OP-104 [Bee Health]
A novel non-invasive method to characterize the gut microbiome and viral profile of honey bee queens

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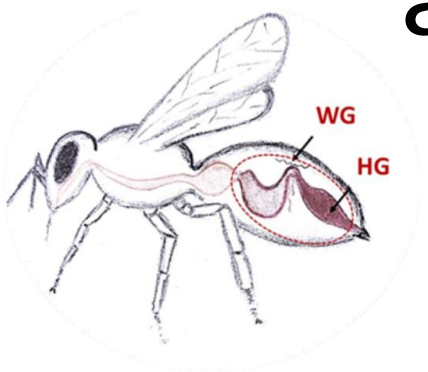
The advent of next generation sequencing has paved the way to develop an extensive body of research on animal gut microbiome interactions, including their critical roles in immunity, nutrition, and reproduction. With the high mortality observed among honey bee colonies in commercial beekeeping operations globally, honey bee health, particularly queen health is at the forefront of challenges facing apicultural industries worldwide. Queen honey bees are not living as long as previously and are failing at unprecedented rates. The causes of queen failure are often unclear but can result from a combination of factors including poor mating, viruses and pathogens, temperature stress, and poor nutrition. The gut microbiome of queen honey bees may shed some light on potential environmental stressors impacting queen health, life span, immunity, and reproduction. Characterizing the gut microbiome of honey bees to date has been destructive, where evaluated queens are killed and therefore cannot be subsequently used to head colonies. In this study, we developed a non-destructive method to characterize queen honey bee gut microbiomes by sampling queen feces. In addition to presenting a detailed protocol for collecting feces from queen honey bees, we present data to demonstrate the potential use of queen feces 1) as a proxy for gut microbiome characterization, 2) to signal possible queen exposure to temperature stress, and 3) to screen for honey bee viruses. This method provides a novel opportunity to screen for viruses and gut parasites, and to begin characterizing the gut microbiome of individual queens to better understand how the queen gut microbiome responds to environmental stressors and their downstream impacts on queen health and fertility. Finally, this non-destructive method will help to evaluate queen health prior to colony installation, importation and/or exportation where international trade is limited by the risk of pathogen spread.

A Novel Non-invasive Method to Characterize the Gut Microbiome and Viral Profile of Honey Bee Queens



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HONEY BEE HEALTH RESEARCH

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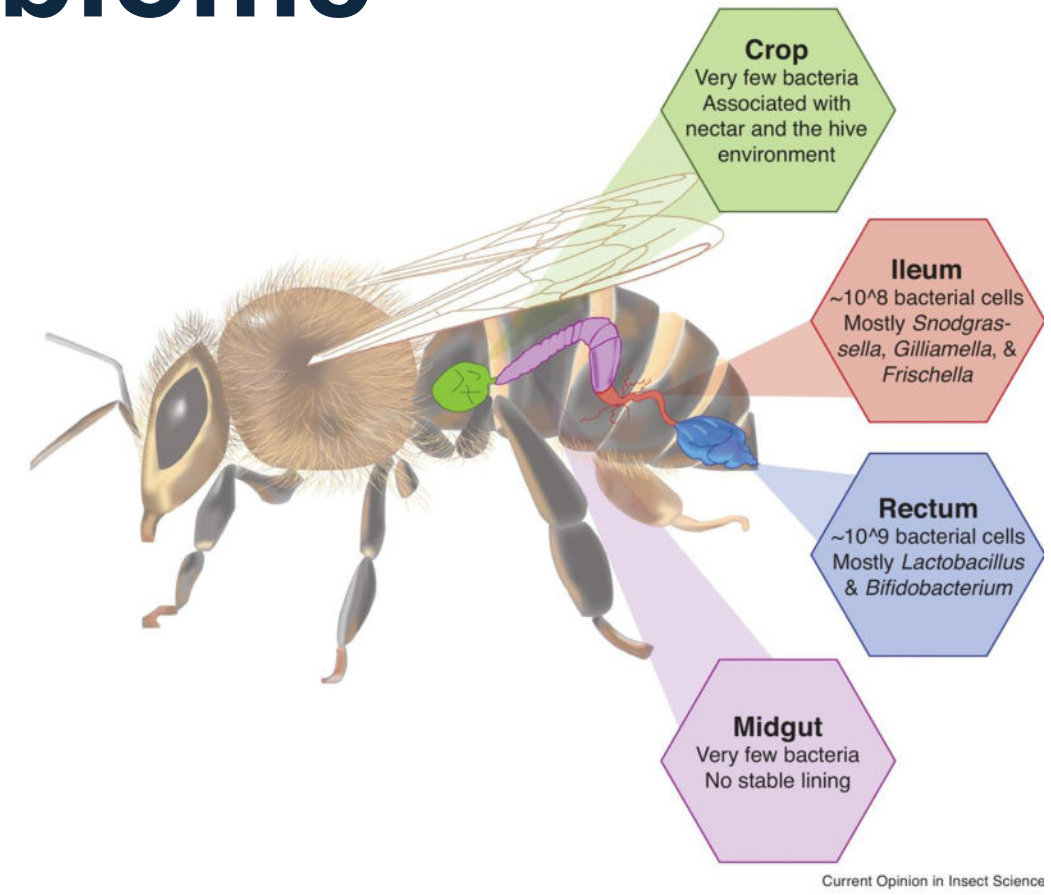
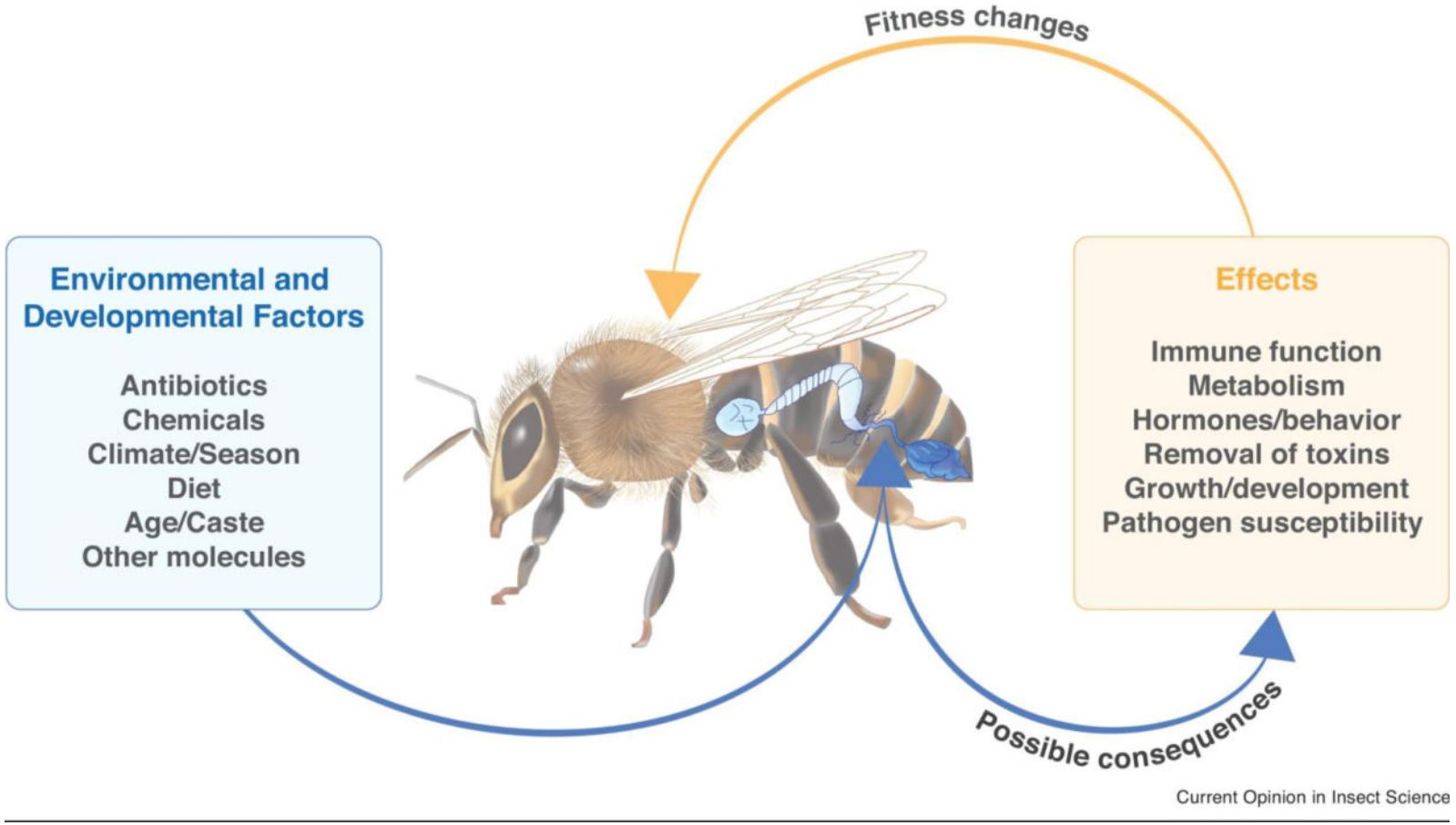
Honey Bee Gut Microbiome

- Simple microbial community
- Highly specialized and conserved
- Responds to environmental and internal factors



Raymann and Moran, 2018

Honey Bee Worker Gut Microbiome



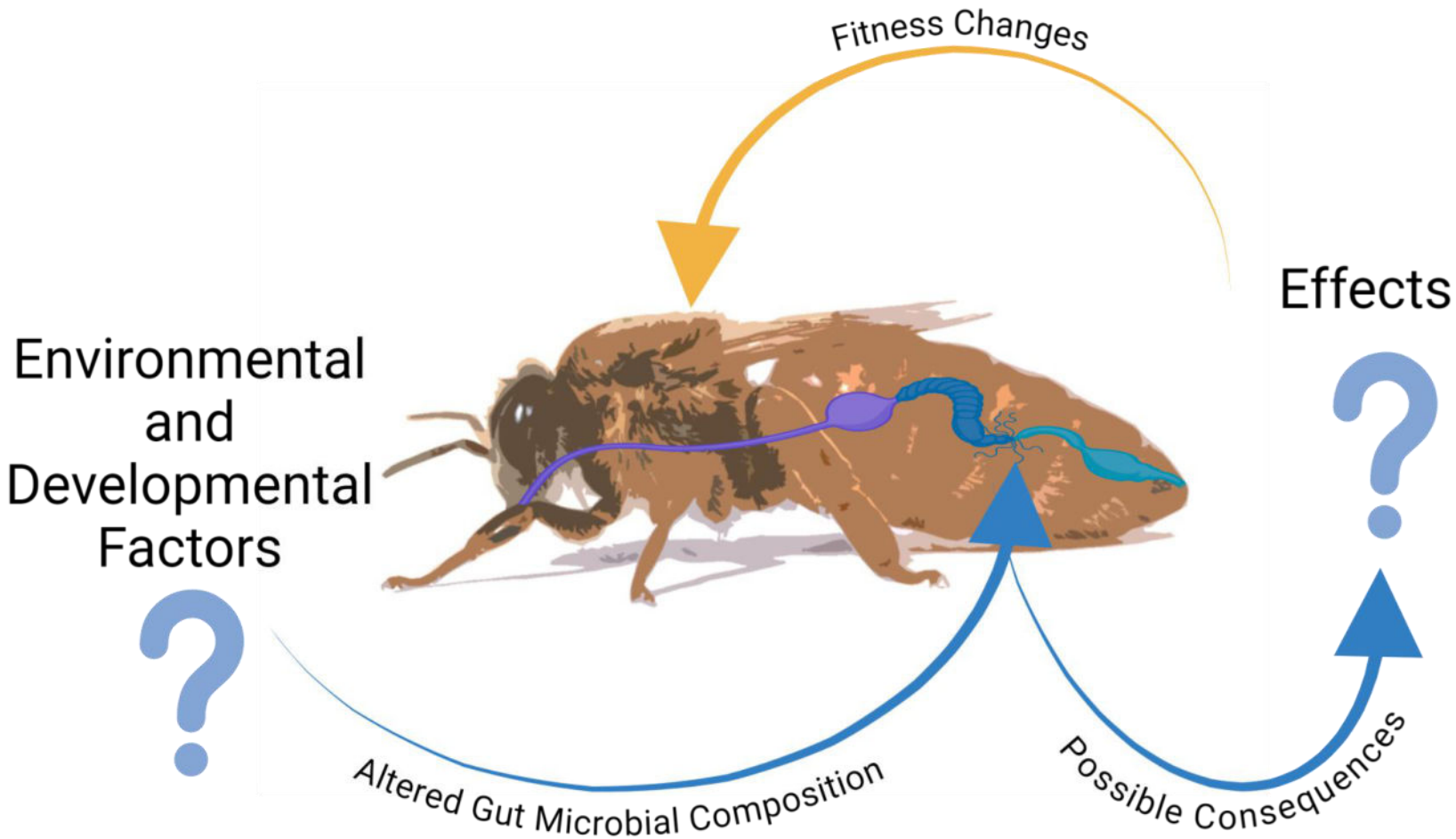
Poster: PP-163 Miriam Bixby

Bee Gut Microbiome Profiling for Diagnosing Neonicotinoid Exposure and Modeling Effects on Colony Productivity and Beekeeper Profitability

Queen Gut Microbiome

Is quite different from worker and drone gut microbiomes

Honey Bee Queen Gut Microbiome



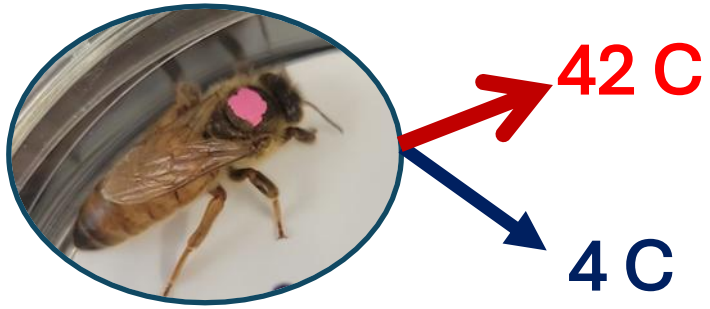
Adapted from Raymann and Moran, 2018; Created in BioRender Holmes, L. (2024)

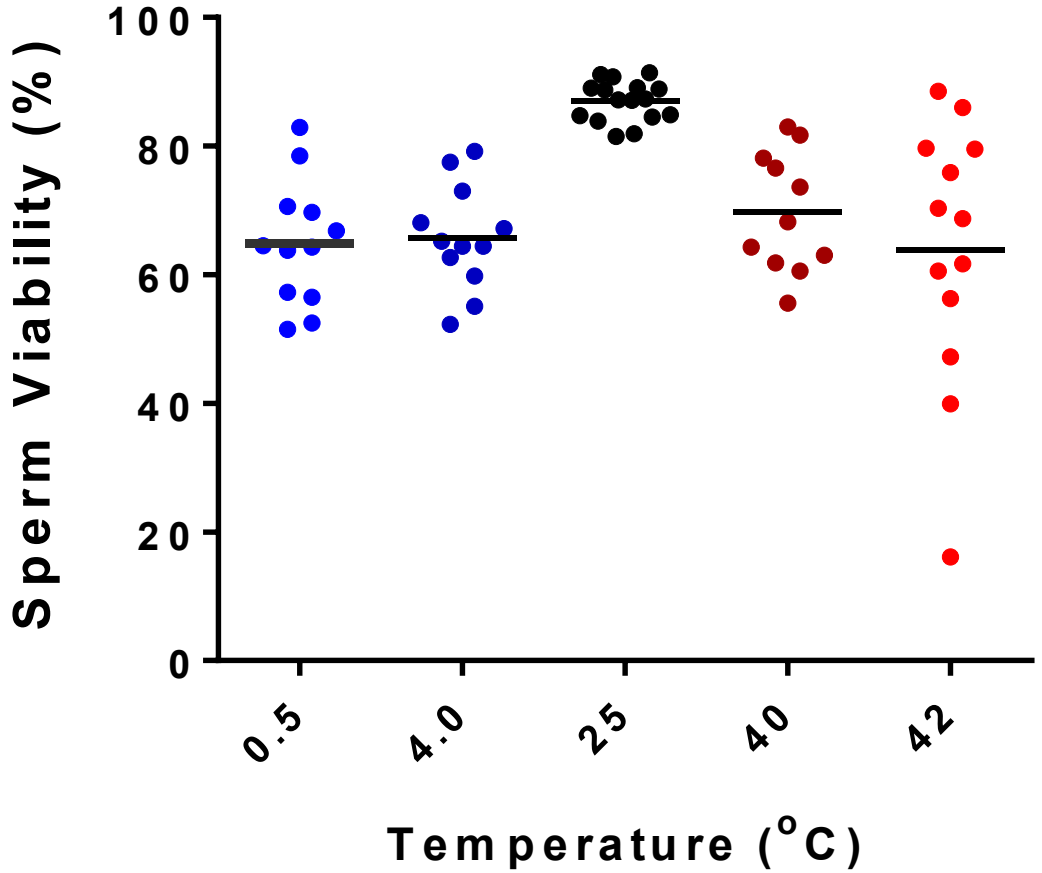
Temperature Stress on Queen Fertility

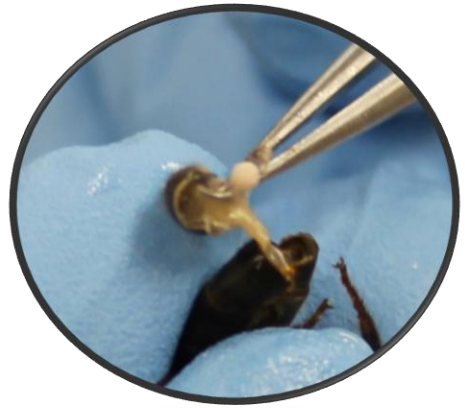
- Exposure to high and low temperatures during shipping.
- Exposure to 40-42˚C or 0-10˚C reduces sperm viability.

Pettis *et al*. 2016, Rousseau *et al*. 2020, McAfee *et al*. 2020. Holmes *et al.* 2023

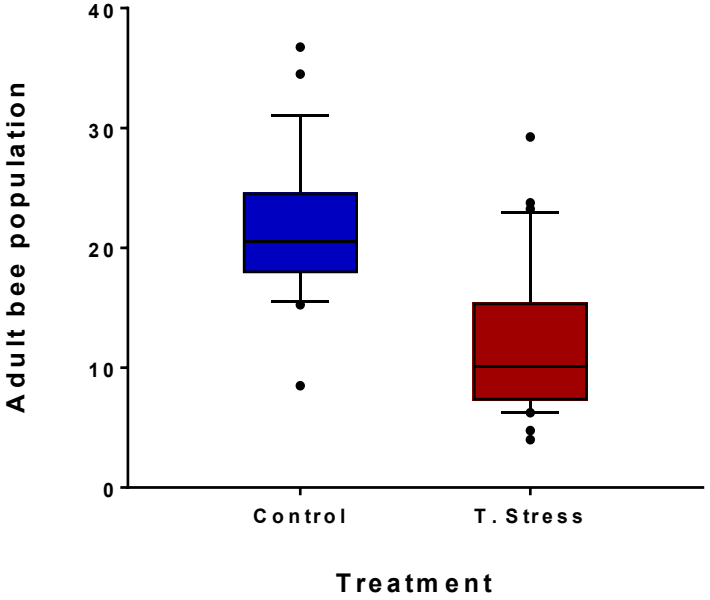
Viability of queen's stored sperm decreases with temperature stress

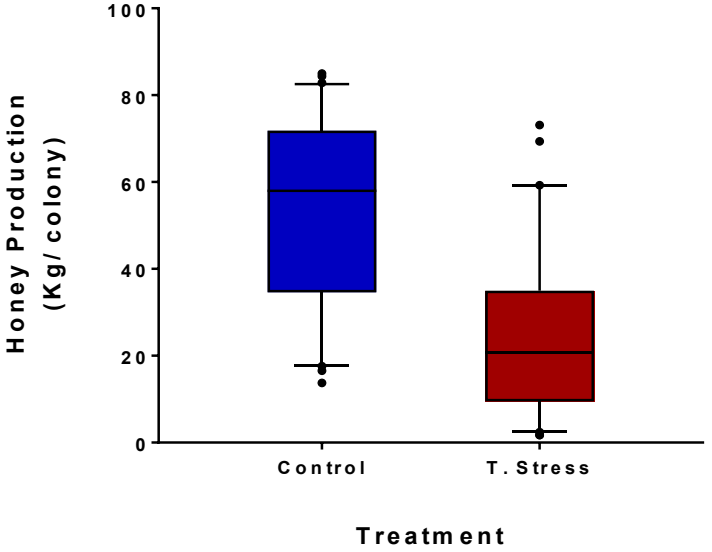


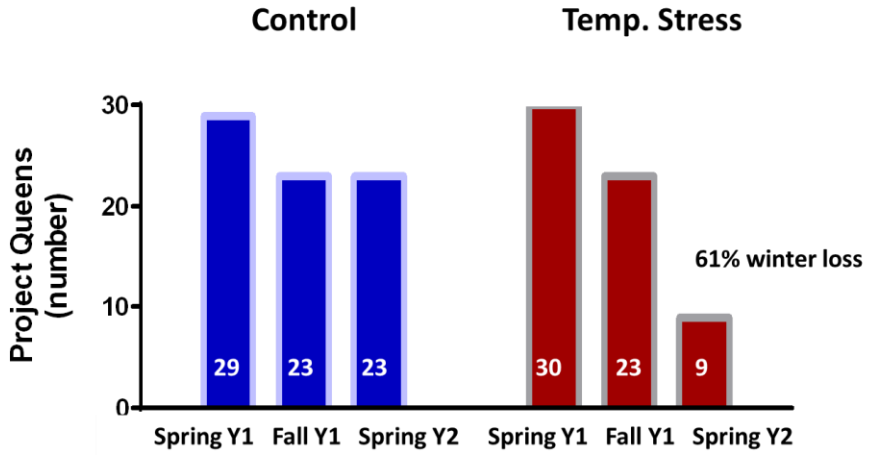




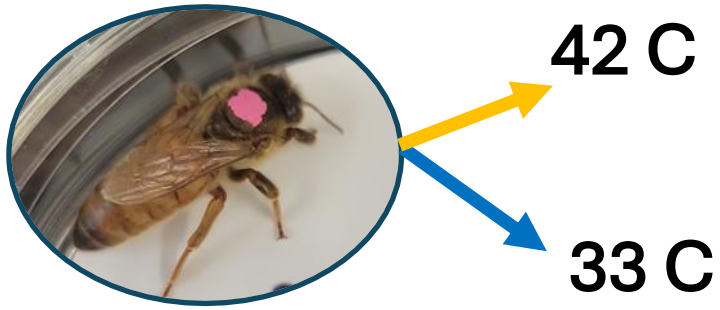
Temperature stress decreases colony productivity & survival

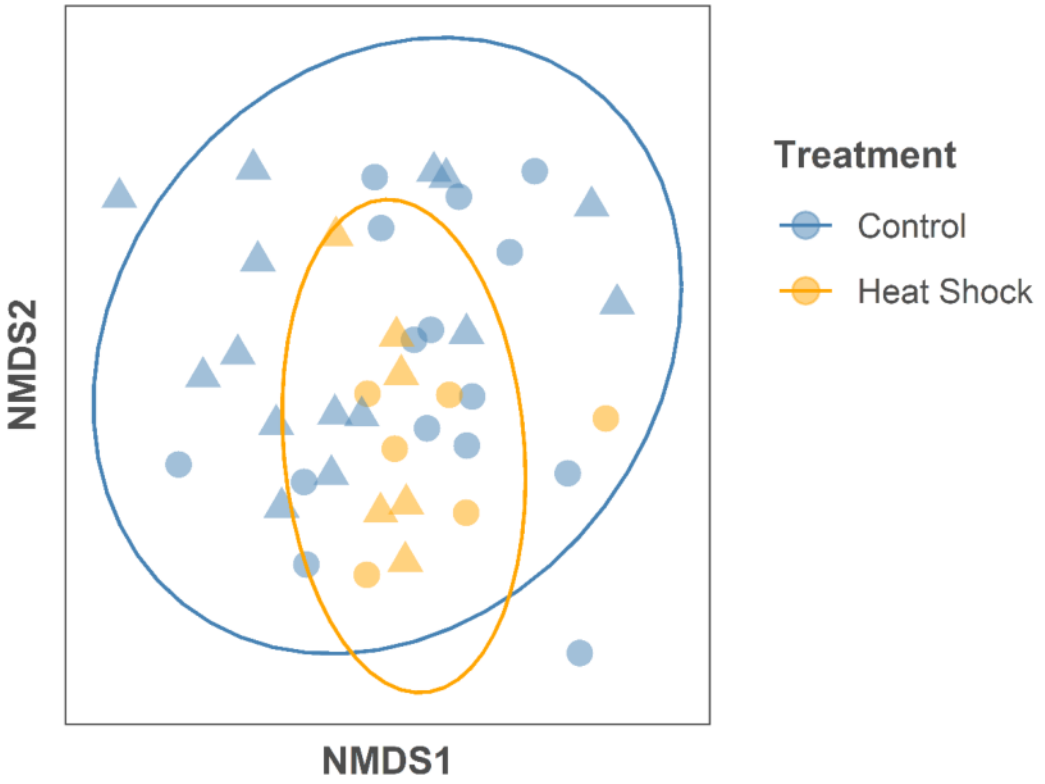






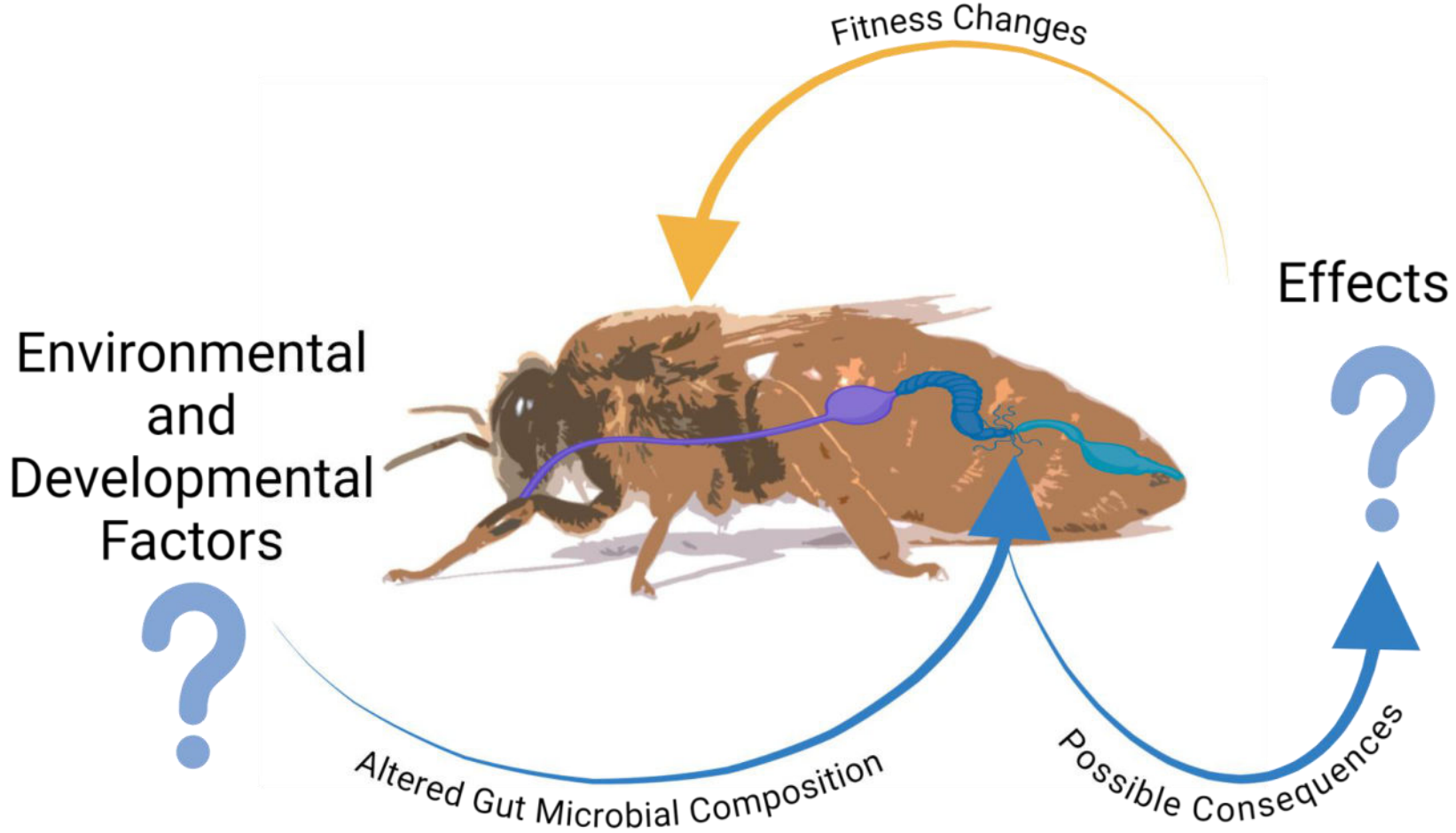
Honey bee queen gut microbiome responds to temperature stress





Honey Bee Queen Gut Microbiome

Challenge: Destructive analysis



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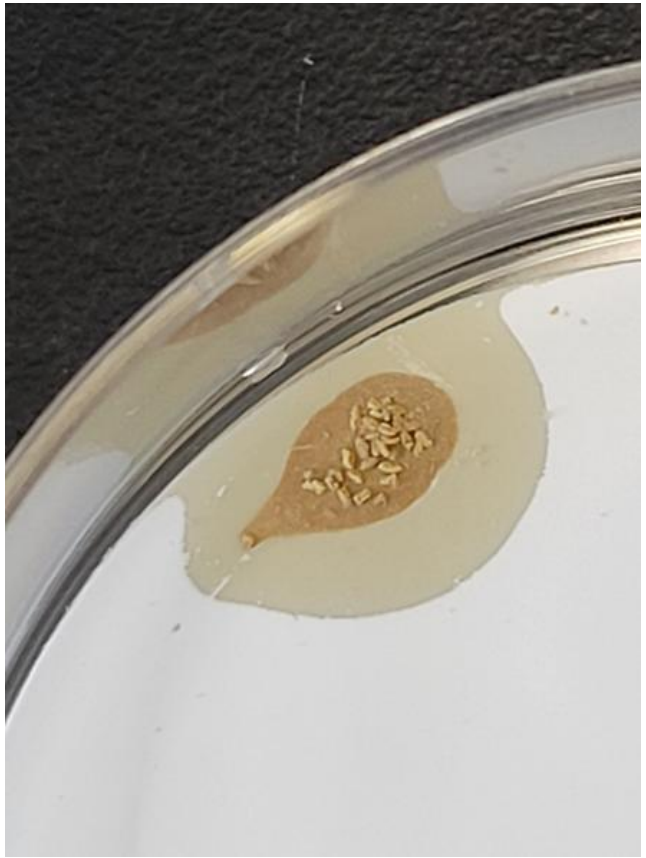
Fecal samples - non destructive alternative?



Using Queen Feces

- Test whether high quality DNA could be reliable obtained for sequencing and microbiome analysis.
- Determine whether queen feces are representative of her gut microbiome.

Collecting Feces from Queens

Detailed Methods

A non-invasive method for profiling the gut microbiome and virome of honey bee queens

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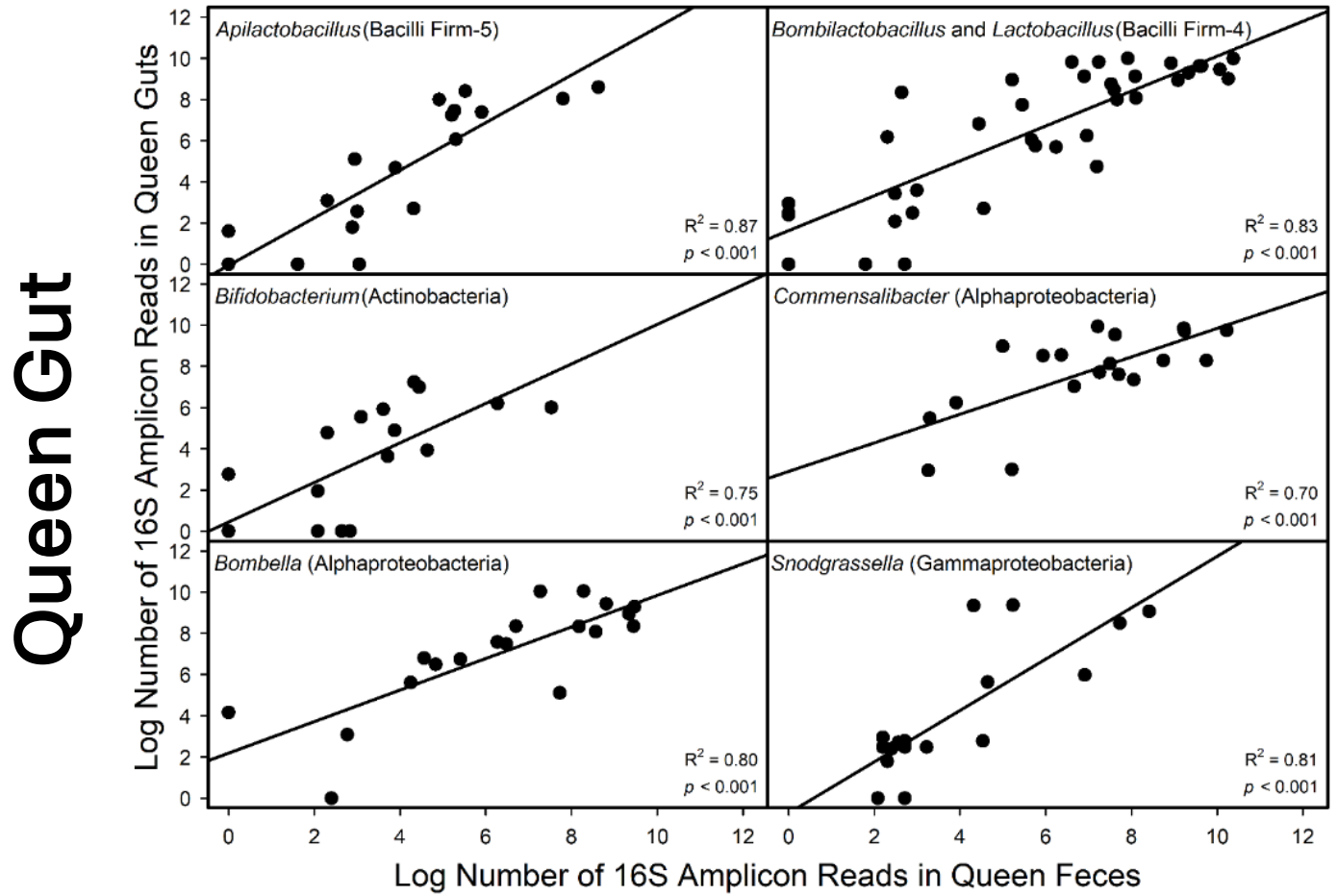
doi: https://doi.org/10.1101/2025.04.15.648983

Are Queen Feces a Good Proxy for Queen Gut Microbiome?



Holmes et al (2025)
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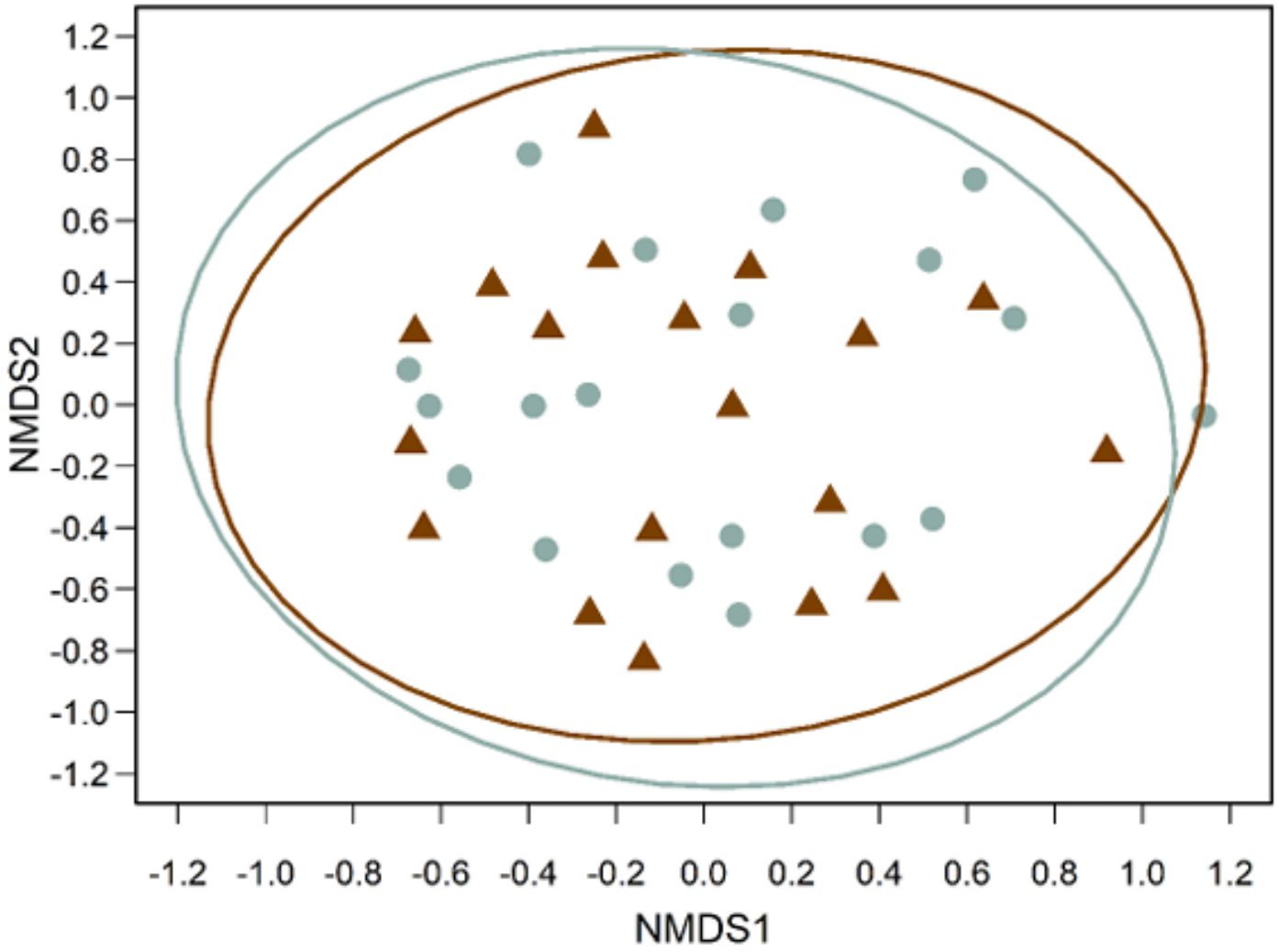
Feces and Gut Microbiomes Share Similar Bacterial Compositions



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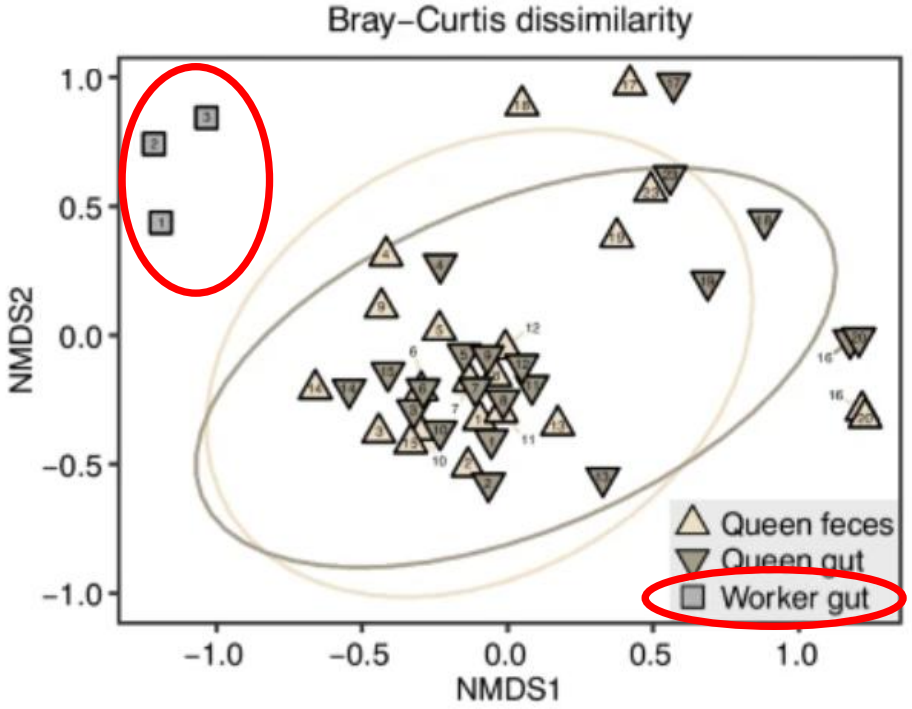
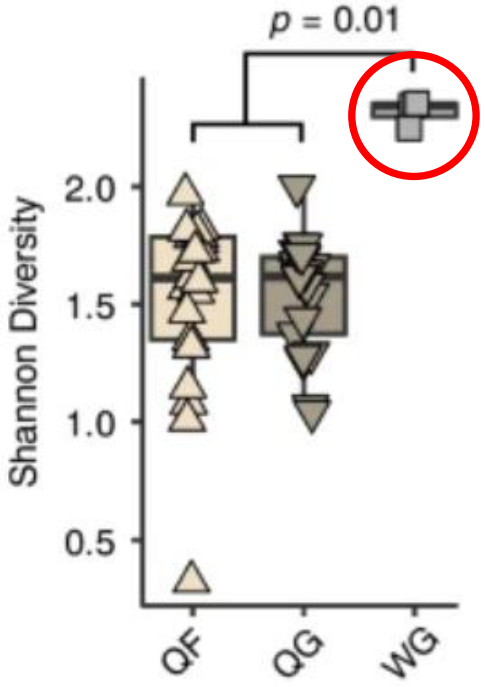
Similarity of Feces and Gut Microbiome Confirmed

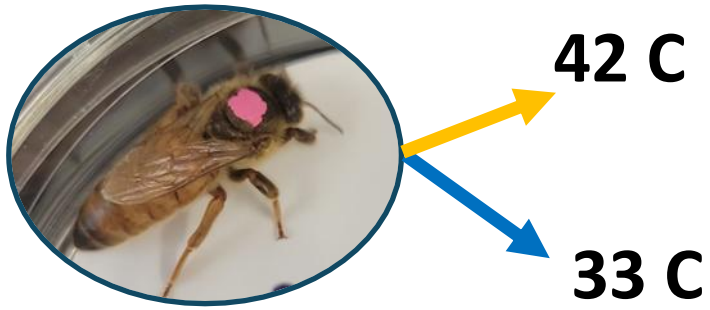


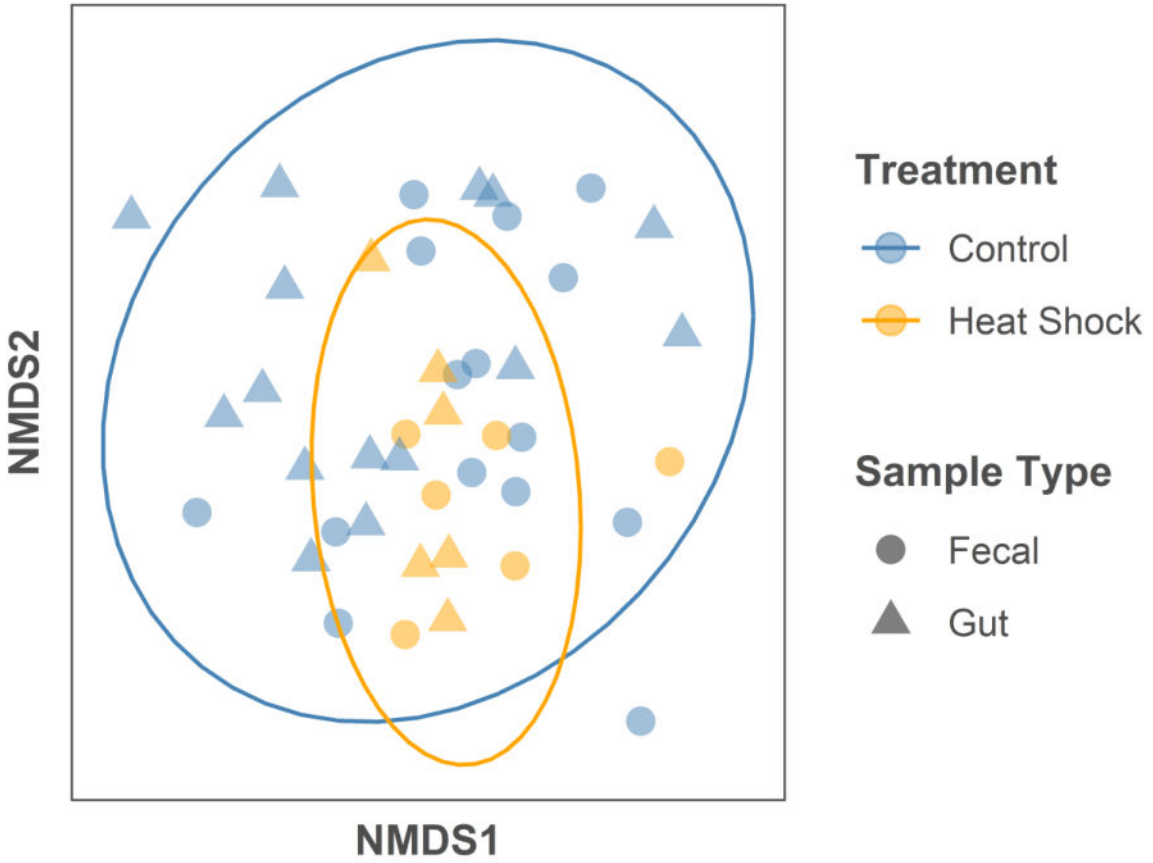


From Erick Motta *et al (2025)*.
https://doi.org/10.1007/s13592-025-01212-w

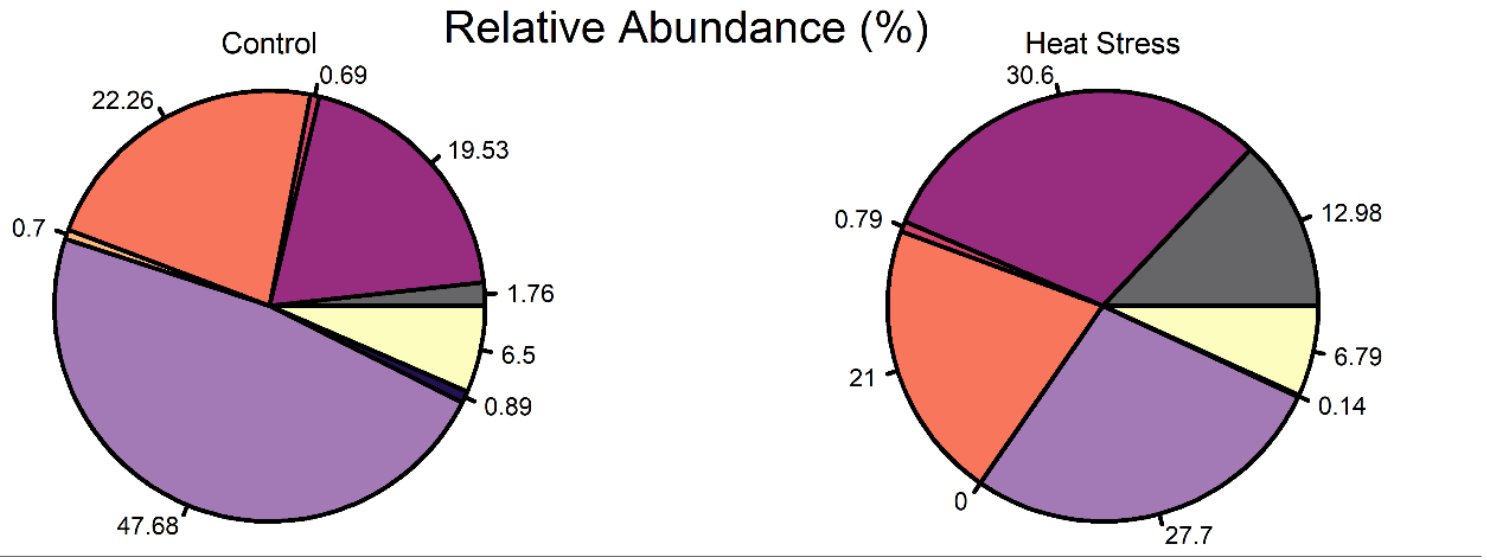
Using fecal microbiome to detect exposure to stress and infection

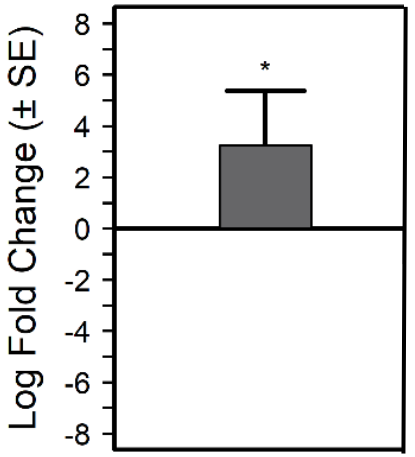
Honey bee queen gut dysbiosis in response to temperature stress





Using feces to study queen gut dysbiosis in response to temperature stress



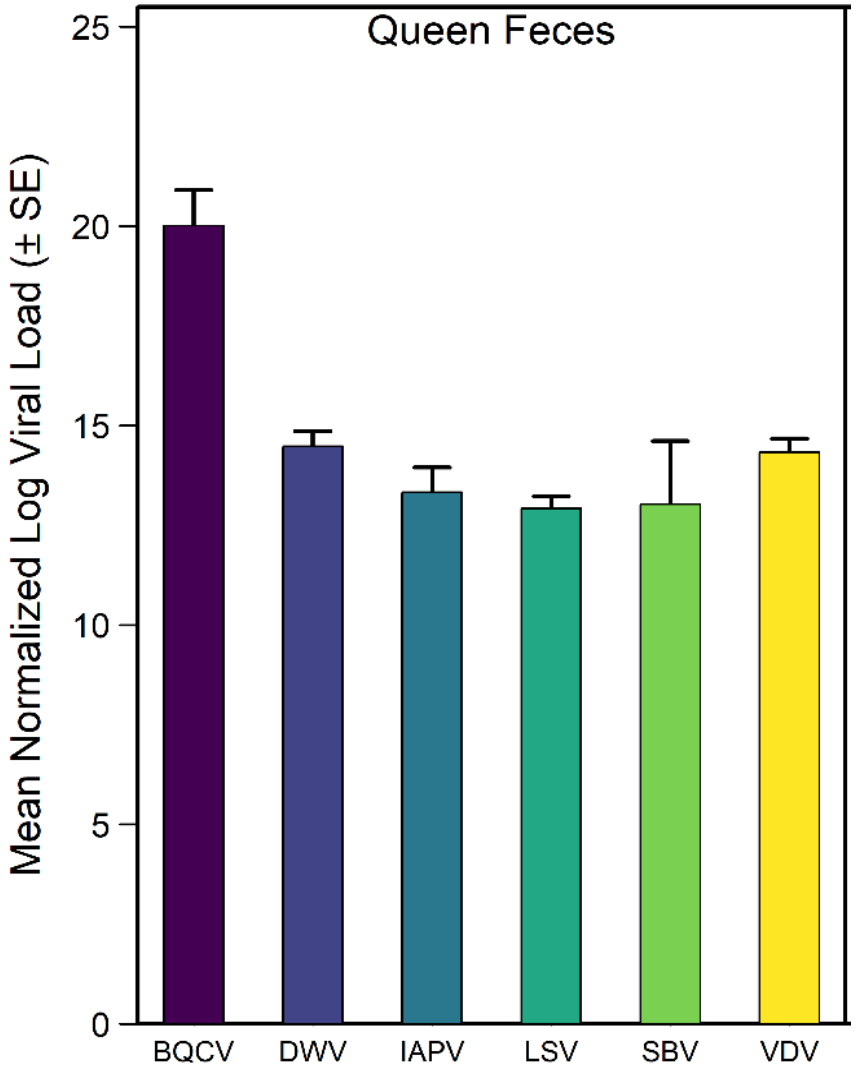


Apilactobacillus
Bifidobacterium
Bombella
Bombilactobacillus
Commensalibacter
Gilliamella
Lactobacillus
Snodgrassella

Detecting pathogens in queen feces

Viruses

BQCV, DWV, IAPV, LSV, SBV, VDV



Conclusions – Fecal Sampling

- High quality bacterial DNA for microbiome analysis from feces.
- Feces are reliable proxy to characterize queen gut microbiomes.
- Temperature stress causes dysbiosis reflected in fecal samples.
- Viruses are detected in queen feces.
- Enables longitudinal microbiome sampling from individual queens.

Thank You!

University of Lethbridge
Jeff Kearns
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Kelsey Gourlie B.Sc.H.

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Carissa Rondeau
Alison Hancharuk
Dawn Laviolette
Elena Battle
Sidney Ferrel

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Driving and supporting science to deliver innovation for healthy bees and bee businesses.

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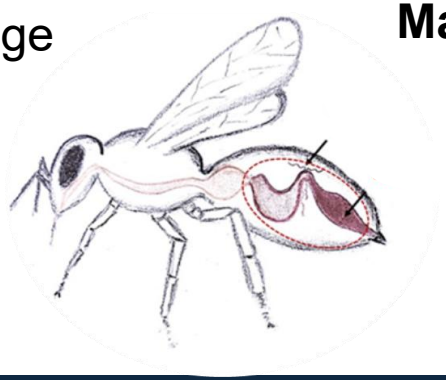

Honey Bee Queens

Poor queens is cited as a top contributing factor to **colony loss** in Canada.

- Beekeepers report replacing half their queens annually.

Canada **imports over 260,000 queens / year**

- Primarily from Hawaii, California, New Zealand, Australia, and Chile.