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Early queen development in honey bees: Social context and queen breeder source affect gut microbiota and associated metabolism

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Review Timeline:

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Reviewer(s): Disclosure of reviewer identity is with reference to reviewer comments included in decision letter(s). The following individuals involved in review of your submission have agreed to reveal their identity: Peng He (Reviewer #2)

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

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April 19, 2022

Dr. Kirk E Anderson
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Re: Spectrum00383-22 (Early queen development in honey bees: Social context and queen breeder source affect gut microbiota and associated metabolism)

Dear Dr. Kirk E Anderson:

Thank you for submitting your manuscript to Microbiology Spectrum. When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

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Sincerely,

Daifeng Cheng

Editor, Microbiology Spectrum

Journals Department
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E-mail: spectrum@asmusa.org

Reviewer comments:

Reviewer #2 (Comments for the Author):

Copeland et al. examined gene expression of oxidative stress and immunity and performed high-throughput sequencing of the gut microbiome across four queen alimentary tract niches. The study is well-designed and the writing is easy to follow. However, some my concerns and errors should be clarified.

Major ones:
1. Why did you select fat body to investigate the immune genes? Any other insect-related papers?
2. Why did you focus on immune genes only? Have you found any other different expression genes?

Minors:

1. Line 154, why did you select 3 weeks as the checkpoint?
2. Line 187, the ® should be the superscript. Check others.
3. Line 197, give the concentrations of primers and templates.
4. Line 214, Bio-Rad. Check others.
5. Line 219, Only single reference gene is not commonly used in today's qPCR assays. Please see the MIQE of qPCR: The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 2009, 55 (4), 611-22.

Staff Comments:

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Re: Spectrum00383-22 (Early queen development in honey bees: Social context and queen breeder source affect gut microbiota and associated metabolism)

RESPONSE TO REVIEWERS
Reviewer comments:

Reviewer #2 (Comments for the Author):

Copeland et al. examined gene expression of oxidative stress and immunity and performed high-throughput sequencing of the gut microbiome across four queen alimentary tract niches. The study is well-designed and the writing is easy to follow. However, some my concerns and errors should be clarified.
REPLY: Thank you for the accolades, we have revised the manuscript for clarity.

Major ones:
1. Why did you select fat body to investigate the immune genes? Any other insect-related papers?
REPLY: Clarified. The fat body is analogous to the mammalian liver, and its function (including the expression of immune genes) is highly dependent on nutrition, which may differ between our treatment conditions: high metabolic demand and low metabolic demand environments. Most of the immune proteins present in the hemolymph are synthesized in the fat body. We also tested gene expression in four separate gut tissues; mouthparts, midguts, ileums, and rectums, and much of this gene expression was significant between time one and two but only a few genes differed by treatment. Specifically, in the midgut and ileum there was a significant increase in expression of oxidative stress and AMP genes from time zero to time one regardless of treatment. Other gene expression in gut tissues are consistent with LMD (queen bank) queens experiencing more oxidative stress relative to HMD (colony) and time zero queens.

2. Why did you focus on immune genes only? Have you found any other different expression genes?
REPLY: Clarified. We were interested in the relationship of the gut microbiome with early immune training in queen phenotypes. We hypothesized that early immune training may be disrupted by the queen bank environment. We also ran a variety of genes involved with oxidative stress and two genes associated with nutritional status.

Minors:

1. Line 154, why did you select 3 weeks as the checkpoint?
REPLY: Now clarified in the methods. We chose (predicted) this time period based on our past work with queen microbial succession wherein we examined 4-6 month old and 16-18 month old queens (Anderson et al. 2018), and another paper highlighting the queen mating process (Tarpy et al. 2014). Collectively these papers indicate that establishment/succession can happen relatively quickly. It also represents enough time for the metabolic pressures and microbiota associated with each social environment to acclimate or establish.

2. Line 187, the ® should be the superscript. Check others.
REPLY: Thank you, we have made this correction.

3. Line 197, give the concentrations of primers and templates.
REPLY: Thank you, we now provide this information. Each primer=0.5ul / 12ul * 10uM =416nM each. Templates were not quantified, because we eluted both DNA and RNA into a single reaction mix, and the spectrophotometer cannot distinguish between RNA and DNA at 260nm. For DNA-based qPCR (most BactQuant, FungiQuant, etc.), we use a 10% dilution of the extraction for Bactquant, but run FungiQuant with the undiluted 100% template.

To create cDNA, we placed 8ul of extracted nucleotides (RNA/DNA) into to 10ul DNAse I treatment (1ul buffer, 1ul enzyme). We then used all 10ul from the DNAse treatment in a 20ul cDNA reaction (per ThermoFisher). Following enzyme activity, we diluted the 20ul cDNA reaction with 180ul H20. RNA yields averaged ~5ng/ul, 8ul=40ng total RNA in our cDNA reactions.

4. Line 214, Bio-Rad. Check others.
REPLY: Corrected throughout.

5. Line 219, Only single reference gene is not commonly used in today's qPCR assays. Please see the MIQE of qPCR: The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 2009, 55 (4), 611-22.
REPLY: Yes, we routinely run both B-actin and an RDP gene, but seems we only ran B-actin in this case.

June 5, 2022

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Daifeng Cheng
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