Supplementary material

Behavioral Ecology and Sociobiology

Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee

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Protocol S1

Success of DWV and *N. ceranae* infections was determined by quantitative PCR using specific primers (table S1). All reactions were performed in a Bio-Rad C1000 Thermal Cycler (Bio-Rad), using SYBRgreen Sensimix (Bioline). Thermal profiles for DWV amplification were: 5 min at 95°C, followed by 40 cycles of 10 sec at 95°C, 30 sec at 57°C, and 30 sec at 72 °C (read); and for *N. ceranae* amplification they were: 3 min at 94°C, followed by 40 cycles of 10 sec at 94°C, 30 sec at 54°C, 30 sec at 72 °C and 20 sec at 78 ^{o}C (read). Samples including all components except cDNA template served as a negative control in each run. Post amplification melting curve analysis was used to check for non-specific amplification (50^{o}C to 95^{o}C with an increment of 0.5^{o}C per second). β-actin (Table S1) was amplified for all samples as an internal reference control. To minimize the risk of false positives, an upper cycle threshold (Cq) of 35 was applied for positive DWV detection (de Miranda et al. 2013).

Table S1. PCR primers used in this study

Target	Name	Sequence	Reference
DWV	DWV-fwd	TTCATTAAAGCCACCTGGAACATC	Forsgren et al. 2009
DWV	DWV-rev	TTTCCTCATTAACTGTGTCGTTGA	"
β-actin	Am-actin2-aF	CGTGCCGATAGTATTCTTG	Locke et al. 2012
β-actin	Am-actin2-qB	CTTCGTCACCAACATAGG	"
N. ceranae	NceranaeF	CAATATTTTATTATTTTGAGAGA	vanEngelsdorp et al. 2009
N. ceranae	NceranaeR	TATATTTATTGTATTGCGCGTGCA	"

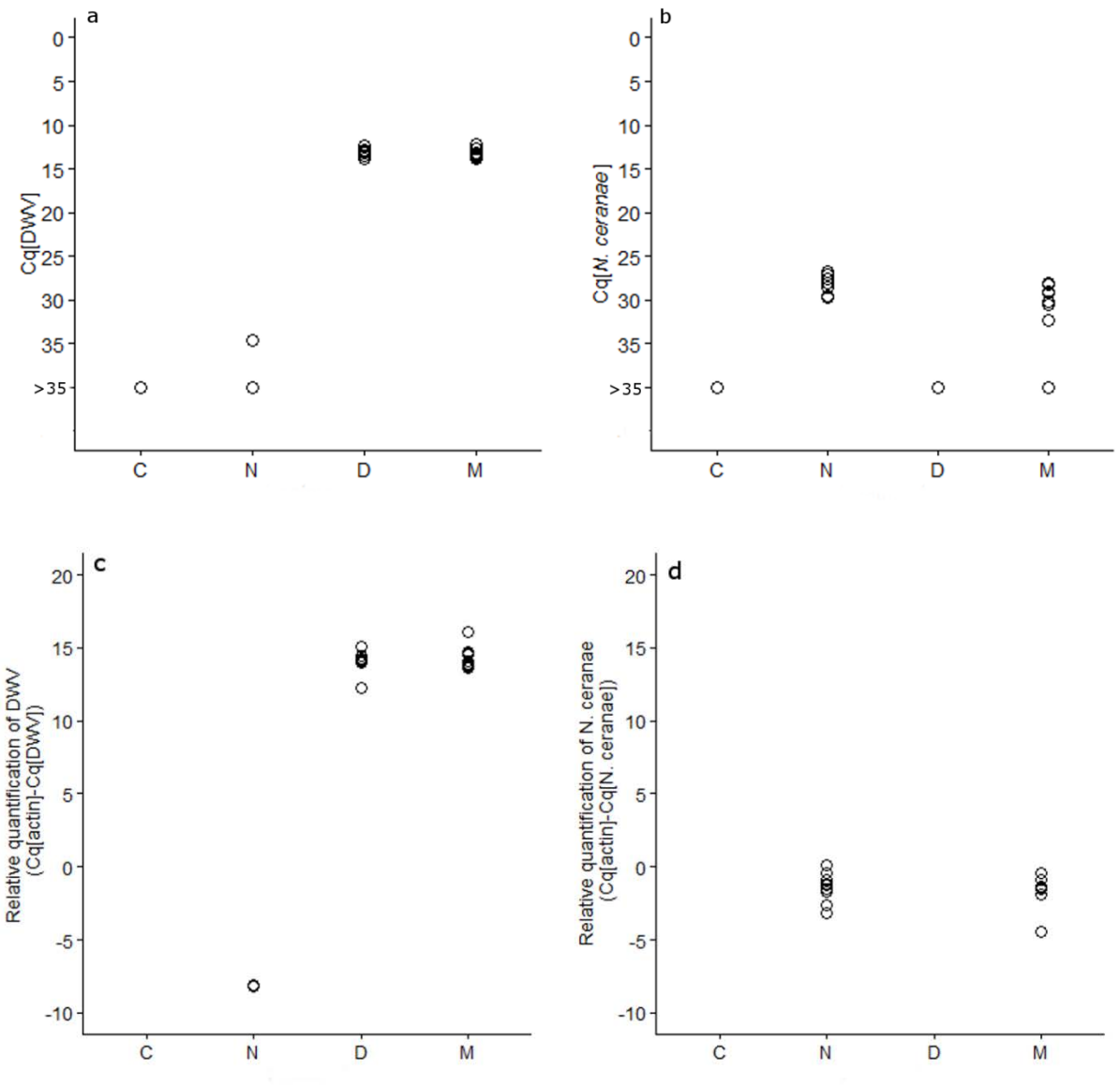

Fig. S1 Amount of **a, c** DWV and **b, d** *N. ceranae* given in either raw Cq values (**a-b**) or normalised to actin (**c- d**), which served as a housekeeping gene (HKG) in a subset of 13-day-old treated bees. Letters on x-axis indicate treatment: C: control, N: *N. ceranae* fed bees, D: DWV injected bees, M: *N. ceranae* fed and DWV injected bees. (Sample size of screened bees: C: n=11; N: n=9; D: n=10; M: n=11). On Y-axis: **a-b** raw Cq ("Cycle of quantification") values. A lower value indicates higher pathogen load. Cq>35 indicates that no amplification was detected; **c-d** pathogen load in terms of comparative quantification

(ΔCq) relative to actin, which served as housekeeping gene. ΔCq values were calculated by subtracting target (DWV or *N. ceranae*) Cq values from their corresponding actin Cq values. Positive values indicate that the amount of target detected was higher than the amount of the housekeeping gene and vice versa.

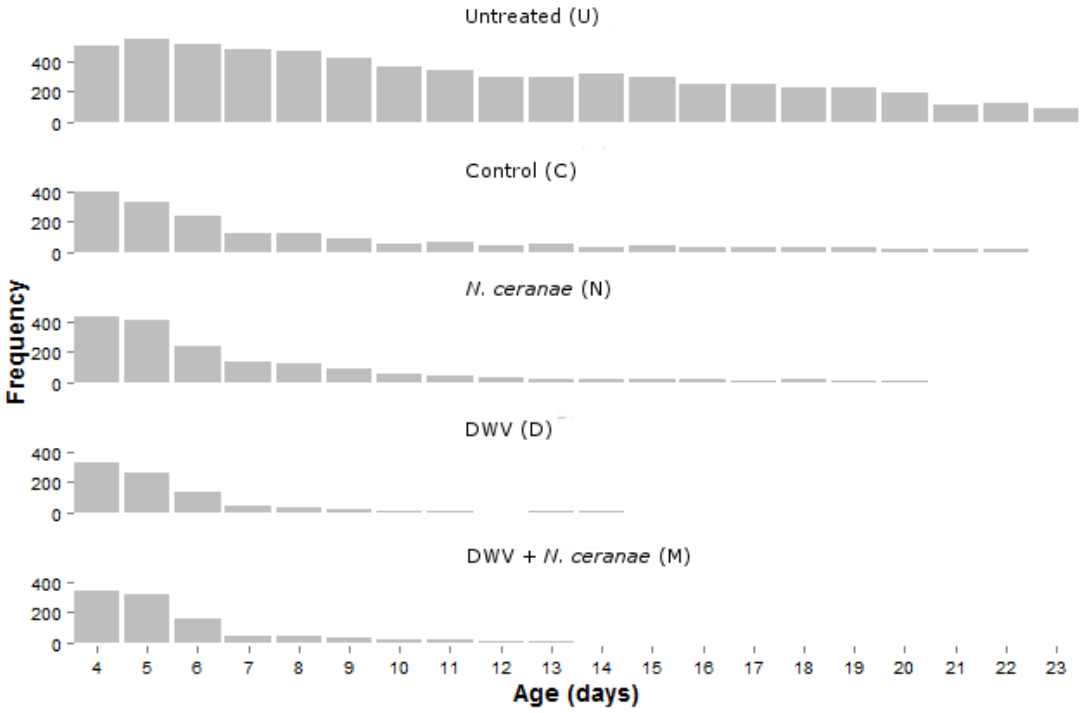

Fig. S2 Distribution of behavioural acts across age (in days) of marked worker bees by treatment.

References for Supplementary material

Forgren E, de Miranda J, Isaksson M, Wie S, Fries I (2009) Deformed wing virus associated with *Tropilaelaps mercedesae* infecting European honey bees (*Apis mellifera*). Exp Appl Acarol 47: 87–97

de Miranda JR, Bailey L, Ball BV et al (2013) Standard methods for virus research in *Apis mellifera*. J Apic Res 52:1–48. doi: 10.3896/IBRA.1.52.4.22

Locke B, Forsgren E, Fries I, de Miranda J (2012) Acaricide treatment affects viral dynamics in *Varroa destructor*-infested honey bee colonies via both host physiology and mite control. Appl Environ Microbiol 78: 227–235

vanEngelsdorp D, Evans JD, Saegerman C et al (2009) Colony collapse disorder: a descriptive study. *PLoS One* **4**, e6481