**Pathogen spillover from *Apis mellifera* to a stingless bee**

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Supplementary Table 1. Location of *T. hockingsi* hives used to collect bees for experimental infection of *T. hockingsi* with *N. ceranae* (1-6), and *T. hockingsi* hives monitored to ascertain *N. ceranae* infection status in wild populations (2-7).

|  |  |  |  |
| --- | --- | --- | --- |
| Colony ID | Locality | Latitude | Longitude |
| CAV1 | Caravonica | -16.8690° | 145.6788° |
| JCU4 | Smithfield | -16.8180° | 145.6847° |
| KOA1 | Koah | -16.8281° | 145.5146° |
| KUR2 | Kuranda | -16.8427° | 145.6261° |
| KUR3 | Kuranda | -16.8314° | 145.6236° |
| SPE3 | Speewah | -16.8734° | 145.6067° |
| KUR4 | Barron River | -16.8194° | 145.6385° |

**Inflorescence processing for spore counts and PCR**

We tested flowers for *N. ceranae* spores following Graystock et al. [1]. Briefly, we removed each flower from its stem and placed it into a sterile 1.5 ml Eppendorf tube containing 1ml of 98% ethanol and vortexed. We removed 20µl of this solution and stored it at -20°C for spore counting. We then centrifuged the vial at 14 000 G for 5min. We discarded the upper 800µl of solution, and homogenised the remaining 200µl with a sterile micro-pestle. The homogenised sample was then washed by adding 800ml of Tris-EDTA buffer, vortexed for 30s and centrifuged at 14 000 G for 5min, after which we discarded 800µl of the supernatant. We repeated this wash procedure a further two times, with 970µl of supernatant being removed on the final occasion. We then centrifuged the vial at 14 000 G for 5min to form a pellet, after which we carefully removed the remaining supernatant. We added 200µl of InstaGene™ matrix to the pellet to absorb cell lysis products and incubated it at 56°C for 20min. We then vortexed each sample for 10s and placed the tubes in a 100°C water-bath for 8 min. Each sample was then vortexed again, followed by being centrifuged at 12 000rpm for 3min. We then used 20μl of the resulting supernatant per 50μl PCR reaction.

**PCR**

We adapted our methods for DNA extraction, PCR and gel electrophoresis from Peng et al. [2] as reported in Ferguson et al.[3].

**DNA extraction**

To prepare an extraction buffer, we combined 0.5g PVP-40, 35 mL double-distilled water, 35 mL 0.5 M EDTA (pH 8), 5 mL 5.0 M NaCl, and 5 mL 1M Tris (pH 8) in an autoclaved bottle. We then combined 500 μL of extraction buffer with 40 μL of bee ventriculus homogenate along with two 2mm stainless steel beads. The tubes were loaded into a mixer mill and shaken at 25 Hz for 90 seconds, inverted and shaken for another 90 seconds. We spun down each tube to remove liquid from the lid and added 66 μL of 10 % SDS to precipitate the proteins, mixing by inversion for 10 seconds before centrifuging at 20800 G for 25 minutes at 4 °C. We continued to centrifuge the tubes in 2 minute intervals as needed until proteins had precipitated. Using new sterile 1.5 mL tubes, we added 445 μL of isopropanol and 500 μL of the supernatant, avoiding carrying over any resuspended proteins. We discarded the tubes containing proteins, and mixed the new tubes by inversion for 10 seconds before placing on ice for at least 15 minutes and centrifuging at 20800 G for 15 minutes at 4 °C. This process formed DNA pellets, which we washed by carefully discarding the supernatant, adding 500 μL of 70% ethanol and gently inverting. We then centrifuged the tubes at 20800 G for 15 minutes at 10 °C and removed the supernatant by pouring off the majority of the liquid and using a 200 µL pipette to remove any residual ethanol. The tubes were left open and placed under a fume hood for 10-20 minutes before we resuspended the DNA pellets in 20 μL of double-distilled water. We used a NanoDrop 2000 to confirm the amount of DNA in the samples and stored them at -20 °C.

**PCR and gel electrophoresis**

To prepare primers (see Table 1) for PCR, we added 250 μL of double-distilled water to 0.025 μmol primer powder to make a 100 mM solution, diluting at a ratio of 5 μL to 45 μL double-distilled water to make 10 mM primers for use. We prepared a PCR master mix in a 1.5mL tube by adding 2.4 μL sterile double-distilled water, 4.0 μL 5xGoTaq® G2 DNA Polymerase colourless buffer, 0.3 μL 10mM dNTP Mix (Promega), 0.4 μL 10 mM *N. cerenae* forward primer, 0.4 μL 10 mM *N. apis* forward primer, 0.4 μL 10 mM universal reverse primer, and 0.1 μL GoTaq® G2 DNA Polymerase (5U/μL) per sample, taking into account positive and negative controls. For each sample, we then transferred 8 μL of the master mix and 2 μL of DNA template into a labelled PCR tube. If gel electrophoresis could not be performed immediately, PCR products were stored for up to a week at -20 °C.

To conduct gel electrophoresis, we prepared the gel at a ratio of 2 g Agarose to 100 mL TBE based on the volume of the gel tray being used, microwaving in 20 second increments until the solution boiled to dissolve. Once the solution had cooled to approximately 65 °C, we poured it into the gel tray with combs in place and allowed it to cool until set. We removed the combs and placed the tray into the gel electrophoresis machine, ensuring all gel was covered by 1x TBE. We then mixed 1 μL of EZ Vision (10 bp marker, pink) with 5 μL of PCR product. Additionally, we mixed 1 μL of EZ Vision (10 bp marker, pink) with 5 μL DNA ladder (50 bp intervals) per row being used in the gel tray. We loaded 5 μL of each mixed sample into the gel wells, ending each row with controls and a ladder, and ran the machine at 80 V and 400 mA for 60 minutes. Following the 60 minutes, we observed the gel in a UV machine at 364 nm and recorded the results.

Supplementary Table 2**.** Description of primers used

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer** | **Sequence 5’-3’** | **Fragment length** | **Source** |
| Mncerenase-F | CGTTAAAGTGTAGATAAGATGTT | 143 bp | Fries et al, 2013 |
| Mnapis-F | GCATGTCTTTGACGTACTATG | 224 bp | Fries et al, 2013 |

Supplementary Table 3. *N. ceranae* spores detected in inoculated and non-inoculated *T. hockingsi* from day 1 post-inoculation to day 9, the last time point at which spores were detected.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Inoculated | | Non-inoculated | |
| Post-infection (days) | n | Spore range x 103 | n | Spore range x 103 |
| **1** | 5 | 10.5-42.5 | 3 | 2-18.5 |
| **2** | 77 | 12-56 | 4 | 3-13.5 |
| **3** | 98 | 11-58 | 2 | 0.5-10.5 |
| **4** | 45 | 7.5-51.5 | 2 | 1-2.5 |
| **5** | 7 | 15-31 | 0 | 0 |
| **6** | 4 | 14.5-26 | 0 | 0 |
| **7** | 2 | 21.5-37 | 0 | 0 |
| **8** | 1 | 53 | 0 | 0 |
| **9** | 1 | 19.5 | 0 | 0 |

Supplementary Table 4. Number of individual *T. hockingsi* infected with *N. ceranae* and their spore counts out of 15 collected each month by hive and month of collection. Locations correspond with hives 2-7 in Supplementary Table 1.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | June | | July | | August | |
| Colony | Infected bees | Spore count x 103 | Infected bees | Spore count x 103 | Infected bees | Spore count x 103 |
| JCU4 | 0 | 0 | 0 | 0 | 0 | 0 |
| KOA1 | 0 | 0 | 0 | 0 | 0 | 0 |
| KUR2 | 2 | 6, 6.5 | 0 | 0 | 0 | 0 |
| KUR3 | 3 | 7.5-11.5 | 1 | 23 | 0 | 0 |
| SPE3 | 3 | 8-13.5 | 3 | 2.5-4.5 | 2 | 9.5, 10.5 |
| KUR4 | 3 | 6.5-11.5 | 0 | 0 | 0 | 0 |

Supplementary Table 5. PCR and spore counts (x 103) from the floral transmission experiment. Half of the inflorescences had been exposed to inoculated *A. mellifera* (exposed) and half had not been exposed to *A. mellifera* (control).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cage | Treatment | PCR result for *N. ceranae* | Inflorescence spore count | Bee 1 | Bee 2 | Bee 3 | Bee 4 | Bee 5 | Total infected bees |
| 1 | exposed | positive | 460 | 2 | 15.5 | 0 | 0 | 41 | 3 |
| 2 | exposed | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | exposed | positive | 305 | 42.5 | 51 | 69 | 30.5 | 100.5 | 5 |
| 4 | exposed | positive | 130 | 0 | 0 | 6.5 | 0 | 0 | 1 |
| 5 | exposed | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | exposed | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | exposed | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | exposed | positive | 150 | 5 | 0 | 0 | 0 | 0 | 1 |
| 9 | exposed | positive | 85 | 61 | 57.5 | 139 | 0 | 39 | 4 |
| 10 | exposed | positive | 110 | 0 | 34 | 38.5 | 51.5 | 91.5 | 4 |
| 11 | exposed | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | exposed | positive | 120 | 130 | 0 | 0 | 129 | 28 | 3 |
| 13 | exposed | positive | 260 | 0 | 13 | 0 | 0 | 0 | 1 |
| 14 | exposed | positive | 110 | 0 | 0 | 2.5 | 0 | 0 | 1 |
| 15 | exposed | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | control | negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[1] Graystock, P., Goulson, D. & Hughes, W.O.H. 2015 Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. *Proceedings of the Royal Society B* **282**, 20151371.

[2] Peng, Y., Baer-Imhoof, B., Millar, A.H. & Baer, B. 2015 Consequences of *Nosema apis* infection for male honey bees and their fertility. *Scientific Reports* **5**. (doi:10.1038/srep10565).

[3] Ferguson, J.A., Northfield, T.D. & Lach, L. 2018 Honey bee (*Apis mellifera*) pollen foraging reflects benefits dependent on individual infection status. *Microbial Ecology*. (doi:10.1007/s00248-018-1147-7).