Disentangling host-parasite-pathogen interactions in a varroa-resistant honeybee population reveals virus tolerance as an independent, naturally adapted survival mechanism

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SUPPLEMENTARY FILES

Table S1: Effect of co-infection on DWV and ABPV titres. Analysis-of-deviance tables (Type III test) from linear mixed models investigating the effect of the natural background infection level of the other virus on the titres of the infected virus, respectively. Replicate nested within colony ID was included in all models as random effect.

Life stage	Treatment	Response Variable	Explanatory Variables	X^2	Df	AICc	con.R^2	p-value
Adult	DWV (MS+MR)	$DWV^{0.425}$	ABPV	0.85	1	1031	0.40	0.35
	ABPV (MS+MR)	Log(ABPV+1)	DWV	0.33	1	224	0.78	0.56
	ABPV (MS+MR)	$DWV^{0.1}$	ABPV	5.03	1	316	0.74	**0.02**
	DWV (MS+MR)	$ABPV^{0.125}$	DWV	0.004	1	285	0.25	0.94
Larvae	DWV (MS+MR)	$DWV^{-0.225}$	ABPV	1.22	1	-235	0.49	0.26
	ABPV (MS+MR)	$ABPV^{-0.825}$	DWV	0.01	1	-1178	0.00	0.90
	ABPV (MS+MR)	$DWV^{0.15}$	ABPV	2.07	1	241	0.25	0.14
	DWV (MS+MR)	$ABPV^{0.375}$	DWV	0.02	1	395	0.31	0.86

Table S2: Absolute numbers of survival in the adult bee experiments.

Colony history	HPI	Treatment	Dead	Alive
MR	48h	ABPV	4	196
MR	48h	DWV	5	195
MR	48h	NoVirus	0	200
MR	72h	ABPV	14	176
MR	72h	DWV	12	175
MR	72h	NoVirus	0	180
MS	48h	ABPV	22	178
MS	48h	DWV	17	183
MS	48h	NoVirus	2	198
MS	72h	ABPV	25	158
MS	72h	DWV	27	163
MS	72h	NoVirus	1	178

Table S3: Experimental design. Visual representation of the design of the virus infection experiments in larvae and adults. Shown are the population (POP) of origin (MR or MS); the name of the colony (COL), the experimental replicate (REP) for each colony and the samples collected at the different time points for uninoculated, DWV-inoculated and ABPV-inoculated bees (shaded fields), with the live samples indicated by a 'Θ' above the diagonal and the dead samples indicated by a '†' below the diagonal.

LARVAE

POP	COL	REP	NONE						DWV					ABPV				
			0 hr	12 hr	24 hr	48 hr	72 hr	120 hr	12 hr	24 hr	48 hr	72 hr	120 hr	12 hr	24 hr	48 hr	72 hr	120 hr
MR	K4	1	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
	K5	1	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
		2	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
	K8	1				Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
		2	Θ			Θ	Θ				Θ	Θ				Θ	Θ	
	K9	1				Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
		2	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
MS	K1	1	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
		2	Θ			Θ	Θ				Θ	Θ				Θ	Θ	
	K2	1	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
		2	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
	K6	1	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
		2	Θ			Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ
	K7	1				Θ	Θ	Θ			Θ	Θ	Θ			Θ	Θ	Θ

ADULTS

POP	COL	REP	NONE						DWV					ABPV				
			0 hr	12 hr	24 hr	48 hr	72 hr	120 hr	12 hr	24 hr	48 hr	72 hr	120 hr	12 hr	24 hr	48 hr	72 hr	120 hr
MR	B_nuc	1	Θ			Θ	Θ	Θ			Θ	Θ/†	Θ/†			Θ	Θ	Θ
		2	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ/†	Θ	Θ	Θ/†	Θ	Θ
	K5	1	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ/†	Θ	Θ	Θ	Θ	Θ/†
	K8	1	Θ			Θ	Θ	Θ			Θ	Θ/†	Θ			Θ	Θ	Θ
	K9	1	Θ			Θ	Θ	Θ			Θ	Θ/†	Θ/†			Θ	Θ/†	Θ/†
	K5_mix	1	Θ	Θ	Θ	Θ/†	Θ	Θ	Θ	Θ	Θ/†	Θ	Θ/†	Θ	Θ	Θ/†	Θ	Θ/†
MS	C_nuc	1	Θ	Θ	Θ	Θ/†	Θ	Θ	Θ	Θ	Θ/†	Θ	Θ/†	Θ	Θ	Θ/†	Θ	Θ/†
	K2	1	Θ			Θ	Θ	Θ			Θ	Θ/†	Θ/†			Θ/†	Θ/†	Θ/†
		2	Θ			Θ		Θ			Θ/†		Θ/†			Θ/†		Θ/†
		3	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ/†	Θ		Θ	Θ	Θ/†	Θ	Θ/†
		4	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ	Θ/†	Θ	Θ/†	Θ	Θ	Θ/†	Θ	Θ/†
	K6	1	Θ			Θ	Θ	Θ			Θ/†	Θ/†	Θ			Θ/†	Θ/†	Θ

Table S4. RT-qPCR assays. Details of the RT-qPCR assays used in the experiments, including the target name, the sequences of the forward and reverse primers, the product size, the reaction efficiency (*E*), linearity of the dilution standards (r^2) and the melting temperature of the reaction products (T_m).

Assay	Primers	Sequence (5' - 3')	Size (nt)	*E*	r^2	T_m
DWV-A/B	DWV-F1425	CGTCGGCCTATCAAAG	417	1.681	0.997	79.5 ºC
	DWV-B1806	CTTTTCTAATTCAACTTCACC				
DWV-B	VaDV-F1409	GCCCTGTTCAAGAACATG	413	1.723	0.997	80.5 °C
	DWV-B1806	CTTTTCTAATTCAACTTCACC				
ABPV	ABPV-F6548	TCATACCTGCCGATCAAG	197	2.043	0.987	81.0 ºC
	KIABPV-B6707	CTGAATAATACTGTGCGTATC				
IAPV	IAPV-F6627	CCATGCCTGGCGATTCAC	203	1.938	0.998	81.0 °C
	KIABPV-B6707	CTGAATAATACTGTGCGTATC				
KBV	KBV-F6639	CCATACCTGCTGATAACC	200	1.905	0.995	81.5 °C
	KIABPV-B6707	CTGAATAATACTGTGCGTATC				
SBV	SBV-qF3164	TTGGAACTACGCATTCTCTG	335	2.046	0.975	80.3 °C
	SBV-qB3461	GCTCTAACCTCGCATCAAC				
BQCV	BQCV-qF7893	AGTGGCGGAGATGTATGC	294	2.194	0.983	80.5 °C
	BQCV-qB8150	GGAGGTGAAGTGGCTATATC				
RNA250	RNA250-F	TGGTGCCTGGGCGGTAAAG	227	1.962	0.999	84.0 °C
	RNA250-R	TGCGGGGACTCACTGGCTG				
RP49 mRNA	RP49-qF	AAGTTCATTCGTCACCAGAG	205	1.902	0.999	77.6 ºC
	RP49-qB	CTTCCAGTTCCTTGACATTATG				

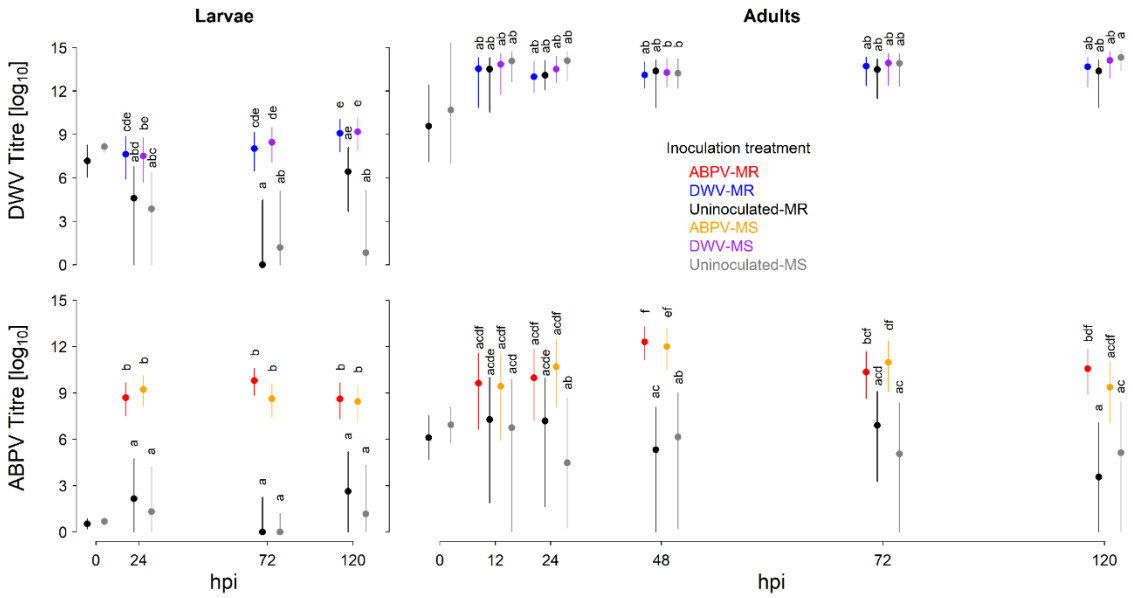

Figure S1: Model predictions of titres of deformed wing virus (DWV) and acute bee paralysis virus (ABPV) found in honeybee larvae (left panel) and adult workers (right panel). Mean values are shown with respect to inoculation treatments (DWV, ABPV, uninoculated) and colony history (MR = mite-resistant, MS = mite-susceptible) over the course of the experiment (HPI = hours post inoculation). Different letters indicate significant differences between predicted marginal means with confidence limits from the respective models that still included all explanatory variables and their interactions.

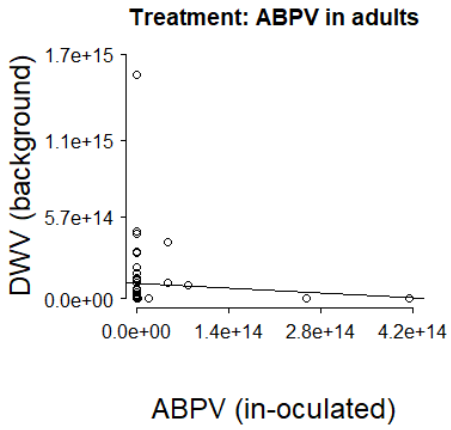

Figure S2: DWV titres depending on APBV titres. Line shows linear prediction.

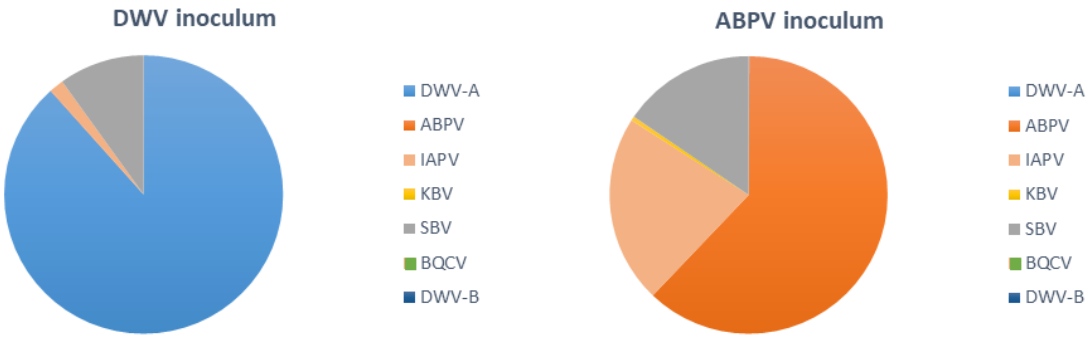

Figure S3. The virological composition of the DWV-A and ABPV inocula, based on the most common viruses that can be propagated by injection.

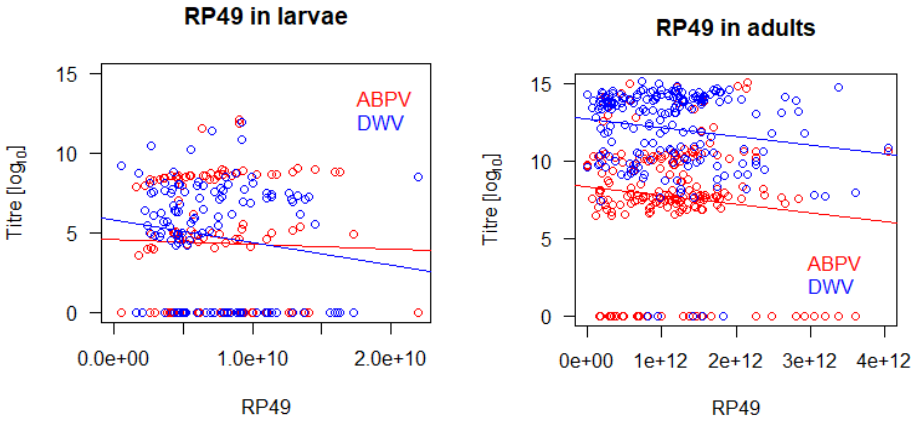

Figure S4: DWV and ABPV titres depending on RP49 titres. Lines shows linear prediction from linear regression models of all data for larvae ($DWV^{0.075}$: F=1.94, p=0.16, R^2 = 0.01; $ABPV^{0.075}$: F=0.02, p=0.87, R^2 = 0.0002) and alive/dead adults ($DWV^{0.125}$: F=3.41, p=0.07, R^2 = 0.016; $ABPV^{0.075}$: F=1.36, p=0.24, R^2 = 0.006).