Jessica L Mullins, James P Strange, Amber D Tripodi, Why Are Queens Broodless? Failed Nest Initiation Not Linked to Parasites, Mating Status, or Ovary Development in Two Bumble Bee Species of Pyrobombus (Hymenoptera: Apidae: Bombus), *Journal of Economic Entomology*, Volume 113, Issue 2, April 2020, Pages 575–581, https://doi.org/10.1093/jee/toz330

Supplementary Methods

Development of a multiplex PCR panel to diagnose major bumble bee parasites in a single reaction

All primer design was conducted within Geneious v.6.1.8 (Biomatters, Auckland, NZ) with the aid of the Primer 3 plug-in (Untergasser et al. 2012). Before being applied to the samples in this experiment, primer sets were tested in PCR both singly and in combination with positives and negatives verified by microscopy to ensure accurate diagnosis. Amplicons from single reactions were sequenced to verify their diagnostic capacities as described below. When PCR products from the multiplex are visualized on a gel, bumble bee samples that produce an amplicon at ~230 bp indicate a successful DNA extraction and PCR. If that is the case, those samples that also exhibit products at ~580 bp can be interpreted as trypanosomatid-positive, samples with products at ~360 bp are *A. bombi*-positive, and those with amplicons at ~270 bp are microsporidia-positive (Figure S1). Reagent concentrations, thermal cycling and electrophoretic conditions, gel staining and visualization are described in the main text.

Bee Control Primers

In the absence of a bee control, samples that produced no products after PCR may be free of parasites or they may represent failed reactions. In order to ensure that samples negative for targeted parasite taxa were indeed parasite-negative, a control marker was developed using a portion of 18S rRNA that would produce a PCR product in the presence of bumble bee DNA. Sequences were obtained for *B. diversus* Smith, *B. ignitus* Smith, *B. mendax* Gerstaecker and *B.*

ussurensis Radoszowski (Genbank accession numbers HM750223, KC413667, HM750222, KC413696, respectively). The primers Apid18SF (5'-GTCCTATCGGTGGGCTTAGC-3') and Apid18SR (5'-ATGCCCCCATCTGTCCCTAT-3') were developed to produce an amplicon 233 bp long. Sequences from eight species of bumble bees were identical (Genbank Accession numbers MK59973, MK59974, MK59976–MK529981). During tests, we also found that this marker successfully amplified a 233 bp of *Apis mellifera* Linnaeaus (Genbank accession number MK529975, primers trimmed) with four nucleotides that differed from the bumble bee sequence.

Trypanosomatid Primers

Primers to detect trypanosomatid parasites, including those in the genus *Crithidia,* were developed as part of previously published project (Tripodi and Strange 2018). The primer CB-SSUrRNA-F2 (5'-CTTTTGACGAACAACTGCCCTATC-3') (Schmid-Hempel & Tognazzo, 2010) when combined with CB18SR2 (5'-TGCTCCTTTGTTATCCCATGCT-3') will produce a 584 bp product in the presence of *Blastocrithidia, Blechomonas, Crithidia, Lotmaria, Herpetomonas, Lafontella, Leptomonas, Novymonas, Paratrypanosoma,Sergeia, Wallaceina,* or *Zelonia. Crithidia bombi, C. expoeki* Schmid-Hempel and Tognazzo*, C. mellificae* Langridge and McGhee*, "C. mexicana"* and *Lotmaria passim* Schwarz are the only trypanosomatids identified in bumble bees to date, and these primers should amplify all of these taxa, although only *Crithidia bombi, C. expoeki, C. melliface* and *L. passim* have been tested so far (Tripodi and Strange 2018).

*Neogregarine (*Apicystis bombi*) Primers*

A novel set of primers was developed to detect another common bumble bee parasite, the

neogregarine *Apicystis bombi,* using sequences of the 5.8S rRNA, internal transcribed spacer 2 and 28S rRNA region of the genome. *Apicystis bombi* sequences (accession numbers KF322207–KF32216 and KP055606–KP055610) were obtained from GenBank and examined for conserved regions. No other identified neogregarine species overlapped this region, but a very different sequence belonging to an unidentified neogregarine isolated from the West Indian sweet potato weevil *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) (AB748927) shared the same conserved regions on which the primers were based. The primer set Apicyst357F (5'-AGCGATGGATGTCTTGGGTC-3') coupled with Apicyst357R (5'-CCTAGTTAGTTTCTTTTCCTCCGC-3') will produce an amplicon 357 bp long in the presence of *A. bombi* DNA. The unidentified weevil neogregarine used to develop this set of primers would result in a product that was 370 bp long. Although this indicates that the primer set may be capable of detecting a range of neogregarines, *A. bombi* is the only neogregarine detected in bumble bees to date and there is little genetic data available for other members of this parasite group. Sequences were deposited on Genbank (accession numbers MK529991–MK529995)

Microsporidia Primers

A novel set of primers was designed to detect a wide range of microsporidia using sequences of a portion of the small subunit (16S) representing all of the known bee-infecting microsporidia (*N. apis* (Zander)*, N. bombi, N. ceranae* (Fries, et al.)*, N. neumani* Chermurot, et al. and *Tubulinosema pampeana* Plischuk, et al.). Sequences (Genbank accession numbers AY741101, AY741104, AY741105, AY741111, DQ235446, DQ374656, DQ673615, EU025027, EU045844, FJ789796, GQ254295, KF188740, KF188742, KF188744, KF188746, KF188750, KF188760, KF188770, KM883008, MF882996, and NAU26534) were obtained and examined

for regions conserved across microsporidian species. A novel primer pair (MSporF2: 5'-AGTGGTGCATGGCCGTTTTC-3' and MSporDegR: 5'- GGTGTGTRCAAAGAACAGGG-3') was designed that results in the following amplicon sizes: *N. apis* and *N. neumani* 271 bp, *N. bombi* 274 bp, *N. ceranae* 280–281 bp and *T. pampeana* 316 bp. Although species cannot be discerned visually on a typical ethidium bromide-stained gel, the presence of an amplicon within approximately 300 bp is indicative of the presence of microsporidia. The only species we could verify with these primers were *N. bombi* and *N. ceranae,* but samples of both bumble bees and honey bees with *Nosema* spores visible at 400X were used to verify the diagnostic capacity of MsporF2-MsporDegR. Sequences of each species were deposited at Genbank (accession numbers: *N. bombi:* MK529983–MK529987 and *N. ceranae* MK529982).

Multiplex Panel

Figure S1 demonstrates a typical diagnostic gel after PCR with the newly developed multiplex panel.

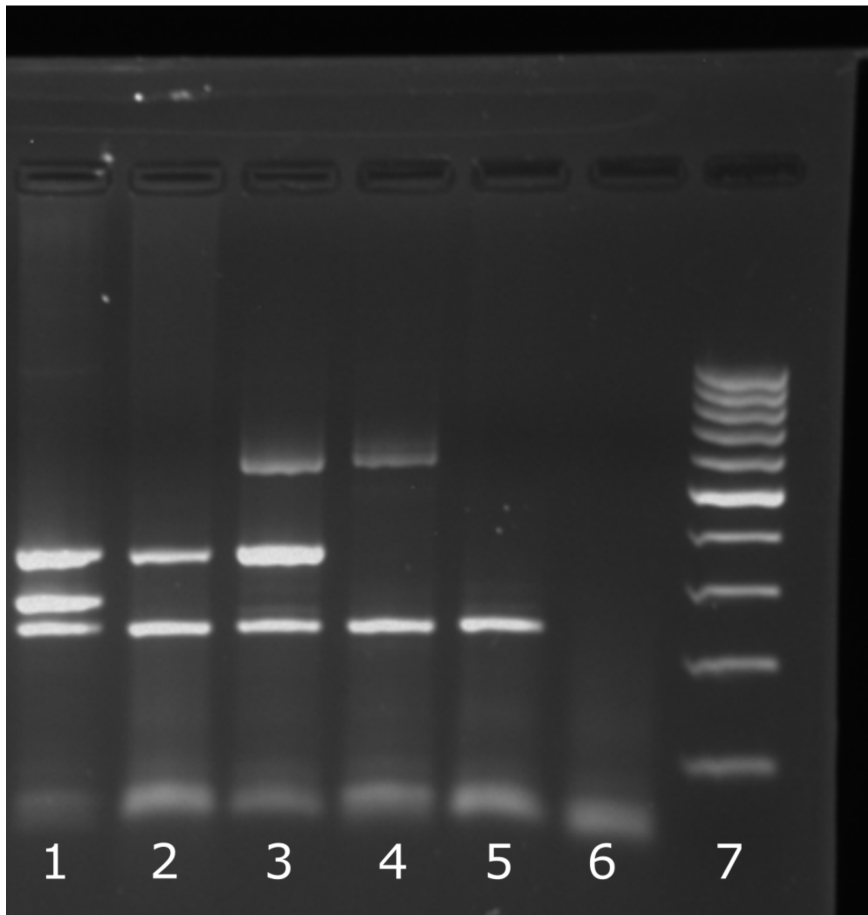

Figure S1. Results of diagnostic multiplex PCR on electrophoretic gel (2% agarose) stained with 2.5X Gel Red and visualized under UV light. Samples in each lane were diagnosed as positive for the following: Lane 1) bee, microspordia and neogregarine; 2) bee and neogregarine; 3) bee, neogregarine and trypanosomatid, 4) bee and trypanosomatid; 5) bee; 6) nothing. Lanes 1–6 were loaded with PCR product. Lanes 1–5 used DNA template that was extracted from bee abdominal cavities and Lane 6 used water in place of template in the PCR. Lane 7 is a 100-bp ladder. The faint product visible above 1,000 bp in Lane 1 is a non-specific artifact and was ignored in the diagnosis.

References

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