Investigating host-environment-microbiota interactions in shaping bee health

Introduction: Honey bees (*Apis mellifera)* are cornerstone pollinators and contribute nearly $20 billion to the U.S. agricultural economy each year[1]. Honey bee populations have drastically declined by an estimated 30-40% in the past three decades, and 2019 marks the largest winter hive loss ever recorded[1]. Bee decline threatens the U.S. economy and food supply, which has driven agricultural stakeholders and the scientific community to investigate reasons for honey bee deaths. A number of factors have already been identified, including habitat and foraging space loss, pesticide exposure, and infection by parasites, fungi and viruses. Bees have a commensal community of microbes aside from pathogens that includes bacterial, viral, and eukaryotic species, collectively known as the microbiome. Like in most organisms, the microbiome in bees plays an important role in nutrition and shaping host health through immunity and disease susceptibility. The extent to which the honey bee gut microbiome influences health outcomes remains unclear and for these reasons the scientific community is diligently working toward the characterization of the honey bee microbiota.

Intellectual Merit/Background: 16S sequencing has revealed that remarkably simple and spatially organized microbial communities of about 8-10 bacterial phylotypes occupy the honey bee gut, consistent across geographic distributions of bees[2]. In-depth metagenomic sequencing and single-cell characterization at the *strain* level revealed that each phylotype spans considerable microbial genomic diversity, leading to substantial polymorphism within and between hives. Such variation between bacterial strains belonging to the same phylotype could result from functional diversification (due to niche partitioning) but also suggests co-divergence and adaptation with host lineages. Bidirectionally, the bee microbiome has been shown to play an important role in modulating the host physiology. Germ-free studies highlighted that native gut microbiota is able to stimulate the immune system in adult worker bees[3] and the use of probiotics to effectively mitigate parasite effects shows promise[4]. However, the contributions of the gut microbiota to host immune pathways and the mechanisms by which the host responds to gut variation has yet to be investigated. Lastly, the genetic architecture of a honeybee colony makes any two daughter worker bees of sister queens mated with a single drone share ¾ of their genes on average[5]. Taken altogether with the consistent microbial phylotypes observed across colonies, this makes honey bees an ideal model for studying host-microbiome interactions. Using a combination of comparative genomics and field experiments, I aim to identify possible routes of honey-bee/microbiota co-diversification. **I hypothesize 1)** that the host genotype, diet, and environmental landscape shape the functional capabilities of the honey bee microbial community, and **2)** this microbial community can acutely impact the innate immune system of adult bees, and that this community can be modulated by the addition of probiotics. I plan to test these hypotheses with the following aims:

Aim 1: Determining the contribution of host genetic and environmental landscape in shaping the functional microbiome of the honey bee microbiota. I will rear several hives derived from single drone-mated sister queens from three different subspecies of *A. mellifera*: *ligustica, carnica,* and *mellifera.* These will be replicated in two geographically separated apiaries (collections of hives). Bees in each location will have access to the same respective landscape of flora to forage from and will also be fed identical nutritional supplements (protein supplementation, sugar syrup). Samples will be collected at the initial hive set-up, then once every 3 months for a year. They will be collected from nurse bees (who stay within the hive) and foraging bees (who leave the hive). Use of apiaries, acquisition of bees, and subspecies identification is enabled by collaboration with Dr. Ramesh Sagili of the Oregon State Honey Bee Lab. High-throughput shotgun metagenomic

sequencing will be used to assess and compare the bees' microbial structure at the phylotype and strain level between and within a) subspecies, b) nurses/foragers of each subspecies and at each location, and c) longitudinally, to assess patterns of functional microbiome congruence between the genetically and geographically distinct subgroups of bees. Additional data including winter survival rates per subspecies will be collected. Microbial samples from the flora the bees forage from in the different apiary locations will be collected in an attempt to ascertain the microbes they are exposed to outside of the hive.

Aim 2: Comparative analysis of functional and spatial diversity in the *A. mellifera* gut microbiome following immune system challenge. Germ-free bee studies have found upregulation of antimicrobial peptides in hemolymph (blood analog in bees) to be associated with inoculation by specific gut microbiota members[3]. I will determine if altering the microbial community with probiotics can modulate the immune response of the bees and reduce fatalities due to *Nosema ceranae* (a parasite associated with bee depopulation[6]). To do so I previously collaborated with the Honey Bee Lab and performed a three-week *in vivo* experiment on the addition of probiotics during *Nosema* infection. Microbiome samples were harvested from the midgut and hindgut of single bees (*Nosema* localizes in the midgut). My pilot 16S sequencing confirmed that our methods are sensitive enough to detect distinct spatial microbial compositions (consistent with the literature). Shotgun metagenomics will be performed to compare strain-level functional diversity between experimental groups and gut regions, as well as determine impact of probiotic strains on composition and functional diversity. Functional pathways will be identified in each group and quantitatively compared to ascertain up- or downregulation of antimicrobial peptides and other immune-related genes in the context of infection or probiotic addition. mRNA-seq will be completed to quantify and compare host response to those pathways identified by metagenomics. Pathway predictions will also be confirmed via LC-MS/MS of antimicrobial peptides present in the bee hemolymph.

Broader Impacts – Research Dissemination: The sequences and metadata generated by this project will be made publicly available through the online BeeBiome consortium[2], where there is a pressing need for comprehensive honey bee microbiome data. Results regarding probiotic treatment of honey bee hives will be communicated to bee keepers through local and nation-wide beekeeping meetings and publications. I anticipate submitting a first author paper detailing my findings in spring 2020, as well as presenting my results at the International Society for Microbial Ecology 2020 meeting in Cape Town, South Africa. At OSU, I will utilize opportunities to share my research, such as the bi-annual Center for Genomic Research & Biocomputing conferences, where I have presented research previously.

Broader Impacts – Science Communication: I will share my research with audiences from K-12 children, community members, and faculty. I plan to involve the community in my research, by expanding this project to include bee gut and local flora samples donated by regional beekeepers. This community-driven, crowd-sourced approach is necessary to characterizing honey bee health outside of the lab setting. I also plan to involve high school AP Biology students during the collection and characterization of apiary flora (Aim 1). I currently serve as the Outreach Coordinator for the Micro Grad Student Assoc. and am in the process of developing several opportunities for local K-12 children to learn about honey bees, utilizing hands-on activities at the weekly Saturday farmer's market. This combination provides an optimal platform for me to talk about my research and engage with community members on the themes of how humans rely on bees for our food production, and how the health of honey bees impacts us.

1. Pollinator Health Task Force. 2016. *Report to Congress.* 2. Engel et al. 2016. *MBio*. 3. Kwong et al. 2017. *R. Soc. Open Sci* 4. Khoury et al. 2018. *Frontiers*. 5. Johnstone et al. 2012. *R. Soc. Bio Sci* 6. Rubanov et al. 2019. *Sci. Rep*