SUPPLEMENTAL METHODS

Controlled infection

In order to obtain two groups of adult age-matched bees that were or were not infected with DWV, we first prepared two treatment solutions, which upon injection were designed to increase or keep down DWV virus titers. This required preparing lysates of honeybees that were infected with DWV but none of the other common honeybee viruses, as well as of honeybees that were confirmed to be virus-free. To this end, we produced a lysate of a pool of 10 bees, which based on multiplex ligation-dependent probe amplification (MLPA) analysis and the use of specific PCR primers were confirmed to be entirely virus and pathogen-free or infected only with DWV (see below) [1]. These pools of bees were obtained following an initial screening of 10 individuals each from a set of 10 *A. mellifera carnica* colonies available in our apiary in Leuven, as well as based on the screening of individuals with overt symptoms of DWV-infection (i.e. displaying crippled wings). This screening also allowed the selection of the previously mentioned virus and pathogen-free donor colony. DWV lysate and virus-free control lysate were extracted from the selected set of DWV-infected and virus-free bees by homogenizing the mass equivalent of five bees (around 500 mg) of the selected samples (stored at -80°C) after immersion in liquid nitrogen, and mixing the homogenate with 5 ml phosphate-buffered saline (PBS, pH 7.4). Subsequently, the samples were centrifuged at 3000 r.p.m. for 30 min at 4°C and the supernatant was stored in aliquots at -80°C for future use [2].

The artificial inoculation with a DWV lysate that was used in this study has previously been shown to be effective in short-term, individual-based setups [2]. In our present study, however, the bees stayed in close contact with each other in the observation hive, throughout their adult lives, so that the virus could still be transmitted horizontally during the experiment. To try to mitigate the effects of horizontal transmission from the DWV infected to the group of uninfected bees [3] we therefore opted to also add a double-stranded RNA treatment to our DWV-negative lysate [4, 5]. In particular, DWV-specific dsRNA was added to the control lysate to try to keep these bees DWV-free and prevent DWV cross-infection inside the host colony, whereas control GFP-dsRNA was added to the DWV lysate to control for the possible effects of foreign dsRNA injection on honeybee physiology. DWV and GFP-dsRNA were provided by Beeologics (Israel) and were dissolved in nuclease free water at 3 mg/ml. Prior to injection, both DWV and control lysates were diluted 1:1000 (cf. Iqbal and Mueller [2] in insect saline buffer (ISB, containing 150 mM NaCl, 10 mM KCl, 4 mM CaCl2, 2 mM MgCl2, and 10 mM HEPES; pH 7.0), after which dsRNA was added at a dosage of 5 µg per bee to obtain the final treatment solutions. Given that bees with overt symptoms have been reported to contain between 1.8×10^{10} and 6.9×10^{11} DWV genome equivalents per bee [6], we estimate that our protocol resulted in the injection of between 1.2×10^{4} and 4.6×10^{5} DWV copies per bee, which is well within the infection loads reported for bees with covert infections (1.4×10^{3} – 2.4×10^{9} copies per bee, [6]). Hence, our DWV inoculation treatment adequately mimicked covert DWV infection levels.

RFID data analysis

Raw RFID tracking data were analyzed with the Track-A-Forager Java application, which filters out rapid-succession scans of the same scanner, labels ingoing and outgoing flights by tagged workers, and corrects occasional errors in the data, including the possible occurrence of missed scans [7]. The

setup options were adjusted based on the foraging type and entrance/exit system that was used in the experimental setup, namely 'natural foraging' and 'joined two scanners'. In the data filtering phase, the default time constraint settings were applied to filter out rapid-succession scans of the same scanner, determine 'IN' and 'OUT' events of tagged foragers and reconstruct forager trips. The output of the application generated the complete list of all the scans of each RFID tag at each scanner with their corresponding time stamp, the reconstructed trips per tag with their corresponding time stamp and the durations of reconstructed trips with the age of each individual at the time of the trip. The resulting data was then joined with the transponder information database consisting of the unique RFID tag code, the treatment, colony and date of introduction of each individual.

To test for differences in the proportion of workers of each treatment group that survived to foraging age, we used a binomial GLM in which 'colony', 'treatment' and their interaction effect were included as fixed factors. ANOVAs with 'colony', 'treatment' and their interaction effect included as fixed factors were used to assay the difference in the age at onset of foraging, life expectancy and activity span. To assess the number of reconstructed trips made by individuals of each group we used a quasipoisson generalized linear model (GLM) with 'colony', 'treatment' and their interaction effects included as fixed factors and 'activity span' included as a covariate. Finally, the duration of the reconstructed trips was analysed with a gamma distributed generalized linear mixed model (GLMM) with the unique RFID tag specified as a random factor and 'colony' and 'treatment' plus their interaction effect specified as fixed factors. All statistical analyses were carried out using *R* v. 3.2.2 and the *lme4* 1.1-9 (for generalized mixed models) and *effects* 2.3-0 packages (to produce effect plots).

MLPA-based screening for viral infections and treatment validation

Multiplex ligation-dependent probe amplification (MLPA) was used to identify virus-free donor colony candidates, select honeybee samples that were best suited for preparing the lysates for the two treatments and to validate the effects of these treatments. MLPA was performed as described in De Smet *et al.* [1], using MLPA probes and RT-primers designed for six virus targets, covering the ten most common honeybee viruses: chronic bee paralysis virus (CBPV), deformed wing virus (DWV, including A, B, i.e. VDV-1, and C type virus, [8]), acute bee paralysis virus (ABPV)/Kashmir bee virus (KBV)/Israeli acute paralysis virus (IAPV), black queen cell virus (BQCV), slow bee paralysis virus (SBPV) and sacbrood virus (SBV) (Table S1). The amplified MLPA products were analyzed by electrophoresis on a 4% high resolution agarose gel with a specific MLPA ladder.

Table S1. Primers and half-probes used for detecting the positive strand of different honeybee viruses and virus species complexes through RT-MLPA. Adapted from De Smet *et al. [1]*.

VIRUS	FUNCTION	SEQUENCE (5'-3')	SIZE (bp)
CBPV	(-)cDNA	GCCCCGATCATATAAGCAAA	88
	(+)MLPA-LPO	gggttccctaagggttggaCCGTAGCTGTTTCTGCTGCGGT	
	(+)MLPA-RPO	P-ACTCAGCTCAGCTCGACGCTAGAtctagattggatcttgctggcac	
DWV	(-)cDNA	TCACATTGATCCCAATAATCAGA	95
(A+B+C type)	(+)MLPA-LPO	gggttccctaagggttggaTGACCGATTCTTTATGAGCGAGCTCT	
	(+)MLPA-RPO	P-TACGTGCGAGTCGTACTCCTGTGACAtctagattggatcttgctggcac	
ABPV	(-)cDNA (ABPV)	CAATGTGGTCAATGAGTACGG	104
KBV	(-)cDNA (KBV&IAPV)	TCAATGTTGTCAATGAGAACGG	
IAPV	(+)MLPA-LPO	gggttccctaagggttggaCTCACTTCATCGGCTCGGAGCATGGATGAT	
	(+)MLPA-RPO	P-ACGCACAGTATTATTCAGTTTTTACAACGCCCtctagattggatcttgctggcac	
BQCV	(-)cDNA	CGGGCCTCGGATAATTAGA	122
	(+)MLPA-LPO	gggttccctaagggttggaCTTCATGTTGGAGACCAGGTTTGTTTGCCGACTTACGGAA	
	(+)MLPA-RPO	P-TGTCGTTAAACTCTAGGCTTTCCGGATGGCTTCTTCATGGtctagattggatcttgctggcac	
SBPV	(-)cDNA	CGCAAACACGACGAATTTTA	131
	(+)MLPA-LPO	gggttccctaagggttggaCGTTCAATGGTCGAGATAGAAGCCACAGTAGAAGTATTACGCGCT	
	(+)MLPA-RPO	P-TCTTGTGTTTTGGCTTATGGGCGTGGGCCTGATCTTCATTCAGCtctagattggatcttgctggcac	
SBV	(-)cDNA	TGGACATTTCGGTGTAGTGG	140
	(+)MLPA-LPO	gggttccctaagggttggaCGTTGATCCAATGGTCAGTGGACTCTTATACCGATTTGTTTAATGGTTGG	
	(+)MLPA-RPO	P-GTTTCTGGTATGTTTGTTGACAAGAACGTCCACCTTCAGCCATTCAGCtctagattggatcttgctggcac	

Samples to derive DWV lysates from were selected for further use if they tested DWV positive but negative for the other tested viruses. To identify suitable honeybee samples, RNA was first extracted from collected bees with and without deformed wings and screened them for pathogens using MLPA. 20 sets of ten adult bees from different colonies without and where possible with overt symptoms of DWV were pooled and whole bodies were homogenized in liquid nitrogen. 80-100 mg of the homogenized tissues were then mixed with 1 ml Qiazol reagent in MagNA Lyser Green Beads sample tubes (Roche, Belgium) for RNA-extraction with the RNA lipid tissue mini kit (Qiagen, Germany). Leftover tissue was kept and stored at -80°C for lysate preparation. Pooled tissues in Qiazol were further disrupted and homogenized on a MagNa Lyser Instrument (Roche, Belgium) for 30 sec at 6500 Hz. The total RNA was isolated according to the kit's instructions and eluted in a final volume of 50 µl. The quality and concentrations of RNA was checked using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Belgium).

To validate the effects of the two treatment solutions, 150 additional eclosed workers were injected with each treatment solution, paint-marked and introduced into a fourth observation hive without the RFID setup. Every four days, 20 individuals of each treatment were sampled from this colony and frozen in liquid nitrogen for storage at -80°C. Subsequently, RNA extraction and MLPA analysis was carried out as described above. We should note that this method produces binary data and scores bees as containing DWV titers that are above or below the MLPA detection threshold. Several quantitative qPCR-based approaches were also tried, but unfortunately failed – likely due to a mismatch in the primer sites.

Confirmation of disease-free status of donor colony using specific PCR primers

To test that our DWV-free donor colony identified before was also free of most other common honeybee pathogens, we collected three sets of ten bees each, which were each homogenised in 5 ml PBS in MagNA Lyser Green Beads sample tubes (Roche, Belgium) on a MagNa Lyser (30 sex at

6500 Hz), extracted their DNA from 120 µl supernatant using the DNeasy Blood & Tissue Kit (Qiagen), and used the specific PCR primer sets given in Ravoet *et al.* [9, 10] to detect the possible presence of for the microsporidian parasites *Nosema apis* and *N. ceranae*, the fungal parasite *Ascosphaera*, the Mollicute pathogen *Spiroplasma* spp. and the Trypanosomatid parasites *Lotmaria passim* and *Crithidia mellificae*. None of the PCR reactions resulted in a PCR product of the expected size, thereby demonstrating that our DWV-free donor colony was not only free of DWV, but that it was also free of most other major known honeybee pathogens (although evidently we cannot exclude their presence at a low rate in a small subset of the workers). We should note that the presence of pathogens in any of the host colonies was not determined, as with *n*=3 host colonies, the statistical power to relate the presence of particular pathogens to variation in the impact of DWV would have been too low, and that such differences would also have been intrinsically confounded with genetic and environmental differences between those colonies.

Characterization of experimental inocula via ultra-deep sequencing

Five bees that were inoculated with DWV lysate, collected 12 days post injection, were subjected to ultra-deep sequencing to determine the DWV strain type the bees were injected with as well as to confirm the MLPA and PCR-based results showing that the bees were free of any other major known honeybee pathogens. For these analyses, total RNA was conducted as described above with an additional on-column DNase digestion with the RNase-free DNase set (Qiagen, Germany) to remove DNA contamination. The quality and concentration of the RNA samples was assessed using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Belgium). Subsequently, the libraries were subjected to 90 bp pair-end RNA sequencing on the Illumina Hiseq2000 platform at BGI, using random hexamer primed cDNA synthesis and a 200 bp short insert library. Adaptors, contamination and low-quality reads were removed by BGI and quality control was performed using FastQC [11]. This produced between between 9 and 10 million filtered, high-quality reads per sample, and a total of 88 936 091 reads from our DWV inoculated bees.

To determine that only a single strain of DWV was present in our inoculated bees, and that no other major pathogens were present, we used Bowtie v. 2.2.6 [12] to align the pooled 88.9 million reads to the reference genome sequences of a set of major honeybee pathogens, which included deformed wing virus types A, B and C (accession nrs. NC_004830.2, AY251269.2 and CEND01000001), acute bee paralysis virus (NC_002548.1), aphid lethal paralysis virus (NC_004365.1), black queen cell virus (NC_003784.1), chronic bee paralysis virus-1 (NC_010711.1), chronic bee paralysis virus-2 (NC_010712.1), Israeli acute paralysis virus (NC_009025.1), Kashmir bee virus (NC_004807.1), sacbrood virus (NC_002066.1), the Trypanosomatid parasite *Lotmaria passim* (GCA_000635995.1), the microsporidian parasites *Nosema apis* (GCA_000447185.1) and *Nosema ceranae* (GCA_000182985.1) and the Mollicute pathogen *Spiroplasma apis* (GCA_000500935.1) and *Spiroplasma melliferum* (GCA_000236085.3) as well as the *Apis mellifera* genome, in order to filter out host RNA (using the latest version 4.5 NCBI assembly). Out of the obtained 88.9 million sequence fragments, 78% (69.2 million) did not map onto the host genome and out of these non-host fragments, 73% (50.7 million) mapped onto one of the included pathogens. Out of all the pathogen-mapped fragments, 99.91% mapped to deformed wing virus type B, whereas the remaining reads (0.09%) mapped onto DWV type A. Nevertheless, the latter had such low counts that they were likely caused by either sequence mismatches between our inoculate and the reference DWV type B strain,

sequencing error, or mapping mistakes. In addition, no reads mapped onto any of the other pathogens, thereby confirming our MLPA and PCR results that the donor colony was indeed free of any of the major known honeybee pathogens.

The interpretation that only a single strain of DWV was present in the inoculate was confirmed based on a de-novo assembly of all the fragments that mapped onto any of the RNA viruses, obtained using the Vicuna viral assembler version 1.3, which was developed to characterize possibly heterogeneous virus populations [13]. As expected if only a single DWV strain was present, the Vicuna assembly returned only one single 5941 nt long contig that mapped with very high fidelity (99.0% high sequence similarity) to the 5' end of the DWV type B reference strain. As coverage across the complete DWV genome was highly variable, however, the Vicuna pipeline was not able to recover the full DWV genome. To obtain the full genome sequence, and given the very high sequence similarity of the obtained fragment with the type B reference strain, we therefore used Bowtie v. 2.2.6 [12] instead to map all virus-mapping reads to the DWV type B reference genome (using option –very-sensitive-local, to allow for a maximum number of sequence mismatches), after which *samtools mpileup* and *bcftools call* was used to obtain the consensus sequence of the position-sorted BAM alignment. The resulting full DWV genome of our inoculated strain (accession number KX783225) was 10112 nts long and contained a 2893 AA long polyprotein, which had sequence similarities of 99.28% at the nucleotide level (73/10112 nts substituted) and 99.76% at the AA level (7/2893 AAs substituted) with the DWV type B reference strain (accession number AY251269.2), as well as an identical length, but greater sequence divergence with reference types A or C (Fig. S1). There was no evidence of our strain being a recombinant between strain types [14]. The DWV type B strain was formerly known as *Varroa destructor virus*-1, but is now classified into the deformed wing virus complex as one of three master variants [8], and has recently been found to be an emergent, more virulent strain of the DWV virus [14], which currently appears to be the most common deformed wing virus strain in Britain. As our inoculate was prepared from bees with overt DWV symptoms from a randomly selected hive, our results suggest that this strain is now also common in Continental Europe. Overall per-nucleotide coverage, calculated using *bedtools coverage*, was 902k on average, with a range of 19 to 15 million. Coverage, together with the tentative positions of polyprotein cleavage sites and their resulting products and other genomic features, annotated following Lanzi *et al.* [15], and the overall structure of the virus, based on what is known from other Picornaviridae, are shown in Fig. S2.

Treatment validation results

MLPA analysis of 4-daily sets of samples of 20 individuals per treatment shows that, as expected, there was a strong main effect of treatment on DWV infection rates (binomial GLM, $p = 0.0006$, $z = 3.4$) (Fig. 2), but that DWV infection rates also increased and that the difference in infection rates between the two treatment groups decreased over the course of the experiment (main effect of log(x+1) transformed duration after introduction into the host colony: $p = 3.4E\text{-}6$, $z = 4.6$; interaction effect of log(time+1) and treatment: $p = 0.003$, $z = -3.0$). Based on the calculated 95% confidence limits, however, the difference in infection rate between the two treatments remained statistically significant until the 12th day of the experiment (Fig. 2).

These treatment results imply that in the beginning of our experiment, infection rates strongly differed between the two treatment groups, but that the control workers also may have horizontally

acquired new DWV infections at the later stages of our experiment or perhaps fed on DWV infected pollen or nectar leading to a sizeable virus load in the gut. It is important to note, however, that MLPA is a qualitative technique that only signals the presence or absence of virus particles in the analyzed samples but does not yield any information on the virus titers. Hence, it is possible, and indeed likely, that the DWV titers in control bees that became infected over the later stages of our experiment were still significantly lower than those in the group which were experimentally infected with DWV, even if we did not succeed in formally demonstrating this using a qPCR-based approach. Nevertheless, the fact that we find statistically significant behavioural effects between our two treatment groups up until late into our experiment, when the proportion of DWV positive bees in the control group approached that in the DWV inoculated group, only strengthens our conclusion that DWV has strongly deleterious effects on honeybee foraging behaviour. That is, the total effect could in fact have been even greater if the control group had remained completely uninfected for the full duration of our experiment, and our estimates of the effects on foraging behaviour and mortality should therefore be interpreted as minimum estimates.

That the dsRNA RNAi treatment [4] in the control bees was not fully effective at keeping bees virus-free may have several causes. First and foremost, our setup combined a mix of uninfected and artificially inoculated workers, and this led to an unusually high potential for horizontal transmission inside the colony. Second, in our experiment we only treated the bees one single time, using a single dsRNA injection, to ensure a standardized approach with equal amounts of dsRNA provided to each bee. In real-life applications, by contrast, dsRNA could be administered orally over extended periods in the food [4, 5, 16], and colonies are treated pro-actively, so that colonies may be able to fight off the virus at a much earlier stage than in our experiments. Hence, the potential of dsRNA treatments to combat DWV infections and help to mitigate its associated costs still deserves further study.

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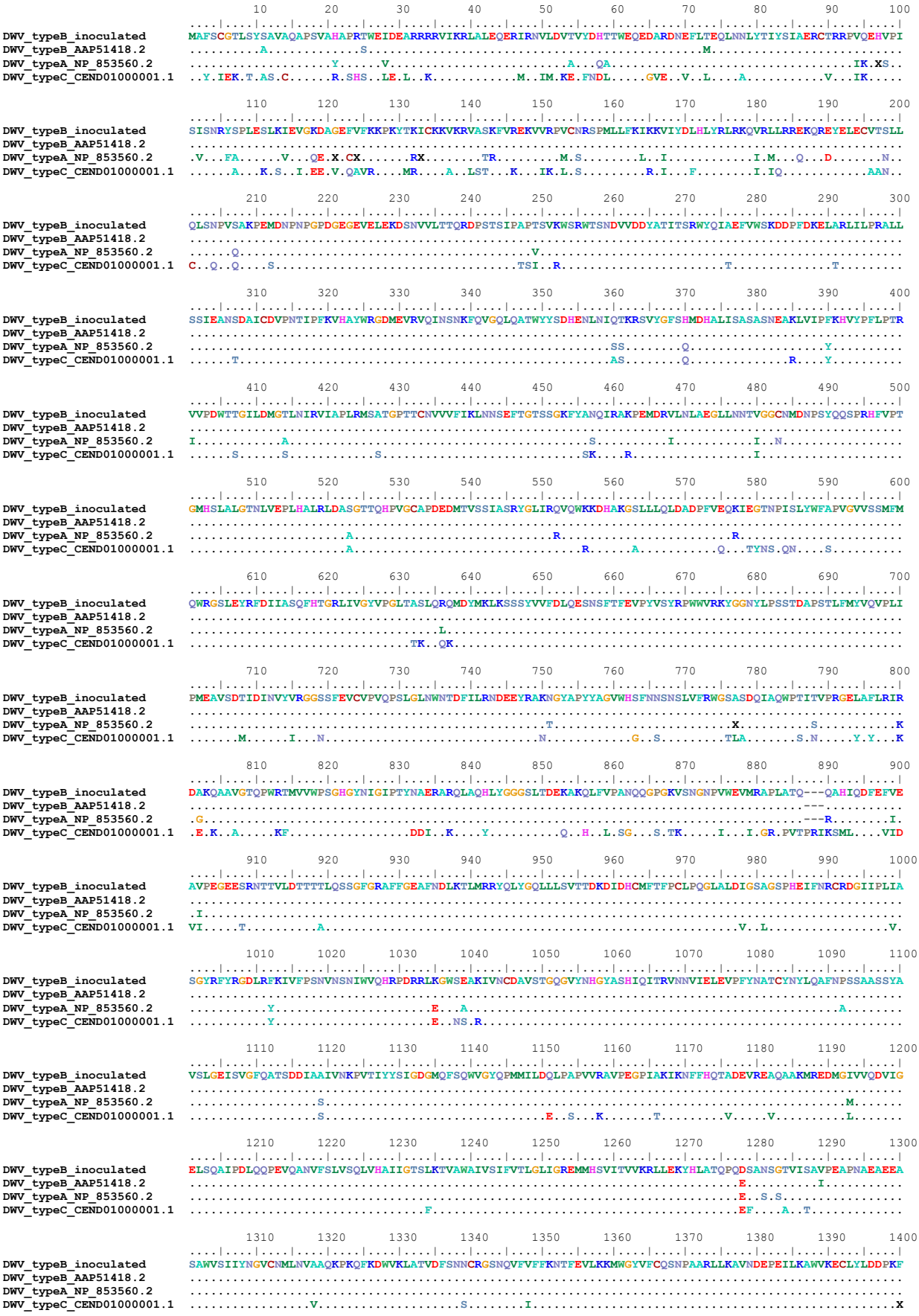
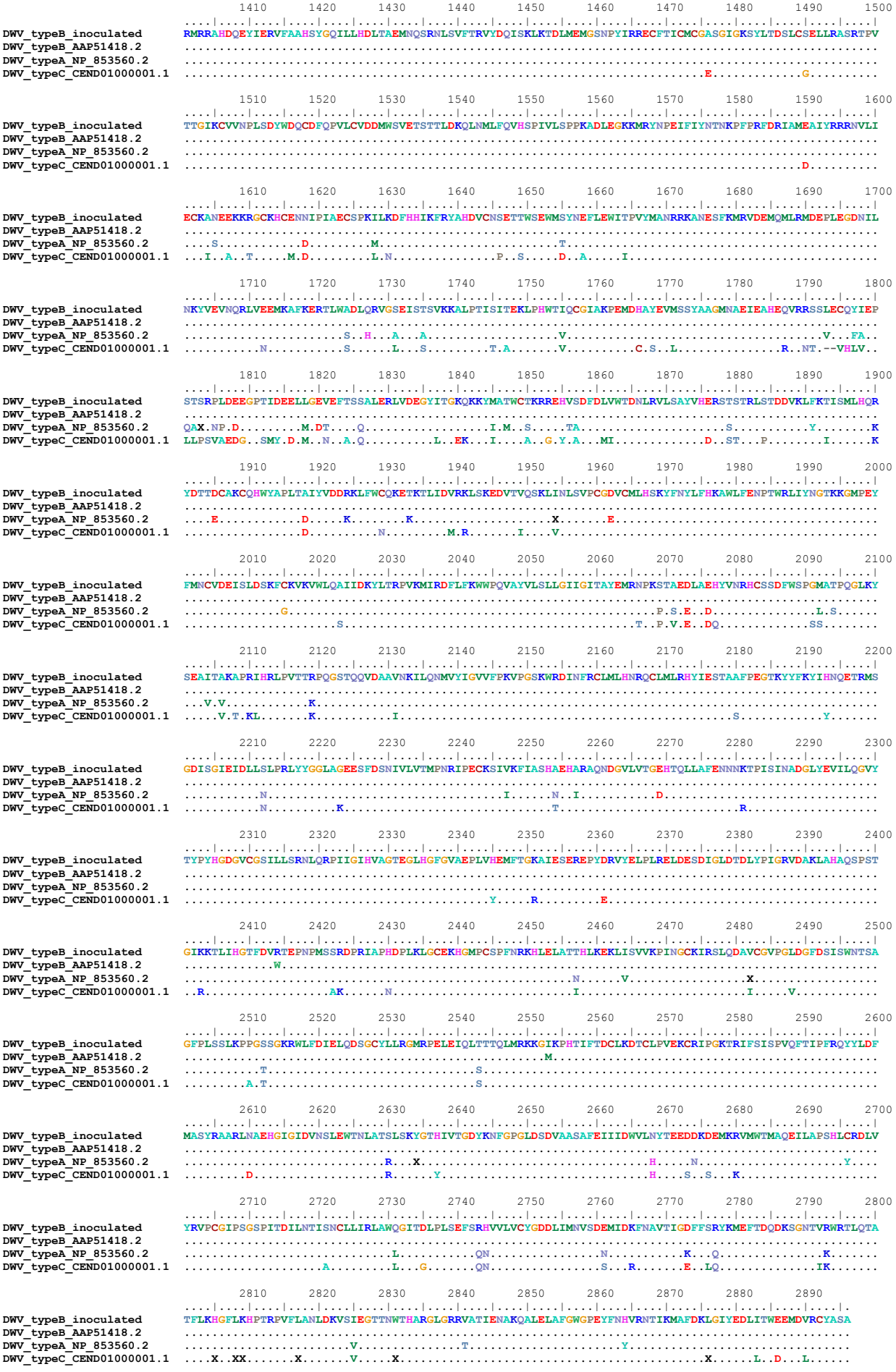


Fig. S1. Amino-acid sequence of the polyprotein encoded by the DWV type B strain that we used to inoculate our bees and comparison to the reference DWV master variants of type B, A and C.



(Fig. S1 continued)

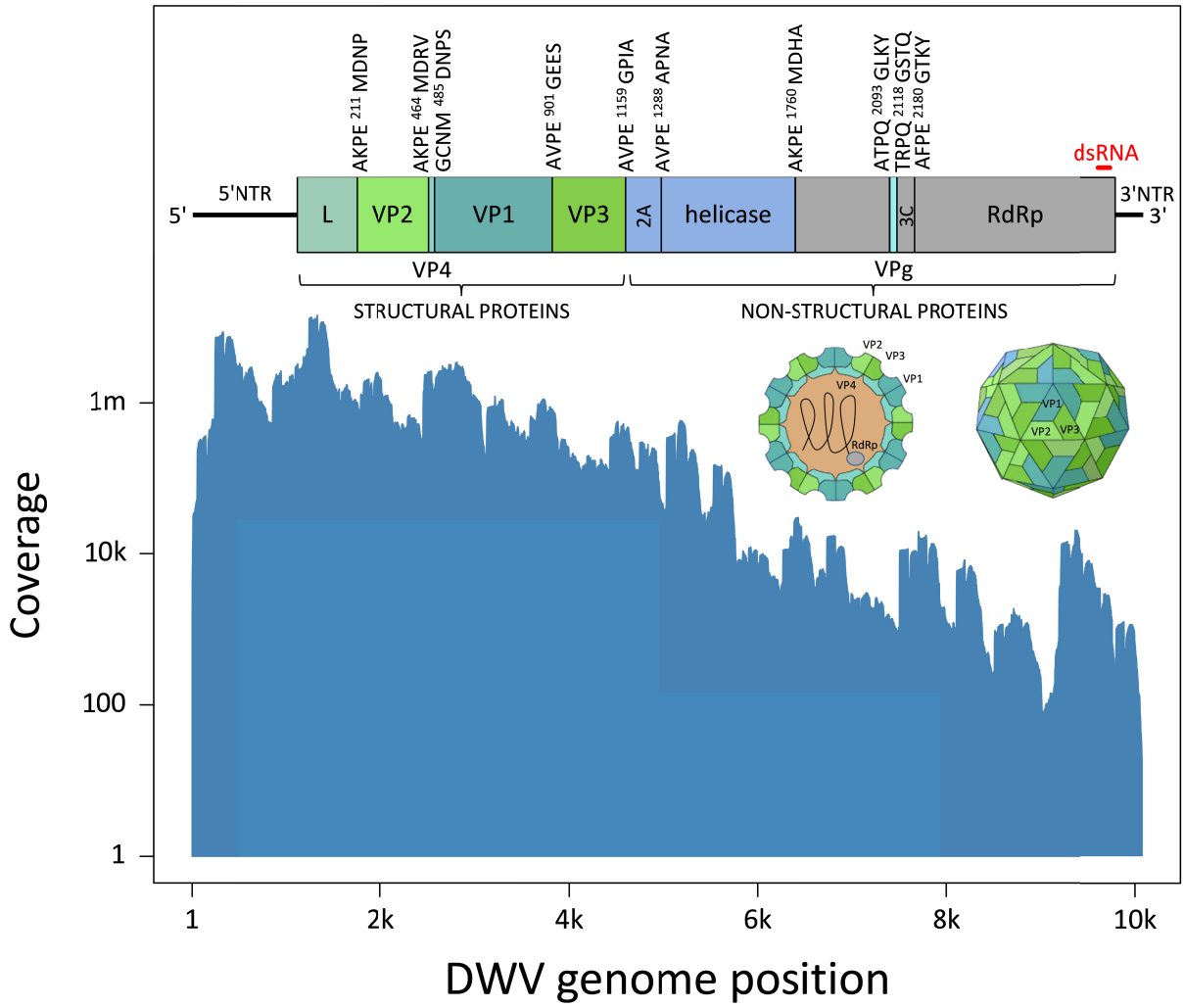


Fig. S2. Fragment coverage over the DWV genome reached in our Bowtie mapping shown together with inferred cleavage sites in the virus's polyprotein and the resulting products, and other genomic features (5'NTR and 3'NTR = non-translated regions, L = L-protein, VP1 to VP4 = capsid proteins, 2A = 2A-like protease site, VPg = VPg protein, 3C = 3C-protease, RdRp = RNA-dependent RNA polymerase, dsRNA = position of dsRNA fragment used in our RNA interference protocol [4], cf. [15]).