



UNIVERSITÀ  
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## Gut microbiome composition and hygienic behavior as indicators of varroa resistance in an Italian honey bee breeding population

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### Background

Honey bee colonies face multiple threats, with *Varroa destructor* among the most serious. Selective breeding for hygienic behavior, enabling bees to detect and remove infested brood, is a key strategy to enhance resistance. Recent research also highlights the gut microbiota's role in bee health and resilience.

This study aimed to investigate the relationship between gut microbiome composition and hygienic behavior in an Italian honey bees population selected since 2015 for docility, productivity, and hygiene. By integrating microbiome profiling and behavioral testing, we explored microbial patterns associated with resistance traits.

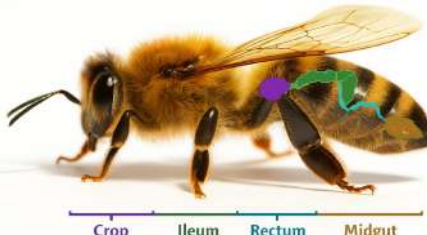


Figure 1. Honey bee gut.

### Materials and Methods



Figure 2. Pin test for hygienic behavior.

Seventy-seven colonies from a breeding population were sampled three times between June and October 2021. For each colony, ten worker bee guts were dissected, pooled, and used for 16S rRNA sequencing (V3–V4 region) via Illumina technology.

Bioinformatic analyses were performed in R, with taxonomic assignment using the SILVA database.

Hygienic behavior was assessed using the Pin Test, where 50 capped brood cells were pierced with a needle and the removal rate after 24h was recorded (Figure 2). The test was repeated twice, and scores were averaged.

### Results

A clear, season-dependent association between gut microbiota diversity and hygienic behavior was observed. Colonies classified as highly hygienic (hpt) exhibited increased  $\alpha$ -diversity, as shown in Figure 3. This trend was particularly evident in October, when varroa destructor pressure typically peaks.

From the differential abundance analysis, 64 ASVs were identified as significantly more abundant in the high-hygiene group, as shown in yellow and red in Figure 4.

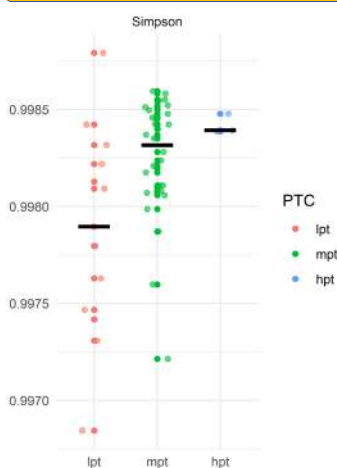


Figure 3.  $\alpha$ -diversity by hygienic group.

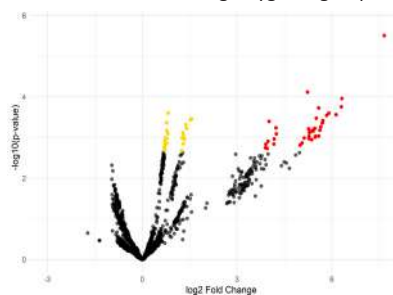


Figure 4. Differential abundance of bacterial taxa.

These ASVs belonged to beneficial bacterial genera, including *Bifidobacterium* and *Lactobacillus*, which are known for their immunomodulatory roles and may contribute to enhanced colony resilience and pathogen defense.

### Conclusions

Our results suggest that a beneficial gut microbiota may support honey bee immune function and enhance hygienic behavior, contributing to greater colony resilience. Understanding microbiome–behavior interactions provides valuable insights for bee health management and highlights the potential of probiotic-based approaches to improve honey bee health and varroa resistance.

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## **Gut microbiome composition and hygienic behavior as indicators of varroa resistance in an Italian honey bee breeding population**

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Honey bees are essential pollinators critical to maintaining biodiversity and agricultural productivity globally, contributing significantly to food security and ecosystem stability. However, honey bee colonies are facing numerous challenges, among which the Varroa destructor mite is one of the most significant threats. Selective breeding programs aimed at enhancing hygienic behavior, a trait that enables bees to detect and remove infected or infested brood, represent a pivotal strategy to improve colony health and resistance to this pest. Recently, attention has also been directed towards the potential role of gut microbiota in enhancing bee health and resilience. In the current research, we investigated the association between gut microbiome composition and hygienic behavior in a population of honey bees selectively bred since 2015 for docility, honey production, and hygienic behavior. A total of 77 colonies were sampled three times between June and October 2021, and hygienic behavior was assessed using the standardized pin test method in March and July. Microbial community diversity was characterized through high-throughput sequencing, and associations with hygienic performance were statistically analyzed. Our results demonstrated a clear but seasonally dependent relationship between microbiome diversity and hygienic behavior. Colonies exhibiting superior hygienic responses showed distinctive microbial community structures, particularly in October, after the varroa infestation typically peaks.  $\beta$ -diversity analyses confirmed significant differences in microbiota composition among colonies varying in hygienic ability. Notably, beneficial bacterial genera, such as Bifidobacterium and Lactobacillus, were enriched in colonies demonstrating high hygiene scores, suggesting their involvement in colony immune function and resistance mechanisms. These findings suggest beneficial gut microbiota may enhance honey bee immune function and hygienic efficiency, thus potentially contributing to improved colony resilience and reduced disease incidence. Clarifying these microbiome-behavior interactions provides valuable insights into bee health management and suggests novel approaches for incorporating microbiome manipulation into selective breeding programs. Consequently, this research opens avenues for future studies investigating probiotic-based approaches to improve honeybee health and varroa resistance.