

Exploring environmental influences on honey bee gut microbiota: a comparative analysis of *Apis mellifera ruttneri* and *Apis mellifera ligustica* across two climates

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The gut microbiota of honey bees plays a crucial role in their health, immunity, and adaptation to environmental conditions. Previous research suggests that *Apis mellifera ruttneri*, the endemic Maltese honey bee, harbours a higher proportion of environmental microbiota than other subspecies, such as *A. m. ligustica*. However, whether this difference is driven by genetic factors or environmental exposure remains unclear.

To investigate this, experimental apiaries were established in Malta (Mediterranean climate) and Italy (continental climate), each housing colonies of *A. m. ruttneri* and *A. m. ligustica* under identical environmental conditions. Worker bees were collected monthly for over a year, and their gut microbiota was analysed using Oxford Nanopore long-read 16S rRNA sequencing, alongside pollen samples to assess environmental microbial influences. Bioinformatics pipelines classified microbial taxa, and statistical analyses determined subspecies-specific microbiota differences.

Findings show that *A. m. ruttneri* harbours a higher prevalence of environment-associated bacteria, often found in soil, including Bartonellaceae, Apilactobacillus, Arsenophonus, Bombella, Commensalibacter, and Pseudomonas, whereas *A. m. ligustica* has a microbiota dominated by Lactobacillaceae (Lactobacillus and Bombilactobacillus). Despite sharing core microbial taxa, their relative abundances differed markedly. These preliminary results suggest that environmental factors play a dominant role in shaping honey bee gut microbiota, with *A. m. ruttneri* exhibiting microbial adaptations potentially linked to the harsh Maltese climate.

By incorporating multi-season sampling and genomic analyses, this study provides deeper insights into the interplay between genetic and environmental factors in shaping honey bee microbiota. Understanding these dynamics is crucial for honey bee conservation and subspecies management, as microbiota composition may influence resilience to pathogens, pesticides, and climate stressors.

For beekeepers, these findings highlight the importance of subspecies-specific microbiota dynamics in colony health and management. If *A. m. ruttneri* naturally integrates more environmental microbiota, this may enhance its resilience to local stressors, reinforcing the need to preserve locally adapted honey bee populations.

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Exploring environmental influences on honey bee gut microbiota:

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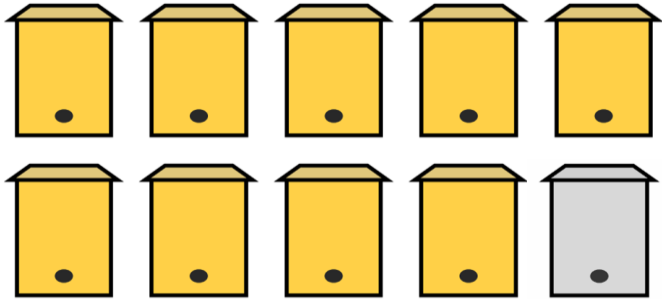


Honey Bee Health in Crisis

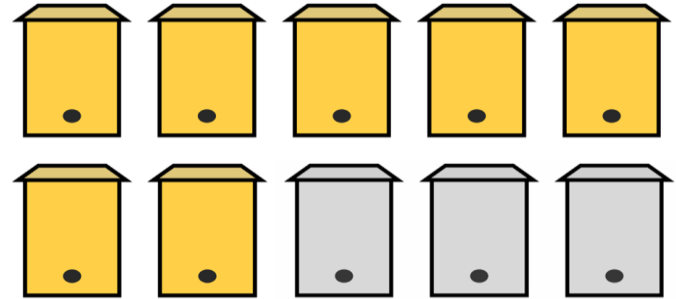
Surveys from this year indicate that commercial beekeepers in the U.S. have lost over **60% of their colonies** in a single season.

Honey Bee Health in Crisis

Pre 2006

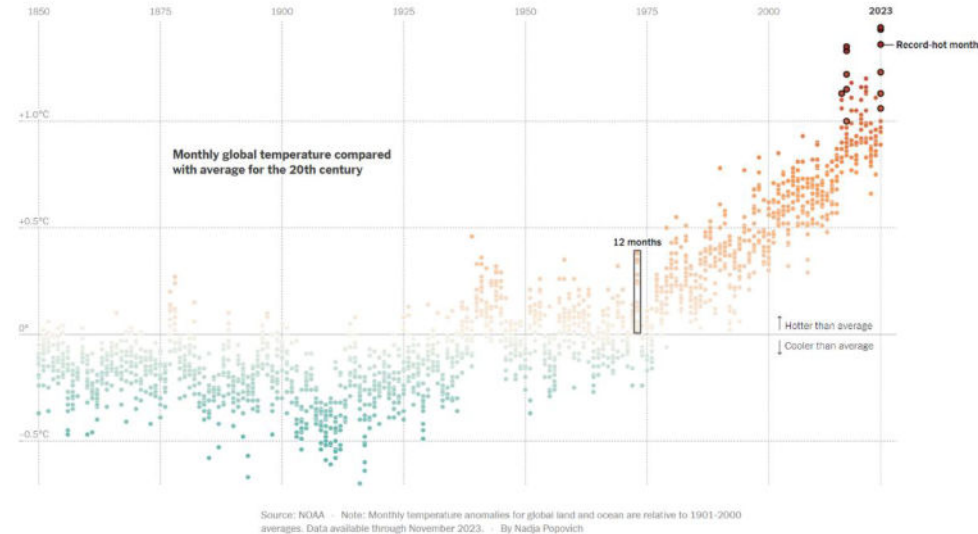


Post 2006



Climate Change: A Growing Challenge

Rapid Climate Shifts: Rising temperatures, prolonged droughts and unpredictable weather patterns are reshaping ecosystems.



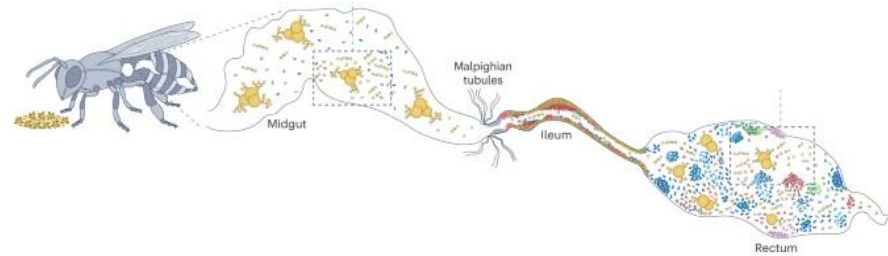
New York Times, 2024

How do we keep honey bees healthy in a changing world?

A Hidden Ally: Gut Microbiota

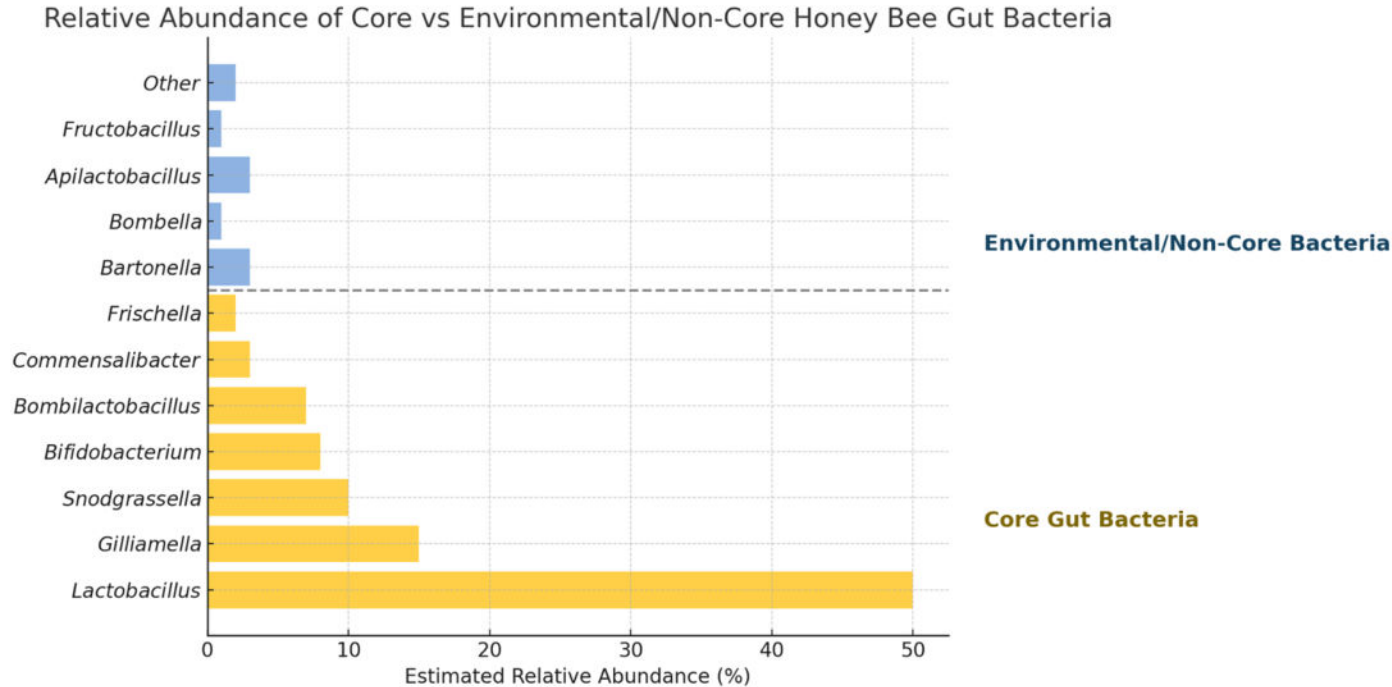
Health: Disruptions to microbial balance can increase susceptibility to diseases and impact honey bee vitality.

Resilience: Critical for resilience to environmental and pathogenic stressors.



Motta and Moran, 2024

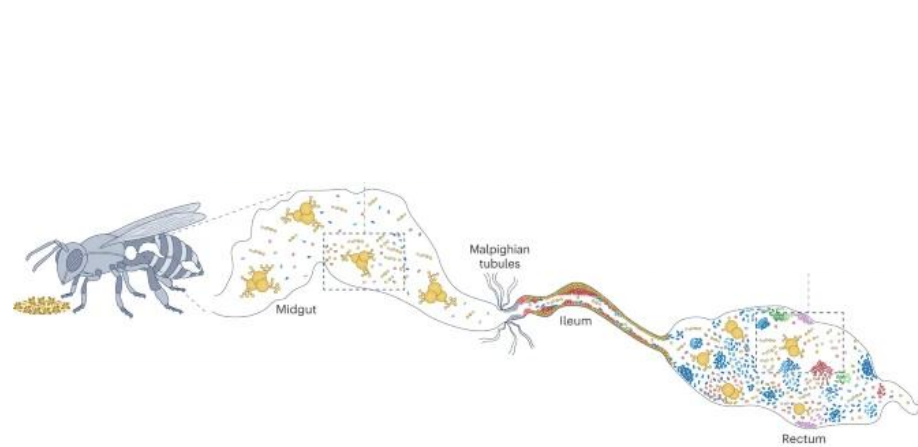
Specialised and Stable

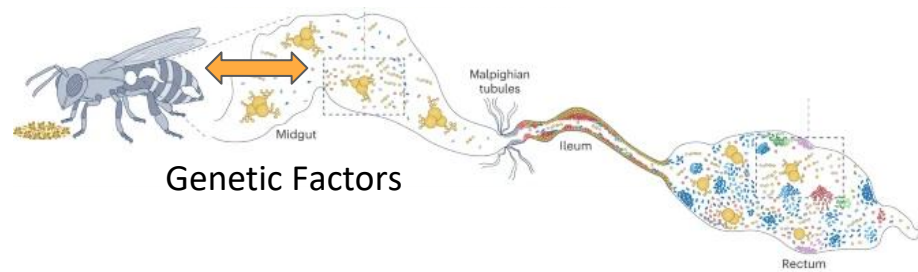


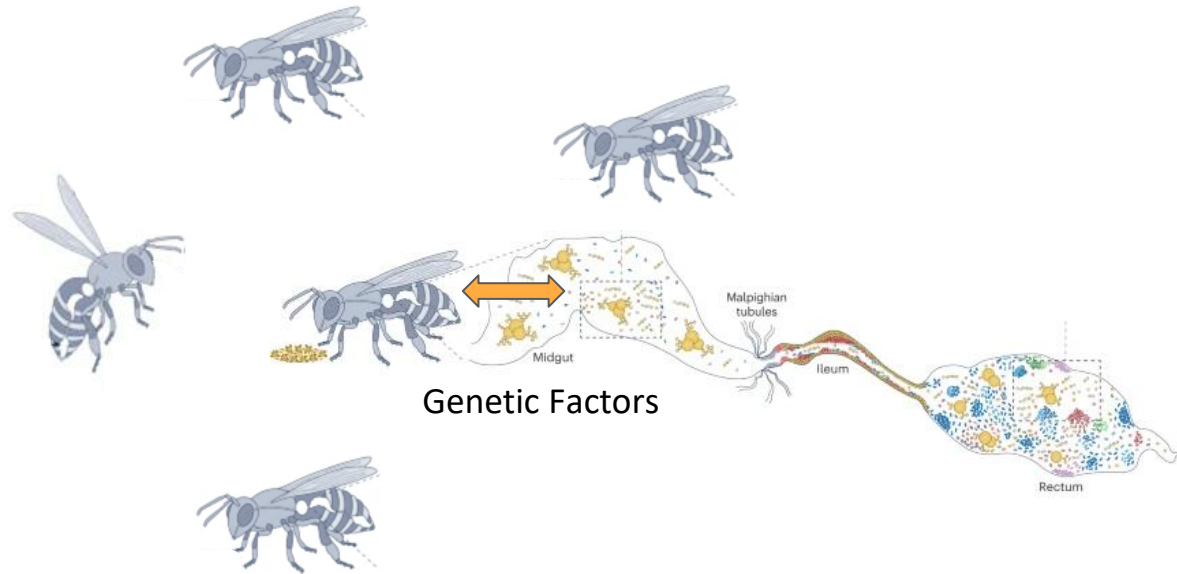
Environmental (non-core) genera, present in much lower abundance, but can increase with environmental stress or exposure to novel habitats.

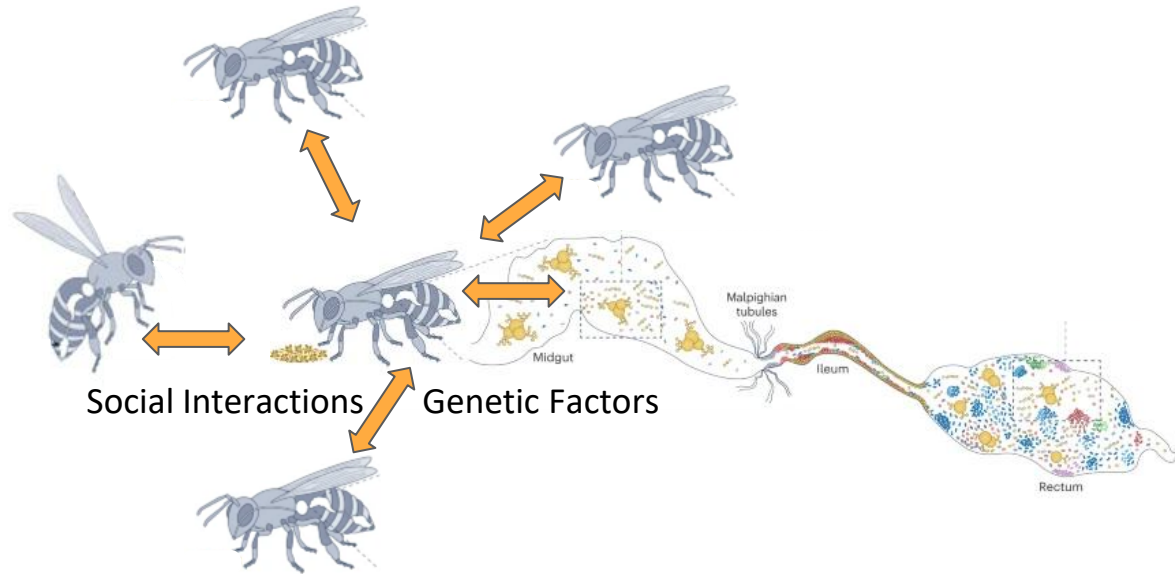
Core bacterial genera, specialised for life in the bee and making up the majority of the community.

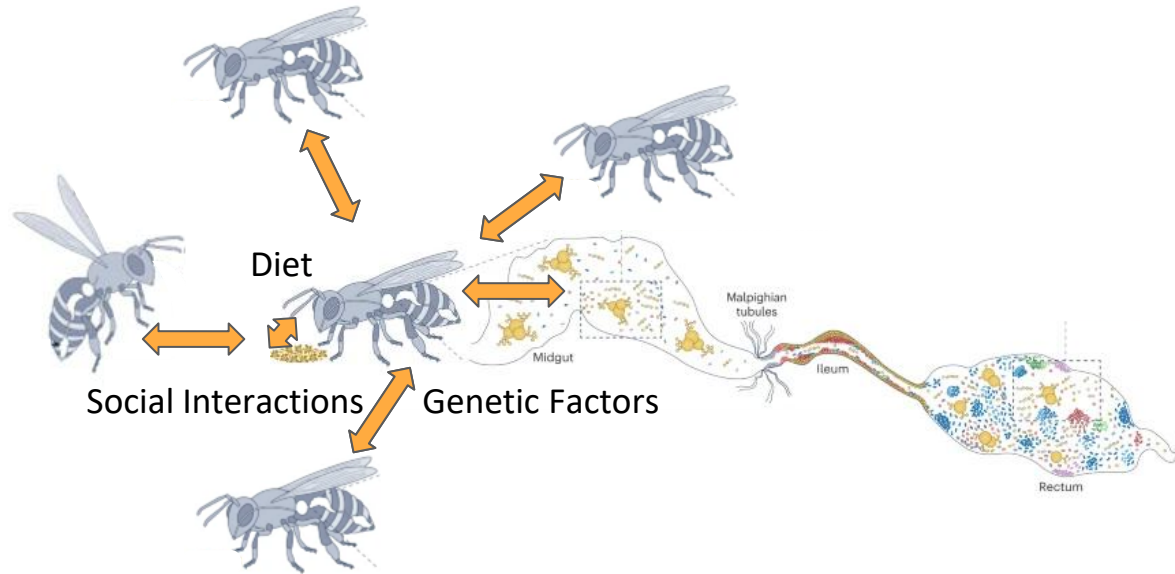
A Web of Interactions



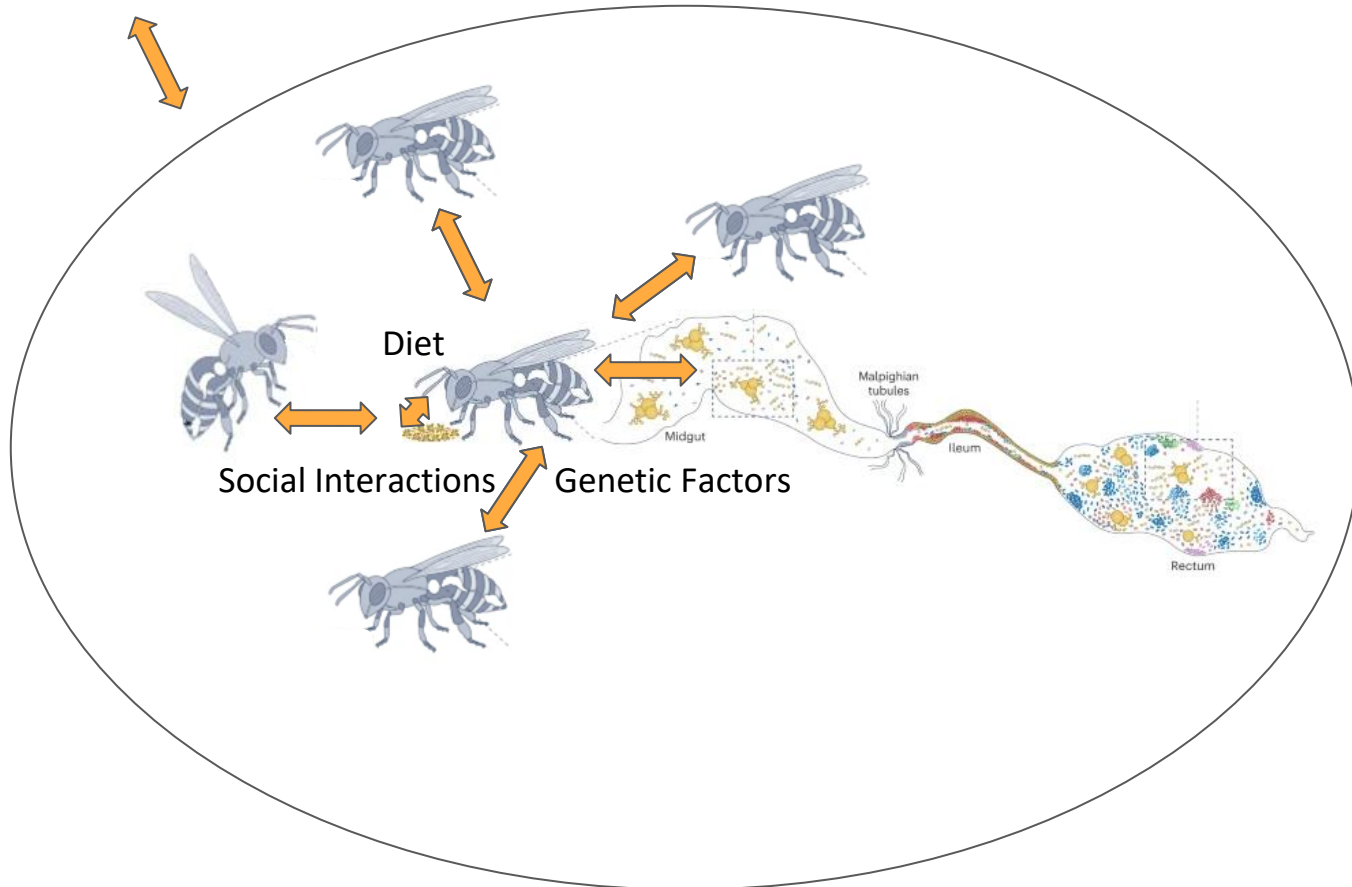




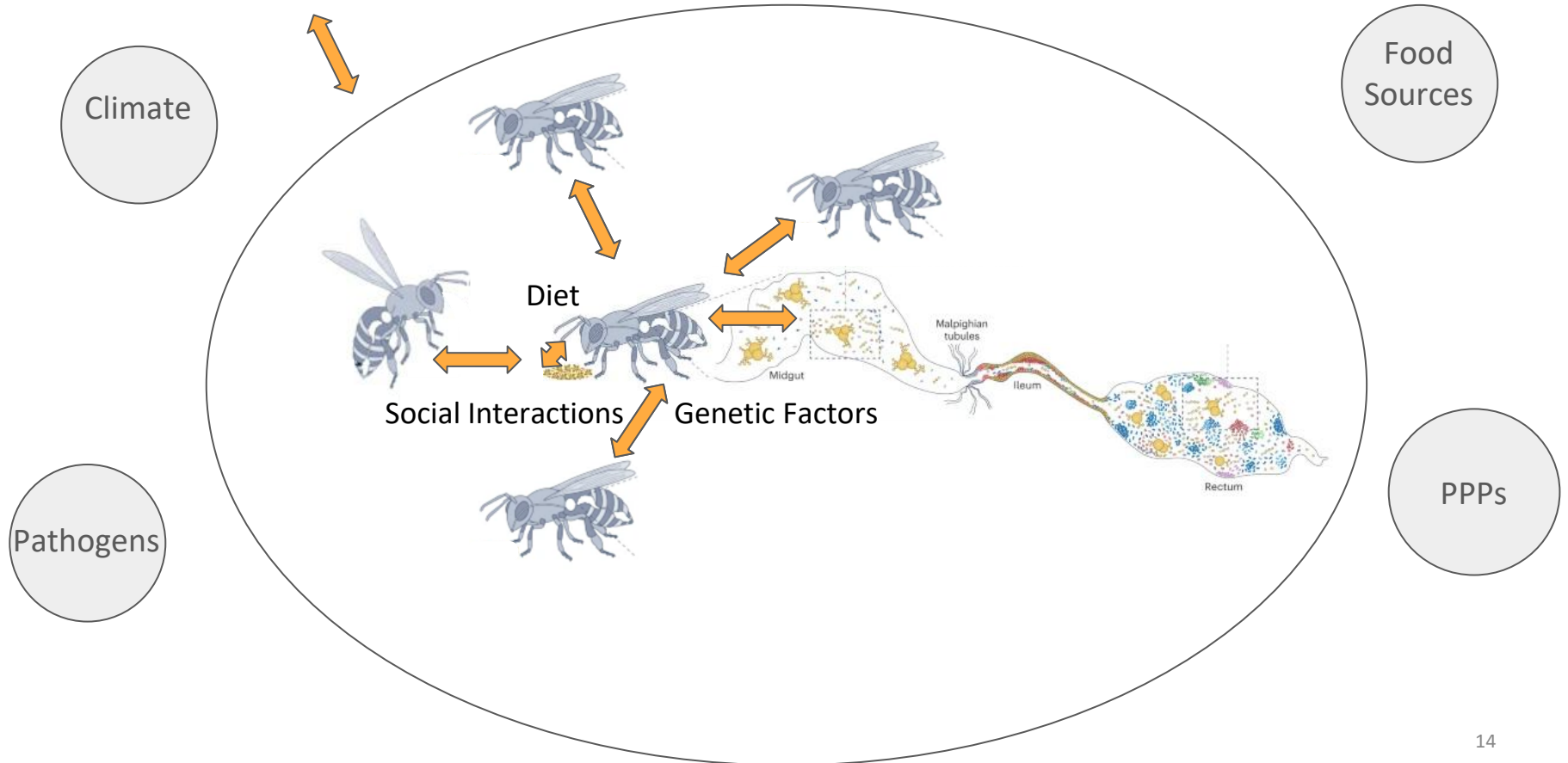




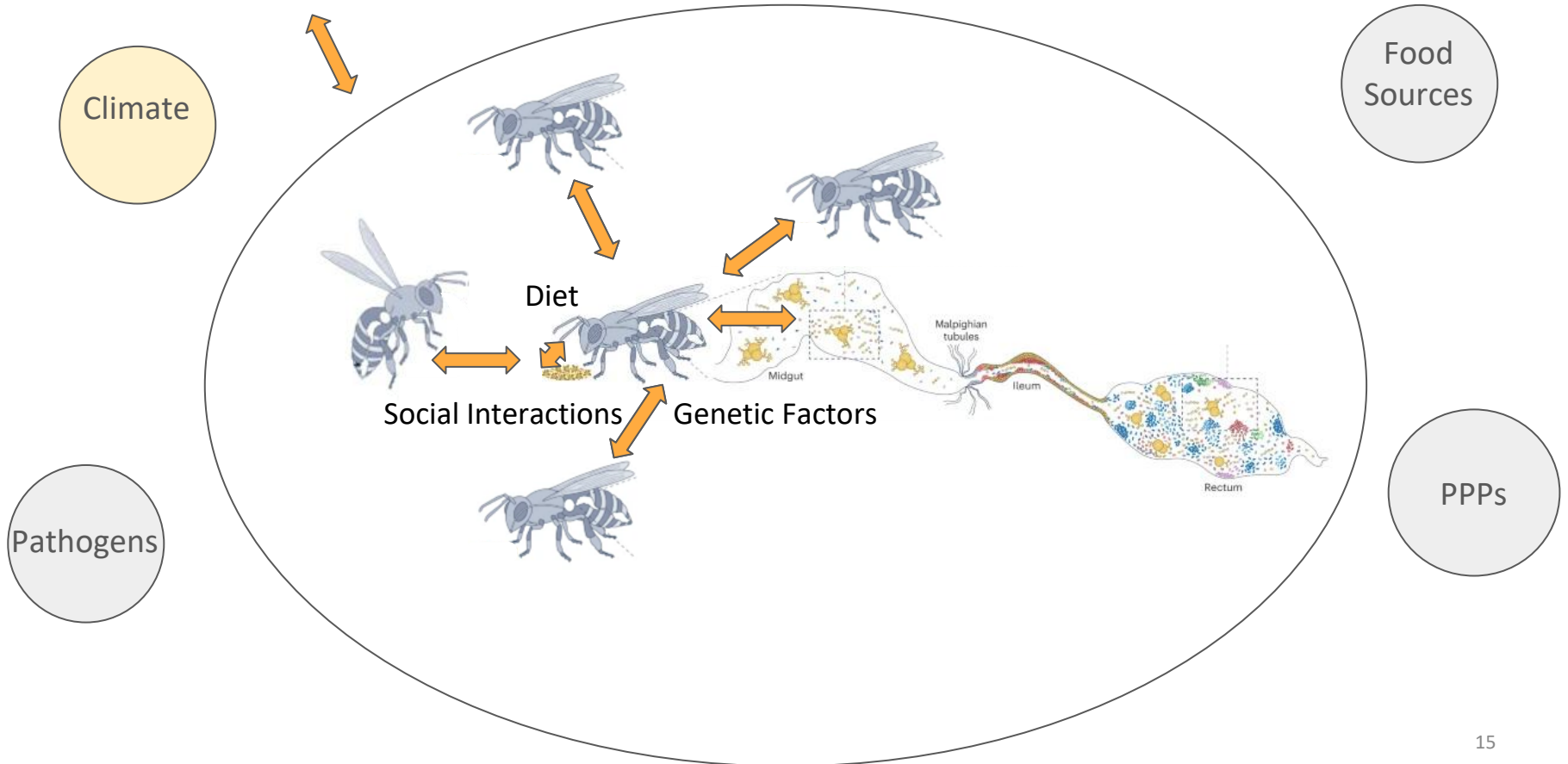
Environmental Context



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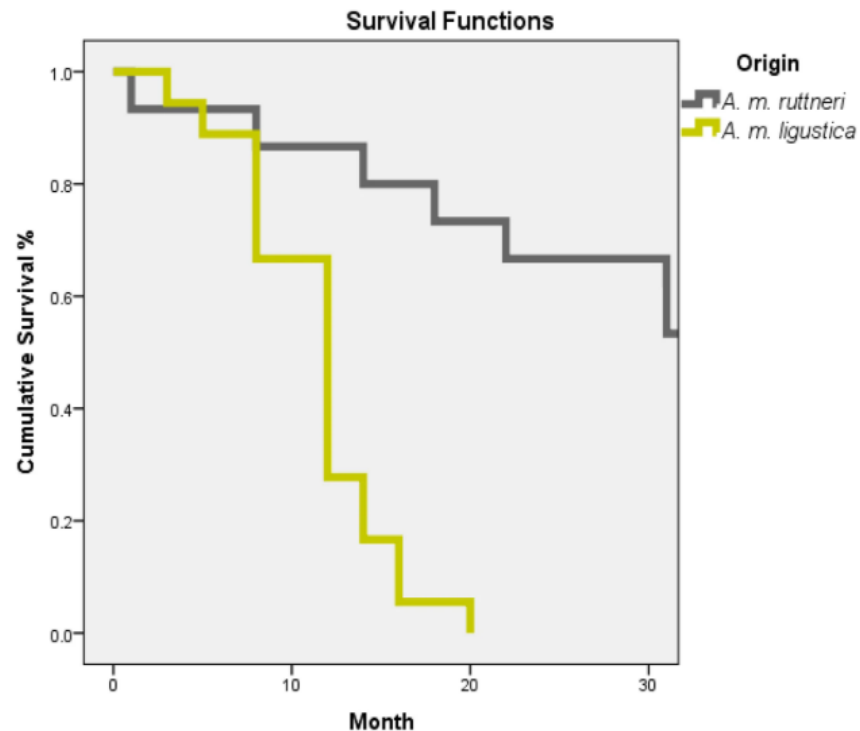
Searching for Clues in Malta

Apis mellifera ruttneri is native to the Maltese Islands, having evolved in isolation. It is uniquely adapted to the region's hot, dry and windy climate.



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Trajectories of colony survival for *A. m. ruttneri* and *A. m. ligustica* colonies (Uzunov et al., 2023).

**Is the Maltese honey bee's gut microbiota adapted to the
unique Maltese environment?**

Study Design

- **Malta (Lineage A, *Apis mellifera ruttneri*):** Għargħur (GH), Campus Msida (CM), Żejtun (ZT).
- **Italy (Lineage C, *Apis mellifera ligustica* & *carnica*):** Bolzano (BZ), Valsamoggia (VS), San Lazzaro (SLS).

At each site, **3 independent colonies** were sampled during **dry summer months**.

15 nurse worker bees per colony were collected.

Bees were **surface sterilised** before gut dissection.

DNA extraction, followed by high-throughput 16S rRNA gene sequencing (Illumina).



Bolzano (BZ)

Valsamoggia (VS)

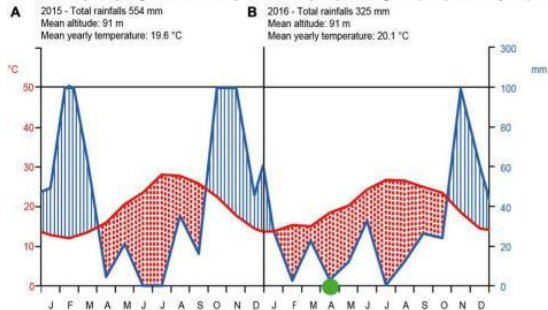
San Lazzaro (SLS)



Foreigners in the Italian Serie A
(2001-02 season)
28 - Brazilians
26 - Argentines
20 - French
9 - Uruguayans
8 - Spanish
7 - Croatians and Yugoslavs
6 - Portuguese
altogether 132 players

Italian domestic records
Most goals scored in a season:
38 - Gianni Perroni (Torino, 1929-30)
392 - Silvio Piola (for Venezia, Lazio, AC, Torino, Juventus and Roma, 1929-52)
Most goals ever:
104 (AC Torino-UC Albano, 1947-48)
670 - Dino Zoff (Juventus, Fiorentina, Napoli and Juventus, 1961-85)
Longest 1st league appearance run:
32 matches - Milan AC (1991-92 - 1992-93)
Highest attendance:
67,798 spectators (Milan AC-Juventus, 1995)

Malta (sampling sites of Campus Msida (CM), Gharghur (GH) and Żejtun(ZT))



Semi-arid Mediterranean climate, with long, dry summers and limited floral diversity.

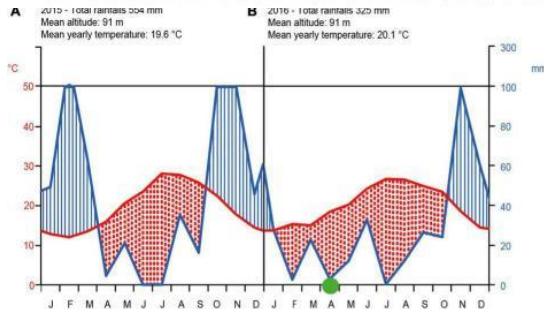
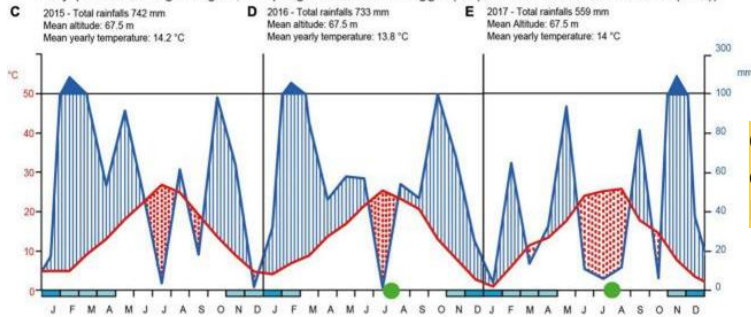
Gharghur (GH)

Żejtun (ZT)

Campus Msida (CM)



Italy (Emilia-Romagna region, sampling sites of Valsamoggia (VS) and San Lazzaro di Savena (SLS))



Semi-arid Mediterranean climate, with **long, dry summers** and limited floral diversity.

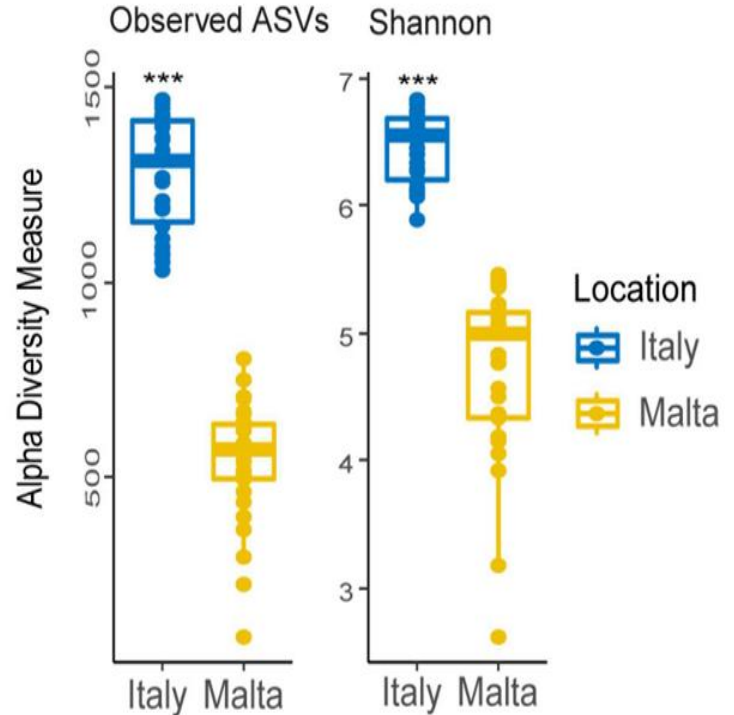
Continental climate, plane landscapes.



Results:

Alpha Diversity

Italian bees (**blue**) show significantly higher microbial richness and evenness compared to Maltese bees (**yellow**).

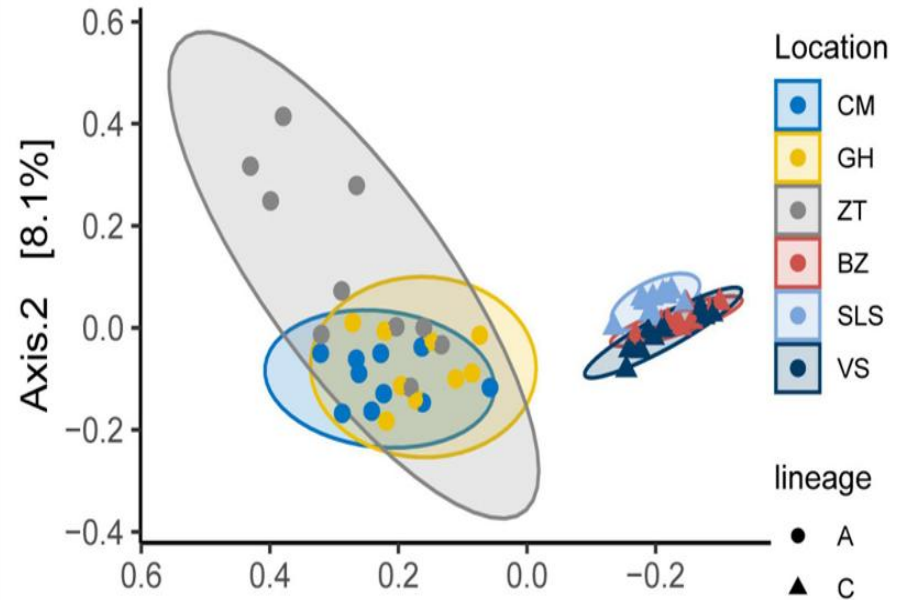


Results:

Beta Diversity

Ordination analysis (Bray–Curtis) shows clear clustering by country:

Maltese (**yellow**/grey/**blue**) and Italian (**dark blue**/**light blue**/**red**) bee gut communities form distinct, non-overlapping groups.

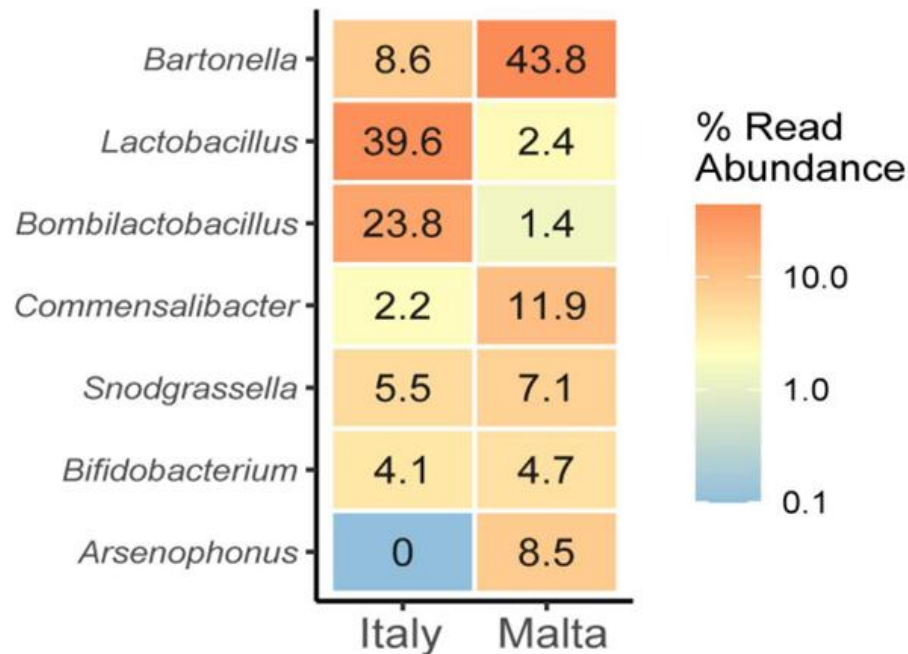


Results:

Microbiota Composition

Maltese bees have gut microbiota with higher prevalence of **environmental bacteria** (*Bartonella*, *Commensalibacter*).

Italian bees have gut dominated by **host-associated (core dominated)** *Lactobacillus* and *Bombilactobacillus*.



Evidence that the **environment** is a major factor shaping honey bee gut microbiota and that the unique microbiome of Maltese honey bees may play a role in their adaptation to a tough Mediterranean climate.

Why it Matters

Understanding how bees, their microbes and their environments interact will help us protect pollinators in a changing world.

Bee microbiomes could be a new target for building resilience.

BeeSustain: Integrative Modelling for Enhanced Beekeeping Carrying Capacity

Combines climate modelling, microbiome analysis and drone imagery to guide colony placement.

Goal: a predictive, real time tool for beekeepers → healthier colonies & sustainable honey yields.

Funded by:



Ongoing work

Aim: Untangle genetics vs. environment

Sample Collection

Samples were collected once a month for 13 months from both the Malta and Italy Apiary.



Nurse
bees
+1,000



Forager
bees +
pollen
+1,000



Honey
+300



Beebread
+300

Variables Observed



Together with sample collection, the following variables are also analysed:

- Botanical sources bees fed on
- Nutritional value of pollen
- Virii load

11th European Conference of Apidology

7-10 September 2026 · Bologna, Italy

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FROM DIVERSITY**

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<https://www.eurbee2026.com/>



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Thank You
Any Questions?

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