

Supplementary Files - Quantitative and qualitative changes in the Deformed wing virus population in honey bees associated with the introduction or removal of *Varroa destructor*.

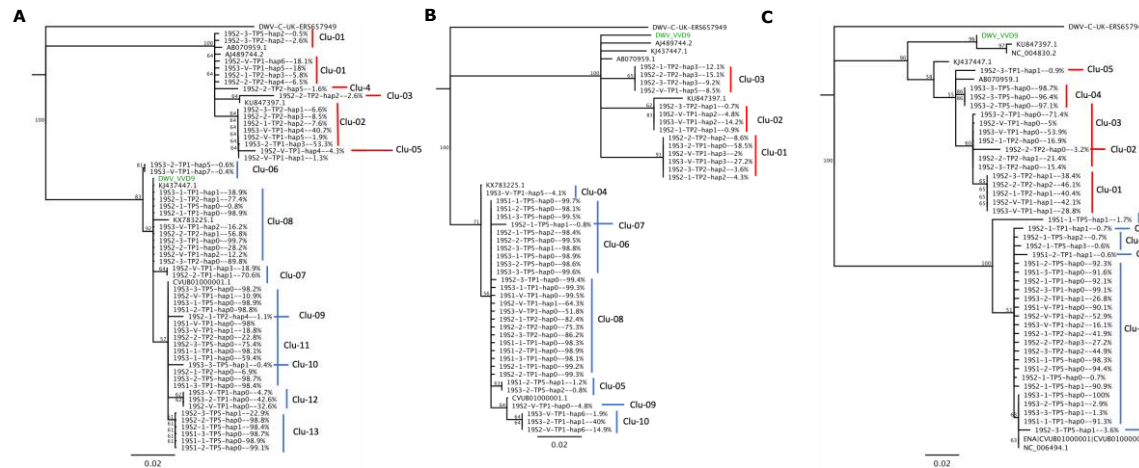


Figure S1: Neighbour joining phylogenetic analysis of ShoRAH haplotypes from 2019 Shook Swarm colonies. The (A) helicase (B) protease and (C) RdRp were analysed. Samples are labelled as 19Sx-y-TPz-hap% where x = colony number, y = sample number and z = time point number. Sample numbers labelled 'V' are from Varroa. The hapx_xx% indicates the haplotype number from ShoRAH and the percentage of the population the sample corresponds to. The blue and red bars indicate the Type B and Type A sequence cluster assignment used to make the bar plots in Figure 2. Reference genomes are shown by their NCBI accession number. The green sequence (DWV_VVD9) was used for the haplotype alignment. The genetic distance model for the phylogenetic analysis was Tamura-nei and no outgroup was included. The phylogeny were generated from 1000 bootstrap iterations and the trees were generated using majority greedy clustering. DWV Type C (ERS657979) was used as the outgroup in each analysis. Sequences were trimmed and aligned and phylogenetic analysis carried out using Geneious Prime 2019.1.

| | Timepoint | 2017 Colonies | | | | 2019 Colonies | | | | Key |
|------|----------------|---------------|---------|---------|----------|---------------|----------|----------|----------|--|
| | | 1-1 | 1-2 | 1-3 | 1-4 | 2-1 | 2-2 | 2-3 | 2-4 | |
| 2017 | July 17/7 | 3.7E+04 | 5.1E+03 | 3.7E+03 | 3.2E+03 | | | | | healthy (10 ⁻¹⁰ ^6) |
| | August 17/8 | 8.2E+03 | 7.5E+02 | 1.7E+06 | 1.4E+03 | | | | | At risk (10 ⁻¹⁰ ^6-10 ⁻⁸) |
| | September 17/9 | 5.4E+05 | 6.3E+04 | 8.0E+04 | 1.7E+05 | | | | | High risk (>10 ⁻⁸) |
| | October 17/10 | 1.6E+10 | 2.9E+09 | 3.0E+07 | 7.93E+06 | | | | | deceased |
| 2018 | May/June 18/6 | | | 1.4E+07 | 2.6E+03 | | | | | |
| | July 18/7 | | | 2.2E+10 | 2.3E+03 | | | | | |
| | August 18/8 | | | | 1.6E+05 | | | | | |
| | September 18/9 | | | | 1.5E+09 | | | | | |
| 2019 | May 19/5 | | | | | 8.81E+02 | 3.00E+02 | 5.01E+04 | 4.49E+04 | |
| | July 19/7 | | | | | 3.86E+09 | 1.01E+07 | 3.89E+04 | 2.00E+06 | |
| | August 19/8 | | | | | 7.70E+06 | 1.04E+07 | 5.46E+06 | 3.83E+08 | |
| | September 19/9 | | | | | 7.02E+10 | 1.23E+06 | 8.76E+09 | 9.33E+10 | |

Figure S2 - Relative viral loads of colonies left in a highly infested apiary over time. Colour scale represents increasing viral load from yellow to red. Black bars represent points where colonies have died. Average quantified viral load shown for each colony at each time point.

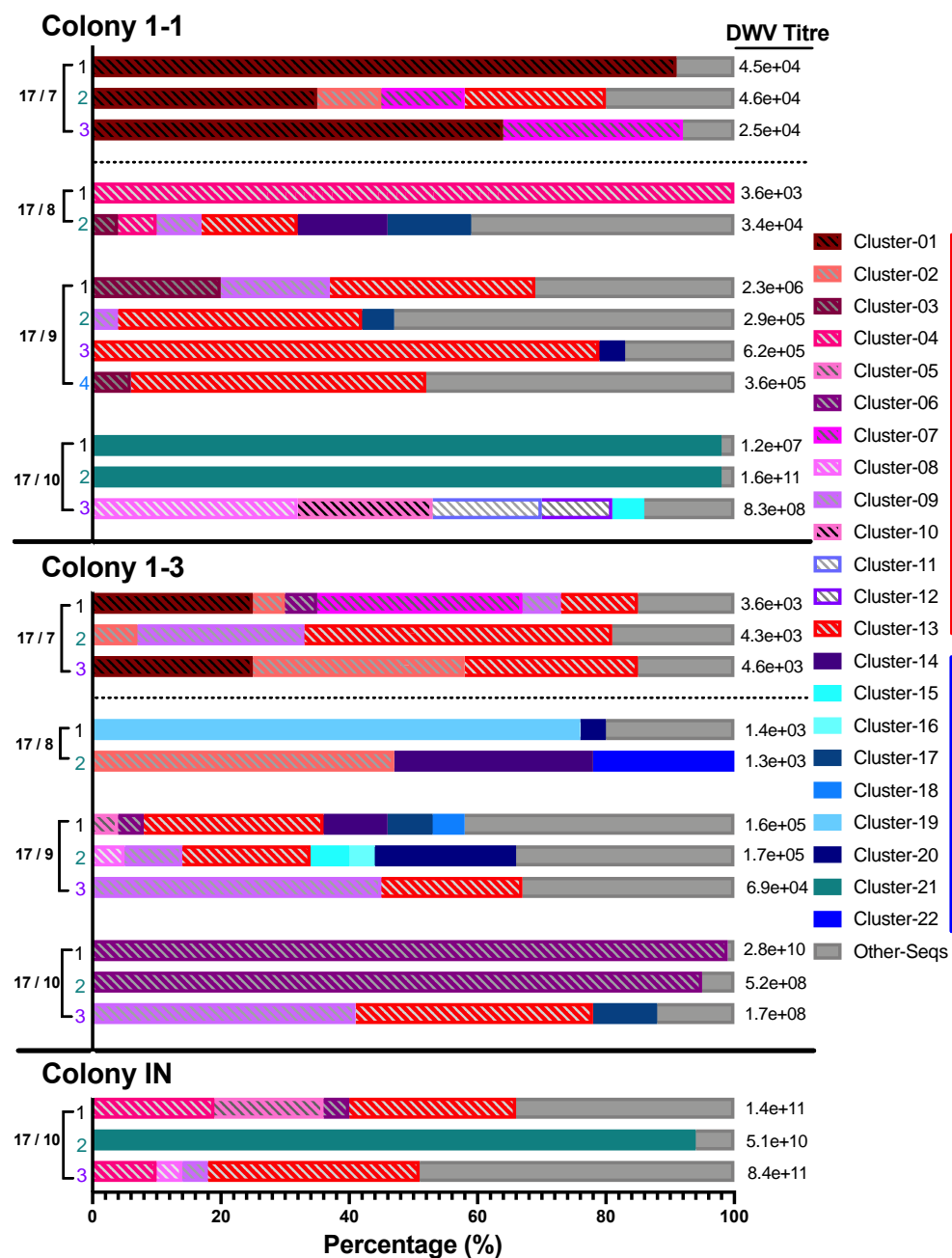


Figure S3 – Analysis of DWV sequence diversity in Varroa-naïve samples using ShoRAH compiled haplotypes. The clusters were compiled in to barplots to visualize changes in diversity over time for colony 1-1, 1-3 and a neighbouring infested colony (IN). The assigned clusters were coloured shades of red if they best matched Type A reference genomes and blue if they best matched Type B reference genomes. All Type A clusters are cross-hatched. ‘Other_Seqs’ are ShoRAH haplotypes below the 3% error threshold determined using a spiked control (data not shown). The quantified viral load of each honey bee is shown beside each bar. The dotted line indicates when the colonies were moved to the infested site.

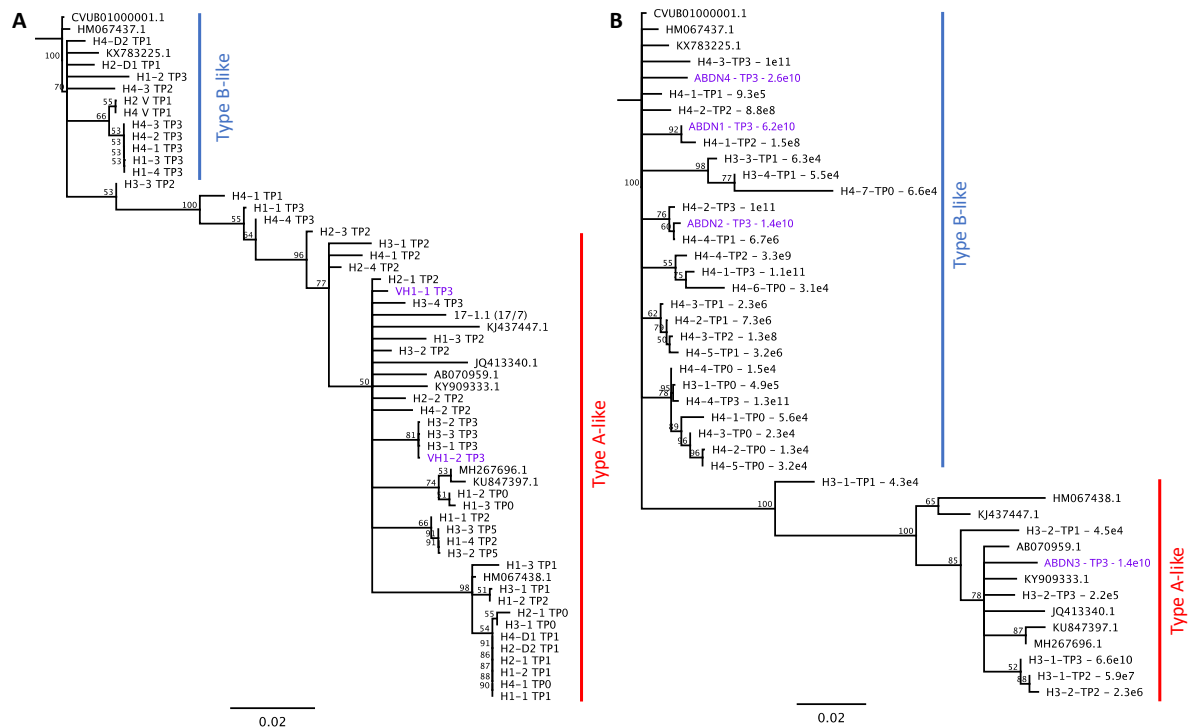


Figure S4 – Neighbour joining phylogenetic analysis of diversity changes over time in *Varroa-naïve* colonies. Sanger sequences of helicase region (4500-5500bp) from Year 1 (A) & Year 2 (B) colonies. Samples are named by colony (Hx), sample number and time point (TPx). Samples from the neighbouring infested colony are shown in purple for each year. Reference genomes are shown by their accession number. The genetic distance model for the phylogenetic analysis was Tamura-nei and the outgroup was CVUB01000001.1. The phylogeny were generated from 1000 bootstrap iterations and the trees were generated using majority greedy clustering. Sequences were trimmed and aligned and phylogenetic analysis carried out using Geneious Prime 2019.1.

Table S1 – Summary of linear model outputs for 2018 and 2019 assessing the change in log DWV titre from the brood samples taken before the shook swarm treatments (May), and subsequent samples.

| Year | R ² (adj) | F value | Fixed effect month | T value | P value |
|------|----------------------|---------|--------------------|---------|---------|
| 2018 | 0.88 | 316.5 | May | 79.7 | <0.001 |
| | | | June | -33.34 | <0.001 |
| | | | July | -29.66 | <0.001 |
| | | | August | -32.57 | <0.001 |
| | | | September | -28.52 | <0.001 |
| | | | October | -27.37 | <0.001 |
| 2019 | 0.62 | 63.44 | May | 39.18 | <0.001 |
| | | | June | -12.95 | <0.001 |
| | | | July | -13.77 | <0.001 |
| | | | August | -11.12 | <0.001 |
| | | | September | -11.66 | <0.001 |
| | | | October | -15.50 | <0.001 |

Table S2 – Summary of mixed effect model outputs for 2018 and 2019 assessing the change in log DWV titre from the brood samples taken before the shook swarm treatments (May), and subsequent samples.

| Year | Random colony (St Dev / Residual) | Fixed effect month | T value | P value |
|------|--------------------------------------|--------------------|---------|---------|
| 2018 | 0.0006 / 1.189 | May | 79.66 | <0.001 |
| | | June | -33.34 | <0.001 |
| | | July | -29.66 | <0.001 |
| | | August | -32.57 | <0.001 |
| | | September | -28.52 | <0.001 |
| | | October | -27.37 | <0.001 |
| 2019 | 0.916 / 2.565 | May | 25.88 | <0.001 |
| | | June | -13.48 | <0.001 |
| | | July | -14.28 | <0.001 |
| | | August | -11.59 | <0.001 |
| | | September | -12.09 | <0.001 |
| | | October | -16.27 | <0.001 |

Table S3 – Summary of linear model outputs for 2017 and 2019 assessing the change in log DWV titre from the brood samples taken before colonies were moved to a mite infested apiary (July 2017 and May 2019), and subsequent monthly samples.

| Year | R ² (adj) | F value | Fixed effect month | T value | P value |
|------|----------------------|---------|--------------------|---------|---------|
| 2017 | 0.73 | 147.4 | July | 23.24 | <0.001 |
| | | | August | -3.96 | <0.001 |
| | | | September | 3.83 | <0.001 |
| | | | October | 15.90 | <0.001 |
| 2019 | 0.39 | 36.9 | May/June | 12.10 | <0.001 |
| | | | July | 6.98 | <0.001 |
| | | | August | 8.04 | <0.001 |
| | | | September | 11.90 | <0.001 |
| | | | October | 7.44 | <0.001 |

Table S4 – Summary of mixed effect model outputs for 2017 and 2019 assessing the change in log DWV titre from the brood samples taken before colonies were moved to a mite infested apiary (July 2017 and May 2019), and subsequent monthly samples.

| Year | Random colony (St Dev / Residual) | effect | Fixed effect month | T value | P value |
|------|--------------------------------------|--------|--------------------|---------|---------|
| 2017 | 0.636 / 2.474 | | July | 18.49 | <0.001 |
| | | | August | -4.06 | <0.001 |
| | | | September | 3.93 | <0.001 |
| | | | October | 16.30 | <0.001 |
| 2019 | 2.32 / 3.78 | | May/June | 6.18 | <0.001 |
| | | | July | 7.70 | <0.001 |
| | | | August | 8.86 | <0.001 |
| | | | September | 13.03 | <0.001 |
| | | | October | 8.18 | <0.001 |