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PP-177 [Bee Health]

The importance of gut flora in the overwintering process for Swedish honey bees (*Apis mellifera*) - effects on bee health?

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Introduction

Winter mortality is one of the biggest challenges for beekeepers, with significant economic and ecological consequences. Most Swedish beekeepers overwinter their colonies on sugar water to maximize honey harvests. However, scientific studies on how this practice affects bee health are rare. Does the winter diet 1) influence the microbiome and 2) affect the ability to survive the long Swedish winter?

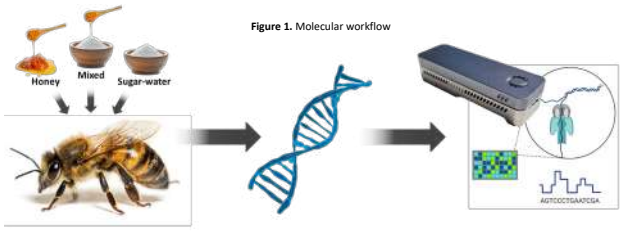

Figure 1. Molecular workflow

Samples
Collection of intestines under sterile conditions from honeybees fed on 1) honey, 2) sugar-water and 3) mixed diet

DNA extraction and PCR amplification
DNA were extracted using the ZymoBIOMICS™ DNA Miniprep kit (Zymo Research) and the 16S gene were amplified using the 16S barcoding kit 24 v14 (Oxford Nanopore Technologies)

DNA sequencing
50 ng of pooled DNA from 12 samples were run for 8 h on a Flongle flow cell in a MinION Mk1B (Oxford Nanopore Technologies) and sequence data were analyzed to the bacteria genus level using EPI2ME software (Oxford Nanopore Technologies)

Figure 2 Dissected bee gut
A) foregut
B) hindgut divided in ileum and rectum

Material & Methods

- A bee yard in Uddevalla, Sweden was sampled on March 4, 2025, after five months of overwintering. 25 bees were collected from each colony. Three dietary groups (Fig. 1) were represented: 1) honey fed, 2) sugar-water fed and 3) mixed fed.
- In total, 9 colonies and 225 bees are under analysis. Preliminary data are presented here (n = 36).
- Each bee gut was dissected under sterile conditions (Fig. 2).
- The rectum was analyzed with molecular tools (Fig. 1).

- Gut community compositions was modeled to estimate diet-specific mean compositions 1) within-diet concentration (φ; higher = tighter) and 2) with a weakly informative prior on log φ (Normal(4.8, 0.6) (Fig. 3-5)).
- Inference via MCMC in brms/rstan.
- As an exploratory complement Bayesian model-based clustering was performed (Fig. 6).

Preliminary results (n = 36, 12 individuals per diet-group)

- ❖ The winter diet strongly influences the bacterial taxonomic composition in Swedish honeybees.
- ❖ Colonies on a sugar diet exhibit the most distinct bacterial profiles (Fig. 5), accompanied by a significant reduction in within-group variability (Fig. 4).
- ❖ The mixed diet results in a bacteria composition that is closer to a honey diet (Fig. 6), but not always (e.g., in Fig. 3 for Enterococcus).
- ❖ The honey diet has the largest variability in the gut bacterial communities (Fig. 4).

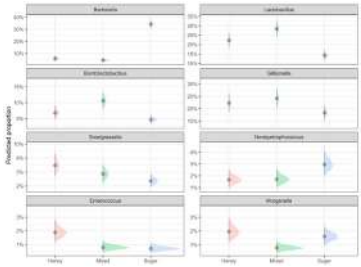

Figure 3. Posterior distributions of predicted taxon proportions (Top-8). Half-violins show posterior distributions per diet; points mark medians and bars indicate 95% credible intervals. Panels display the 8 taxa with the largest between-diet differences in posterior median proportion (remaining taxa not shown). Predictions come from a Bayesian Dirichlet regression fit to all taxa with diet as predictor and diet-specific concentration (ϕ).

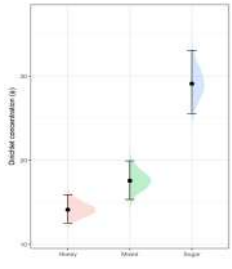

Figure 4. Dirichlet concentration (ϕ) by diet — higher = tighter communities. Half-violins show posterior distributions of ϕ from the Bayesian Dirichlet regression (fit to all taxa with diet-specific ϕ); points mark medians and bars indicate 95% credible intervals. Larger ϕ implies lower within-diet variability in community composition. Sugar shows the highest ϕ (i.e., the lowest within-diet variability).

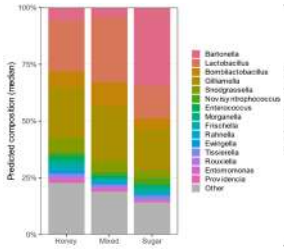

Figure 5. Predicted community composition (posterior medians). Stacked bars show median predicted proportions from the Bayesian Dirichlet regression (fit to all taxa with diet-specific concentration, ϕ). Displayed are the Top-15 taxa with the largest between-diet differences; all remaining taxa are grouped as "Other." Colors indicate taxa.

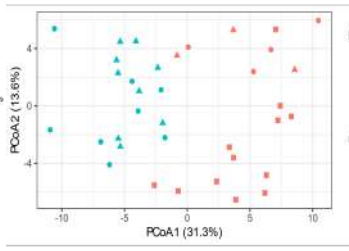

Figure 6. Principal coordinates analysis (PCoA) of Aitchison distances. Points are samples; color = model-based cluster (Gaussian mixture in ILR space; BIC-selected VVI, K=2), shape = diet. Axes show the variance captured by the first two principal coordinates; the plot is for visualization only—clustering was performed in the full ILR space.

Figure 7. Brood frame during spring. Spring development of analysed colonies was monitored from April-June 2025. All frames were photographed, and each brood cell marked and counted (right). Spring development will be combined with the microbiome results and tested for possible correlations.

Conclusions

Winter diet seems to strongly structure the honeybee gut community.

Two microbiome community types were identified: sugar mapped almost exclusively to Cluster 1, whereas honey/mixed predominantly fell into Cluster 2. Thus, a distinct sugar-associated community type seems to be exist, while mixed foder - an alternative for beekeepers - resembles the honey fed community.

We are currently evaluating the microbiome results of a larger data set in which we also tracked the spring development and survival of the different colonies from April-June 2025 (Fig. 7).

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PP-177 [Bee Health]

The importance of gut flora in the overwintering process for Swedish honey bees (Apis mellifera) – effects on bee health?

Sonja Leidenberger, Niclas Norrström, Erik Gustafsson
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Winter mortality of honeybees (Apis mellifera) is one of the biggest challenges in beekeeping, with significant economic and ecological consequences. Although most Swedish beekeepers overwinter their bees on sugar water to maximize honey harvest, there is a lack of scientific studies investigating how this practice affects bee health compared to overwintering on their own honey. Today, it remains unclear how different overwintering diets influence the gut microbiota of honeybees and their ability to survive the long Swedish winter. In general, scientific studies on winter diets are rare, especially those focusing on the microbiome and subsequent spring survival. In this study, we investigated how the overwintering diet affects the gut microbiota as well as the health and survival of the Swedish honeybees. We 1) analyzed the gut microbiome of Swedish honeybees (Apis mellifera) colonies using third-generation sequencing (Oxford Nanopore Technologies), 2) compared the bacterial composition and relative abundance across the three different diets (honey, sugar, honey-sugar mixture) over one winter season (October 2024 to March 2025) and 3) examined whether the overwintering diets affect the bees´ health condition at the beginning of the new season. To assess the bee health, we focused on visible signs of diarrhea and monitored colony development during the spring, including brood and drone development as well as their pollen collection (April to June 2025). Our first preliminary results showed that the microbiome differs depending on the food the honeybees have eaten during overwintering. In addition to characterizing data on the gut microbiome of the Swedish honeybees, this study aims to identify the optimal winter diet to support successful overwintering. Winter mortality is costly, both for the professional beekeeper and the recreational beekeeper, and even a delayed spring start can negatively impact colony productivity. That is why we also analyzed the mixed diet - consisting of honey and sugar - as an interesting alternative, which may help future beekeepers to cultivate honeybee colonies with a healthier and more diverse microbiome flora, which can support the bees' fitness and resilience at the onset of the new season.