

Electronic supplementary material

“Chronic neonicotinoid pesticide exposure and parasite stress differentially affects learning in honeybees and bumblebees”

Saija Piironen & Dave Goulson

Supplementary Table S1. PCR reactions and thermal cycle conditions used in parasite screenings. Changes made to the original PCR reaction and thermal cycle conditions are indicated in bold.

Species	<i>N. ceranae</i>	<i>N. apis</i>	<i>N. bombi</i>	<i>A. bombi</i>	<i>C. bombi</i>	<i>Apidae</i>
Reference	Martin-Hernandez et al. 2007	Gisder & Genersch 2013	Klee et al. 2006	Meeus et al. 2010	Meeus et al. 2010	Meeus et al. 2010
Gene	<i>16s</i>	<i>RPBI</i>	<i>16s</i>	<i>18s</i>	<i>18s</i>	<i>18s</i>
Product size (bp)	218	297	323	260	420	130
PCR mix of amplification:						
GoTaq® Flexi buffer (X) (Promega)	1	1	1	1	1	1
MgCl ₂ (mM)	3	1.5	3.75	1.5	1.5	1.5
Forward primer (μM)	0.4	0.2	0.2	0.5	0.5	0.1
Reverse primer (μM)	0.4	0.2	0.2	0.5	0.5	0.2
dNTPs (mM)	0.4	0.2	0.3	0.4	0.4	0.4
GoTaq® G2 Flexi polymerase (U) (Promega)	1	1	0.25	1	1	1
DNA template (μL)	2	1	2	1	2	2
Total volume (μL)	15	10	10	10	15	15
Thermal cycle program	(94°C for 2min) 1 cycle + (94°C for 15s, 61.8°C for 30s, 72°C for 45s) 10 cycles + (94°C for 15s, 61.8°C for 30s, 72°C for 50s) 20 cycles + (72°C	(94°C for 5min) 1 cycle + (94°C for 60s, 58°C for 60s, 72°C for 60s) 35 cycles + (72°C 10min) 1 cycle	(94°C for 4min) 1 cycle + (94°C for 60s, 50°C for 60s, 72°C for 60s) 35 cycles + (72°C 4min) 1 cycle	(94°C for 2min) 1 cycle + (94°C for 30s, 60°C for 30s, 72°C for 45s) 35 cycles + (72°C 3min) 1 cycle	(94°C for 2min) 1 cycle + (94°C for 30s, 56°C for 30s, 72°C for 45s) 35 cycles + (72°C 3min) 1 cycle	(94°C for 2min) 1 cycle + (94°C for 30s, 56°C for 30s, 72°C for 45s) 35 cycles + (72°C 3min) 1 cycle

7min) 1 cycle

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Supplementary Table S2, Percentage (%) of bees in different treatment groups screened for parasites by PCR. All *N. ceranae* inoculated honeybees were confirmed positive by PCR (spores detected under the microscope) whereas only 3% of the *Nosema* inoculated bumblebees were positive (no spores were detected under the microscope). One bumblebee was positive for *C. bombi* (belonged to the parasite treatment). None of the screened samples were positive for *A. bombi* and *N. bombi* in bumblebees or for *N. apis* in honeybees.

Treatment	Honeybee		Bumblebee	
	%	n/n total	%	n/n total
Control	33.6	40/119	47.9	46/96
Pesticide	35.0	42/120	47.9	46/96
Parasite	32.5	39/120	52.1	49/94
Both	35.0	42/120	52.1	50/96
Total	34.0	163/479	50.0	191/382

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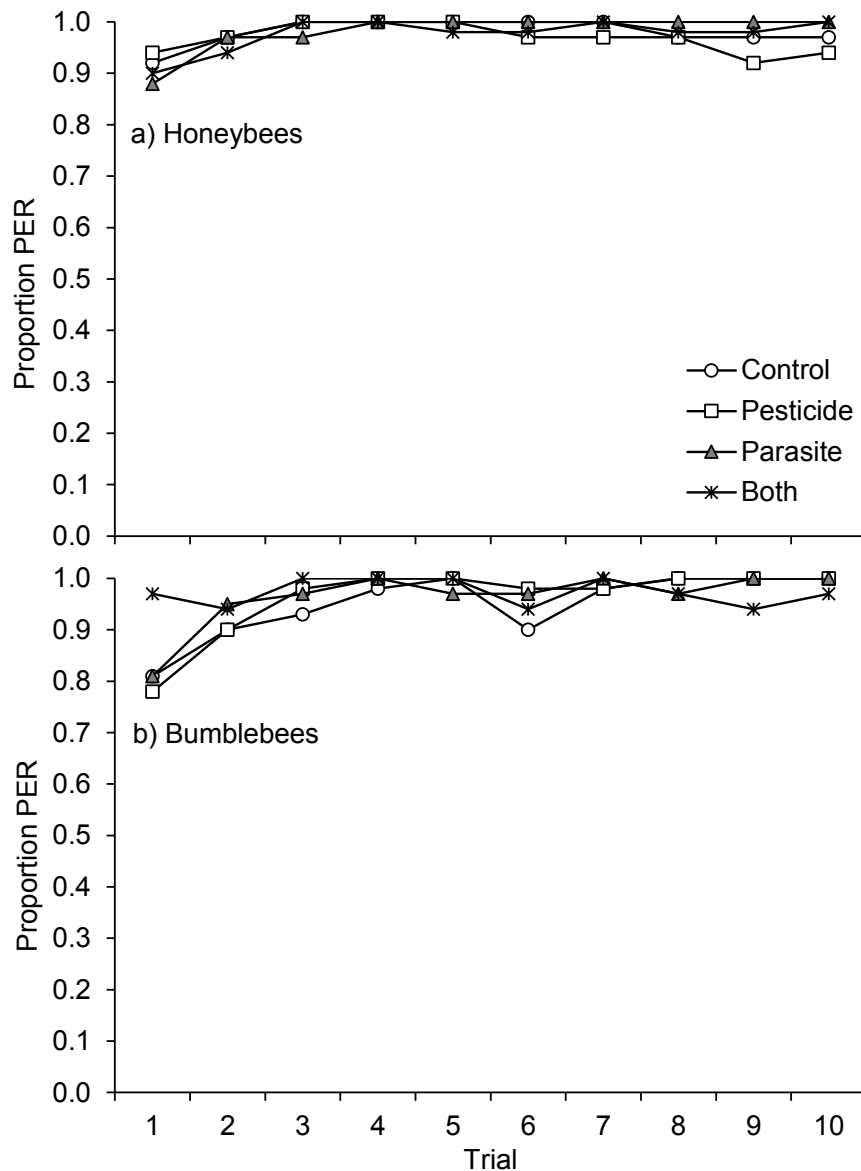
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Supplementary Table S3 Percentages (%) and sample sizes of honeybees and bumblebees in different treatment groups responding to sugar stimulus prior proboscis extension response (PER) training, completing the PER training and tested in the memory test.

Treatment	Honeybee					Bumblebee				
	Response to stimulus		Completed PER		Memory test*	Response to stimulus		Completed PER		Memory test*
	%	n/n total	%	n/n total	n	%	n/n total	%	n/n total	n
Control	65.7	46/70	80.4	37/46	35	78.1	50/64	84.0	42/50	29
Pesticide	75.0	48/64	75.0	36/48	29	76.2	48/63	83.3	40/48	24
Parasite	67.2	43/64	79.1	34/43	29	70.6	48/68	77.1	37/48	29
Both	84.1	53/63	90.6	48/53	43	71.9	41/57	78.0	32/41	17
Total	72.8	190/261	81.6	155/190	136	74.2	187/252	80.7	151/187	99

*The number of bees in the memory test differs from those in PER training as some bees showed a negative response to sugar stimulus after the memory test and were excluded from analysis or died before conducting the memory test.



Supplementary Fig. S1 Proportion of proboscis extension responses (PERs) to sugar stimulus (US), for a) honeybee and b) bumblebee workers exposed to pesticide clothianidin and inoculated with the parasite *N. ceranae* across 10 CS-US trials.