



# Metagenomics and population genomics of honey bees through the direct sequencing of honeys

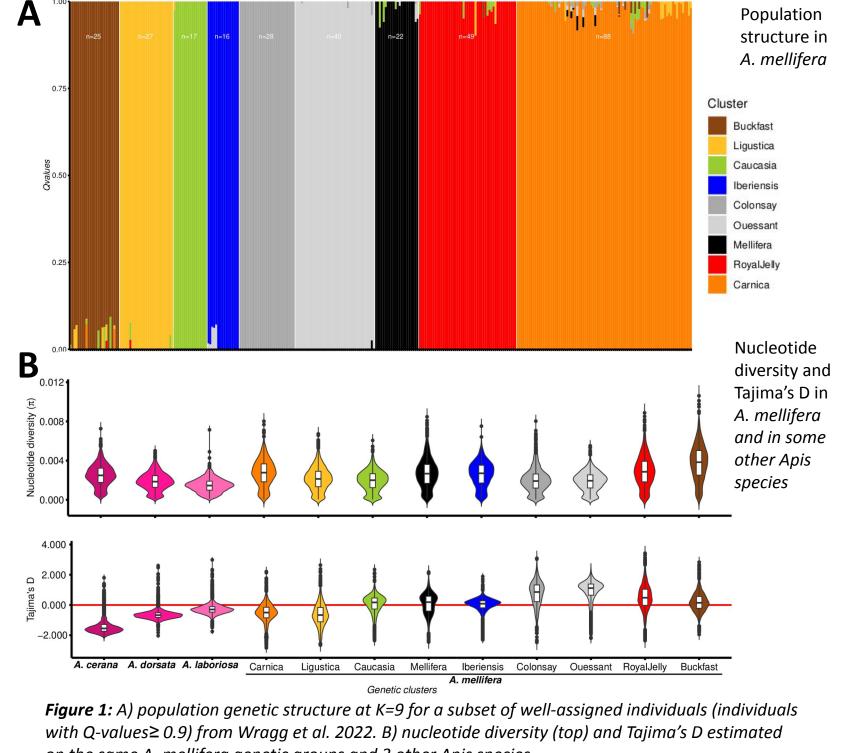
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# Background & general hypothesis

Our research team located at INRAE in Toulouse is specialized in the **description of the genetic diversity** and adaptation of honey bees used by beekeepers.

During the last decade, we have put considerable effort into collecting and whole genome sequencing of hundreds of haploid males to describe the population structure and levels of diversity in Europe.



Collecting haploid males however requires regular visits to the hives, which can be an issue for large sampling. Some campaigns are difficult to organize for practical and/or geopolitical reasons.

**Direct sequencing of honeys could offer a relevant alternative** (easy access given the importance of the worldwide market, non-lethal sampling, low carbon footprint of our research, ...)

# Metagenomics

Our primary objective was to meta-assemble sequencing data from various hive products, primarily honeys, to characterize eDNA present in the samples.

### DNA extraction and shotgun sequencing (Illumina)



### 51 honey samples:

- 46 in-house samples (low coverage: ~500,000 reads/sample)
- 2 from Bovo et al. 2018 (low coverage, ~200,000 reads/sample)

- 3 from Bovo *et al.* 2020 (high coverage, ~ 60 million reads/sample)



# 2 beeswax samples: In-house samples (extrem

In-house samples (extremely low coverage: ~1,000 reads/sample)



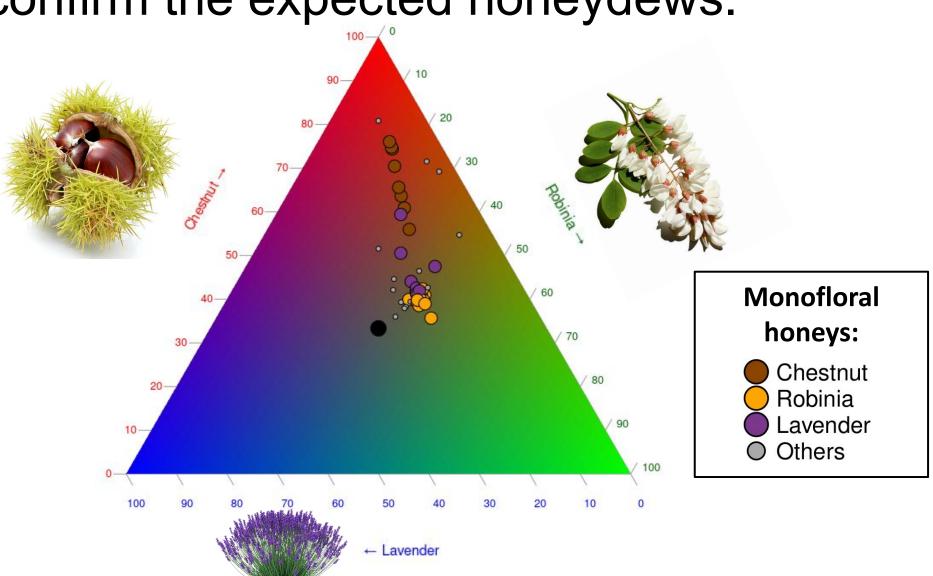
### 2 propolis samples:

In-house samples (low coverage: ~1,000,000 reads/sample)

In total: ~ 242 million paired-end reads

De novo meta-assembly of all sequencing data (MEGAHIT), metagenome binning (CONCOCT) and taxonomic annotation (Blastn, NCBI nr database)

We then evaluate the potential of metagenomics to enhance honey traceability. As an initial test, we explore whether reads corresponding to plants can confirm the expected honeydews.



## on the same A. mellifera genetic groups and 3 other Apis species. **Eukaryotes** (47 MAGs, 19 potential species, total assembly size: 211.2 Mb) 5\_Fungi\_Davidiellaceae\_Cladosporium 06 Fungi Dothioraceae Aureobasidium (0.7 Mb)01 (honey bee) (205.2 Mb) (1.0 Mb)(3.2 Mb)17\_Plant\_Liliaceae\_Asparagus **Bacteria** (65 MAGs, 24 potential species, total assembly size: 86.1 Mb) 01\_Apilactobacillus **Eukaryotes** (47 MAGs) (10.5 Mb) 23 Lactobacillus sp. **DNA viruses** (12 MAGs, ≥4 potential species, total assembly size: 9.6 Mb) **Bacteria** (65 MAGs)

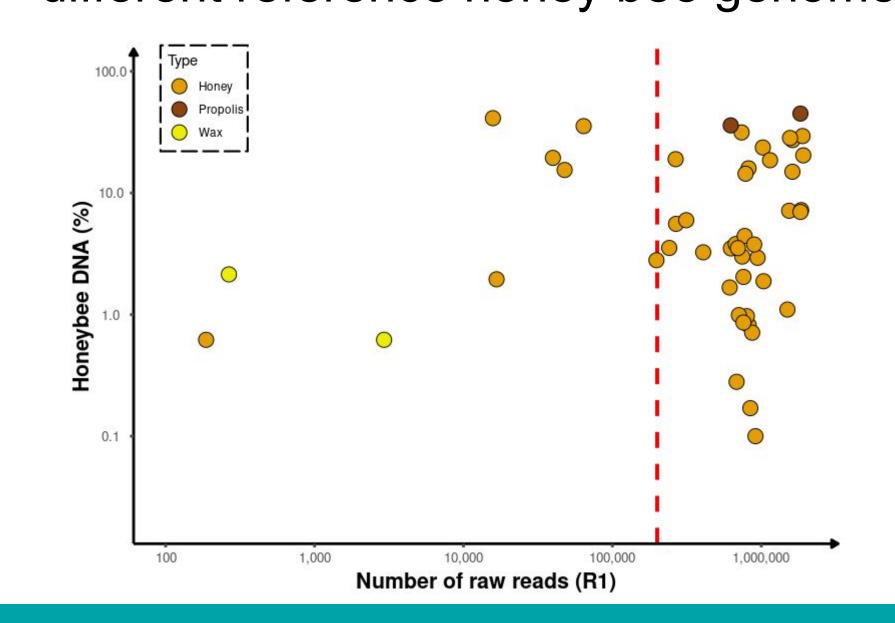
# Population genomics

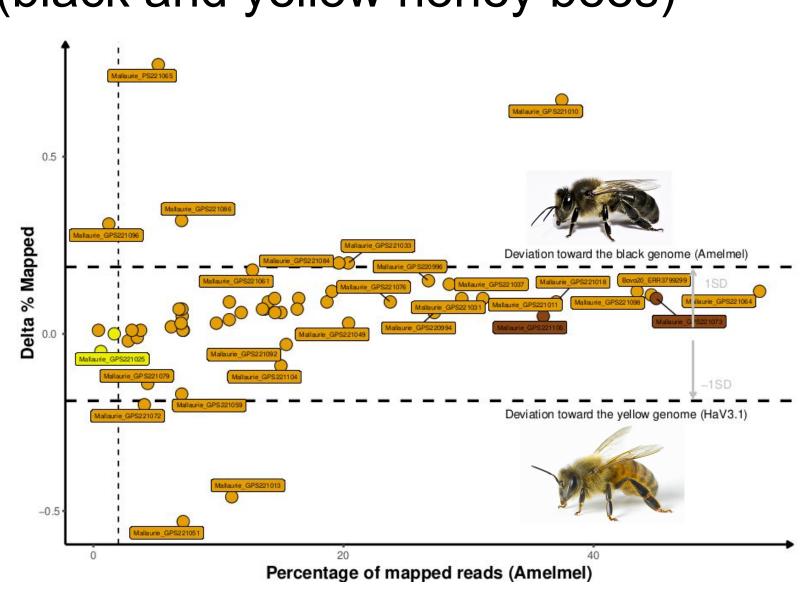
**DNA** viruses

(12 MAGs)

**Jnassigned** 

To explore whether hive products can provide future opportunities for studying the genomics of honey-producing bee populations, we mapped reads to two different reference honey bee genomes (black and yellow honey bees)





To know more or continue to follow the project:
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References:

Bovo, S. et al.. Shotgun metagenomics of honey DNA: Evaluation of a methodological approach to describe a multi-kingdom honey bee derived environmental DNA signature. PLoS One,13(10):e0205575 (2018)

01\_Caudoviricetes\_phage

Bovo, S. et al. Shotgun sequencing of honey DNA can describe honey bee derived environmental signatures and the honey bee hologenome complexity. Scientific Reports 10, 9279 (2020)

Wragg, D. et al. Complex population structure and haplotype patterns in the Western European honey bee from sequencing a large panel of haploid drones. Molecular Ecology Resources 22, 3068–3086 (2022)