWV-A	BQCV CBPV SBV ABPV KBV		Most	Most	5	Yes

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