A Saliva Protein of *Varroa* Mites Contributes to the Toxicity toward *Apis cerana*

and the DWV Elevation in *A. mellifera*

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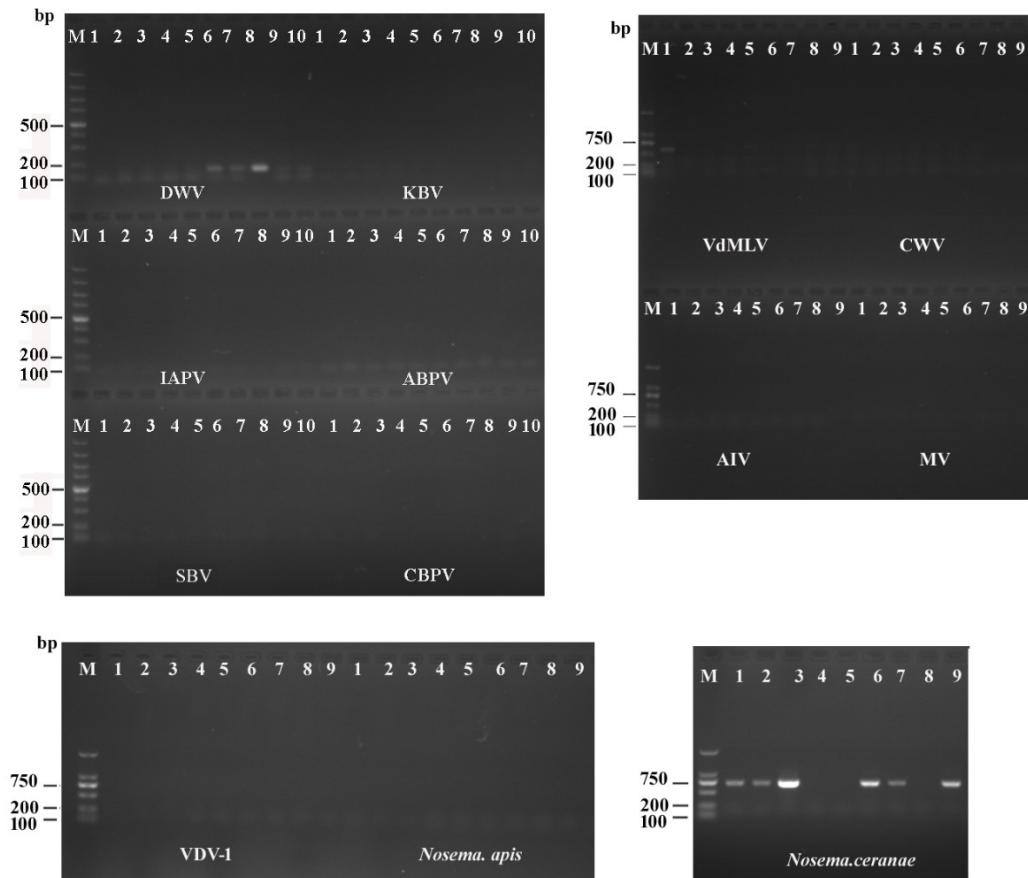
Supplementary Figure S1. Detection of viruses and *Nosema* from worker L5 larvae of *A. mellifera* after VTP injection.1, 9: L5 worker larvae without injection; 2: L5 worker larvae injected with PBS (10 mM, pH 7.4); 3, 8: L5 worker larvae injected with tag protein purified from *E. coli* Transetta pET32(a); 4, 5, 6, 7, 8: the adults developed from worker L5 larvae injected with the purified E-VTP at different concentrations (0.01 ng/μL, 0.1 ng/μL, 1.0 ng/μL, 2.0 ng/μL, and 5.0 ng/μL).

predicted signal peptide

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            10         20         30         40         50         60
  1  ATGTTCAAACTTCTCGTTATCGCGACCCTCGTGGCTGTAGCCATCGCTGATGTGCAGCCA   cDNA
  1   M  F  K  L  L  V  I  A  T  V  V  A  V  A  I  A  D  V  Q  P    Aa
            70         80         90        100        110        120
 61  GCCATTGAGGCTTTAAAGACCGCTATCGACAATCTCGATATTCCCGATGAACGCAAGGCT   cDNA
 21   A  I  E  A  L  K  T  A  I  D  N  L  D  I  P  D  E  R  K  A    Aa
           130        140        150        160        170        180
121  CTTTATAAGGCCAGCGCTGACAAATCGAAGGAATGCCTTGAAGGTGTTGCCGCTGATGCC   cDNA
 41   L  Y  K  A  S  A  D  K  S  K  E  C  L  E  G  V  A  A  D  A    Aa
           190        200        210        220        230        240
181  GGACCAGAACGCATTCAAGACTACATCACCAAGCTGAGCCCGCTCGTCGCTGCTTGCAGC   cDNA
 61   G  P  E  R  I  Q  D  Y  I  T  K  L  S  P  L  V  A  A  C  S    Aa
           250        260        270        280        290        300
241  GAAGAAATCAAAGGTATTGCCCATGATCATGTTGAGGAGCGCAAAACAAAGTTCCAGGAA   cDNA
 81   E  E  I  K  G  I  A  H  D  H  V  E  E  R  K  T  K  F  Q  E    Aa
           310        320        330        340        350        360
301  TGCATGAAGGAAAAGGTTCACGGGGAAGAGTCGACCCTCGACGAAAAGCAGAAGGAGAAC   cDNA
101   C  M  K  E  K  V  H  G  E  E  S  T  L  D  E  K  Q  K  E  N    Aa
           370        380        390        400
361  GTTGTCAAAGTCAAGGCTTGCCTCCAGCAGGCGCTCGCCTCCTAA                  cDNA
121   V  V  K  V  K  A  C  L  Q  Q  A  L  A  S  *                   Aa
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Supplementary Figure S2. The cDNA and amino acid sequences of the *Varroa* toxic protein (VTP).
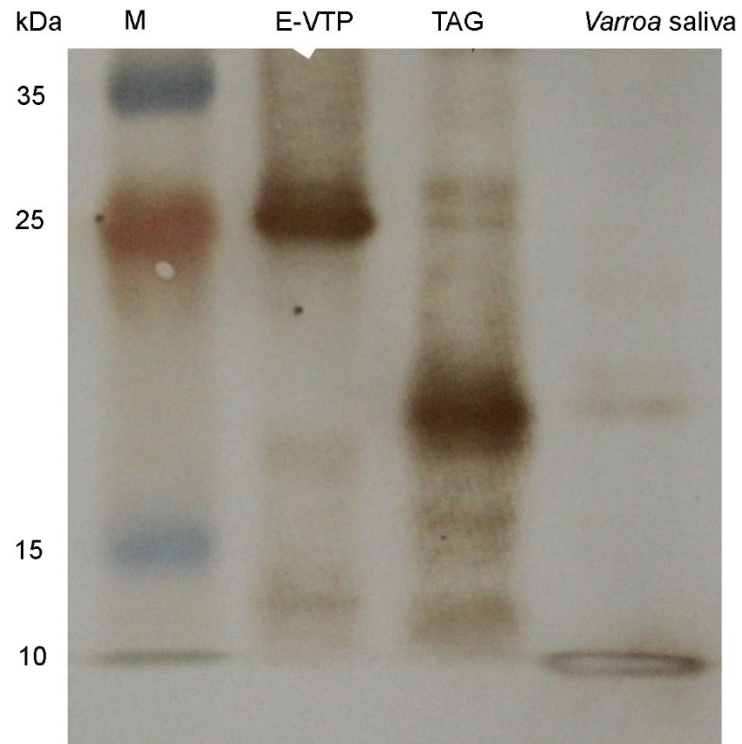
Supplementary Figure S3. Western blot analysis of VTP in crude *Varroa* saliva. M,

Pre-stained protein marker; *Varroa* saliva: crude saliva of *V. destructor*; E-VTP, purified

recombinant fusion VTP; TAG, purified tag protein.
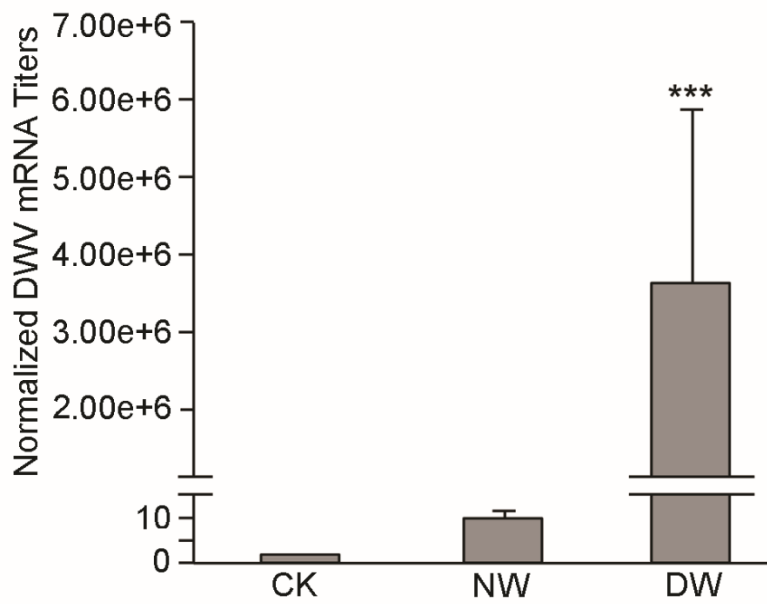
Supplementary Figure S4. Quantitative titers of DWV in *A. mellifera* adults. CK:

Adults with normal wings and without injection at L5 larvae; NW: Adults with normal

wings and PBS injection at L5 larvae; DW: Adults with deformed wings after injected

with E-VTP at L5 larvae.
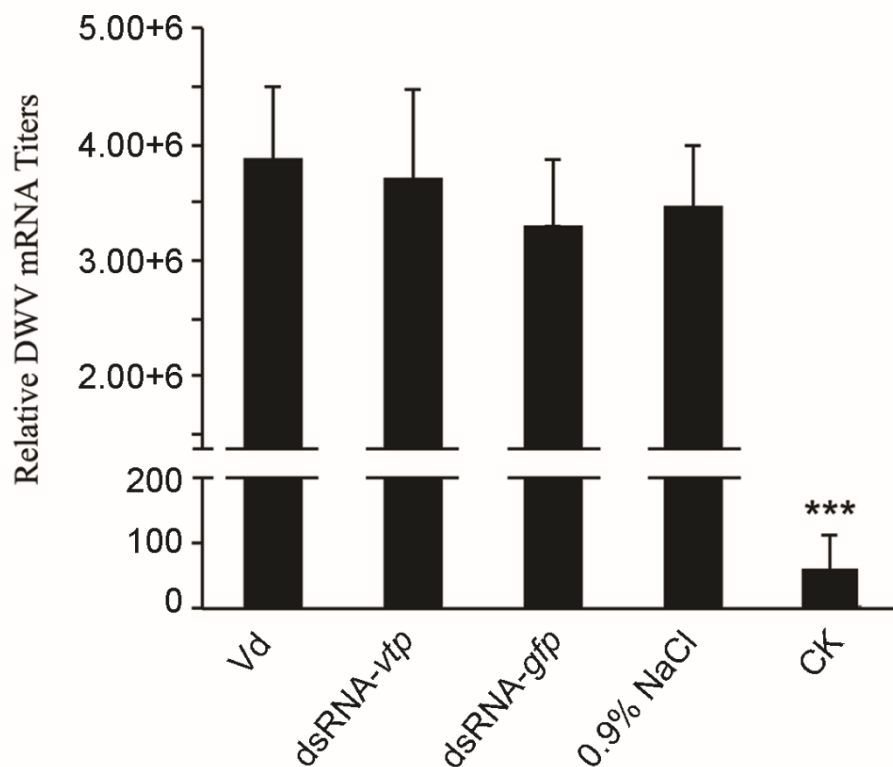
Supplementary Figure S5. Relative DWV mRNA levels in newly emerged worker bees (*A. mellifera*) infected by RNAi-treated *Varroa* mites. *A. mellifera* were infected with mites treated by overnight (approximately 15 hours) immersion in different solutions at 16°C. CK, No mites; Vd, *V. destructor* mites that were not treated; 0.9% NaCl, mites treated with 0.9% NaCl; dsRNA-*gfp*, mites treated with dsRNA-*gfp* (2.5 μg/μL); dsRNA-*vtp*, mites treated with dsRNA-*vtp* (2.5 μg/μL).

Supplementary Table S1. Quantitative titers of DWV in *A. cerana* adults developed from worker L5 larvae.

Treatment	Ct (Average ± SD)	ANOVA (df=8,35; F=0.441; P=0.885)
CK	34.1 ± 0.5	a
PBS	33.3 ± 1.1	a
TAG (2.0 ng/µL)	33.9 ± 0.5	a
E-VTP (0.01 ng/µL)	33.6 ± 0.8	a
E-VTP (0.1 ng/µL)	33.4 ± 1.6	a
E-VTP (1.0 ng/µL)	33.4 ± 1.8	a
E-VTP (2.0 ng/µL)	34.1 ± 0.8	a
E-VTP (5.0 ng/µL)	33.8 ± 0.5	a
NTC	33.2 ± 1.0	a

All the bee samples were collected and the DWV genome equivalents were measured by using qRT-PCR [34, 35]. CK, the adults developed from worker L5 larvae without any injection; PBS, the adults developed from worker L5 larvae injected with PBS (10 mM, pH 7.4); TAG, the adults developed from worker L5 larvae injected with purified tag protein from *E. coli* Transetta pET32(a). E-VTP (0.01 ng/µL, 0.1 ng/µL, 1.0 ng/µL, 2.0 ng/µL, and 5.0 ng/µL), the adults developed from worker L5 larvae injected with the purified E-VTP at different concentrations (0.01 ng/µL, 0.1 ng/µL, 1.0 ng/µL, 2.0 ng/µL, and 5.0 ng/µL). NTC: no template control during the qRT-PCR. Three replicates were established for each treatment. The data were analyzed using a normal one-way analysis of variance (ANOVA) using SPSS statistical software (16.0), and the significance of the between treatment differences was evaluated using Duncan's multiple range test, with significance indicated by a *P* value of < 0.05. The data presented are the mean values ± S.D. The same letters indicated no significant difference.

Supplementary Table S2. Primers used for the detection of viruses and microsporidia parasites.

Organism	Primer sequences	References
Deformed Wing Virus (DWV)	DWV-F: GACAAAATGACGAGGAGATTGTT DWV-R: CAACTACCTGTAATGTCGTCGTGTT	5
Acute Paralysis Bee Virus (APBV)	ABPV-F: TTATGTGTCCAGAGACTGTATCCA ABPV-R: GCTCCTATTGCTCGGTTTTTCGGT	31
Chronic Bee Paralysis Virus (CBPV)	CBPV-F: AGTTGTCATGGTTAACAGGATACGAG CBPV-R: TCTAATCTTAGCACGAAAGCCGAG	31
Black Queen Cell Virus (BQCV)	BQCV-F: TGGTCAGCTCCCACTACCTTAAAC BQCV-R: GCAACAAGAAGAAACGTAAACCAC	31
Kashmir Bee Virus (KBV)	KBV-F: GATGAACGTCGACCTATTGA KBV-R: TGTGGGTTGGCTATGAGTCA	31
Sacbrood Virus (SBV)	SBV-F: GCTGAGGTAGGATCTTTGCGT SBV-R: TCATCATCTTCACCATCCGA	31
Apis mellifera Filamentous Virus (AmFV)	AmFV -F: CAGAGAATTCGGTTTTTGTGAGTG AmFV -R: CATGGTGGCCAAGTCTTGCT	40
Big Sioux River Virus (BSRV)	BSRV-F: RGTGCAGCTTTATGCGTTGCC BSRV-R: CCGCTGTTGAGAATAAGGATATCCAGG	41
Lake Sinai Virus complex (LSV)	LSVdeg-F: GCCWCGRYTGTTGGTYCCCC LSVdeg-R: GAGGTGGCGGCGCSAGATAAAGT	42
Slow Bee Paralysis Virus (SBPV)	SBPV-F: GATTTGCGGAATCGTAATATTGTTTG SBPV-R: ACCAGTTAGTACACTCCTGGTAACTTCG	43
Varroa destructor Macula-like virus (VdMLV)	VdMLV-F：ATCCCTTTTCAGTTCGCT VdMLV-R：AGAAGAGACTTCAAGGAC	44
Israeli Acute Paralysis Virus (IAPV)	IAPV-F: AGACACCAATCACGGACCTCAC IAPV-R: AGATTTGTCTGTCTCCCAGTGCACAT	45
Cloudy Wing Virus （CWV）	CWV-F: ATCAGCGCTTAGTGGAGGAA CWV-R: TCGACAATTTTCGGACATCA	46
Apis Iridescent Virus （AIV）	AIV-F: GGCTAGTAAACGTAGTGGATATGACAAT AIV-R: CACCTGGTGGTCCAAGAGAAG	47
Varroa destructor Virus 1 (VDV-1)	VDV1-R: CTTCCAAGGGCTCATCCATA VDV1-F: CATGGAAATGGGATCAAACC	48
Moku Virus (MV)	MV-F: GTGCGATAGCTAAGCCTGAGATGG MV-R: CAGTGCCCCCTATAGGTGTTGTT	49
Nosema.apis	*N.apis*-F: CCATTGCCGGATAAGAGAGT *N.apis*-R: CCACCAAAAACTCCCAAGAG	32

| *Nosema.ceranae* | *N.ceranae*-F: GACAACAAGGAAGACCTGGAAGTG | 32 |
| | *N.ceranae*-R: TGTGAATAAGAGGGTGATCCTGTTGAG | |