

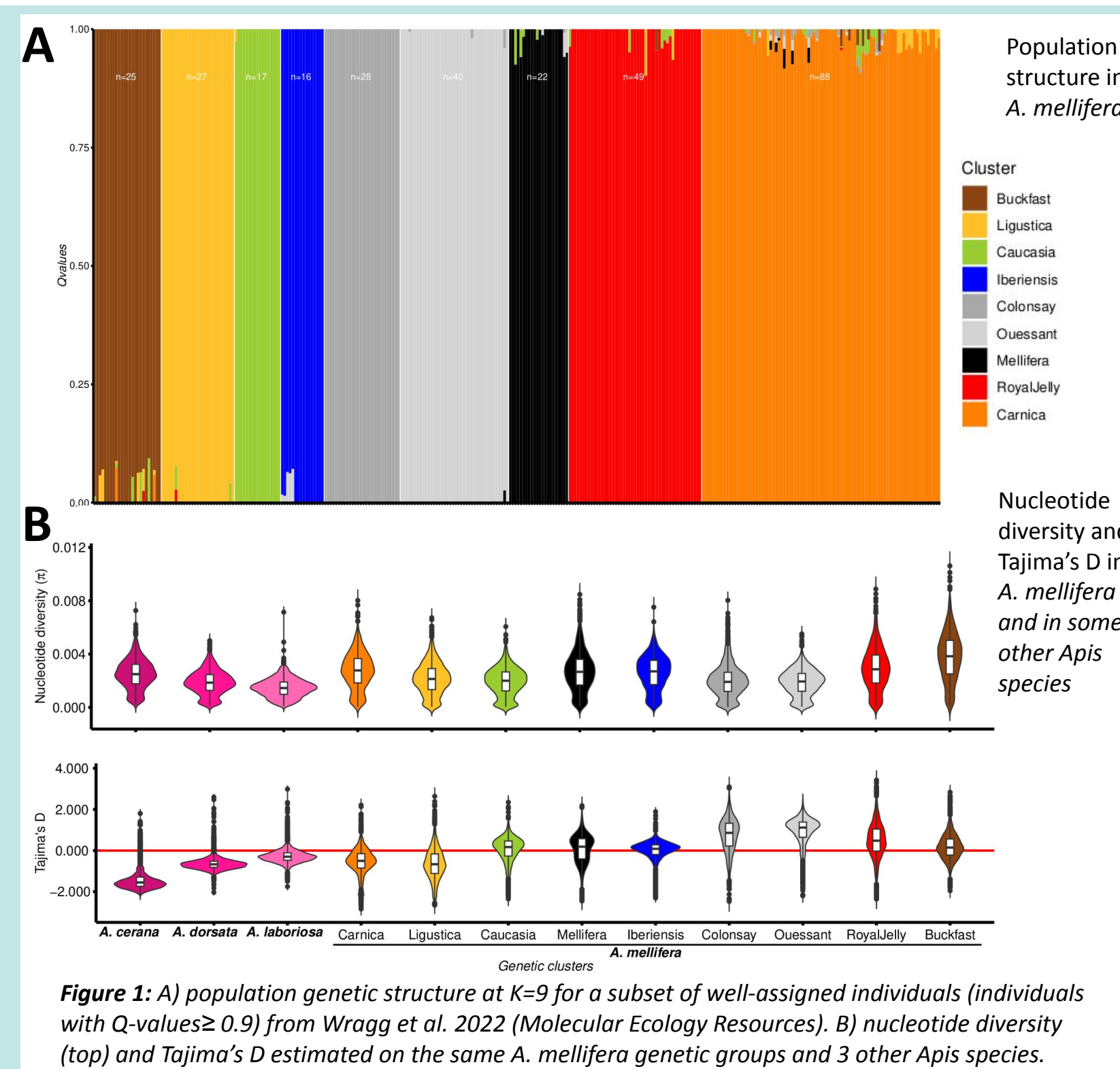


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## BACKGROUND

Our research team located at INRAE in Toulouse, France specializes in the **description of the genetic diversity** and adaptation of honey bees.

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 (Fig. 1).



Recently, Bovo et al (2020) sequenced **three honey samples** allowing access to whole-genome sequences of the contributing honey bees. These results support highly variable yields among the three samples, with **1 to 65% of reads effectively corresponding to bee DNA**. To our knowledge, no study successfully sequenced bee DNA from beeswax samples.

## CURRENT LIMITS

The sequencing of drones has many advantages, including a direct access to haplotypes. **Collecting drones however requires regular visits** to the hives, which can be an issue for large regional sampling. In addition, campaigns could be difficult or **impossible to organize** for practical and/or geopolitical issues.

## MAIN QUESTION

**Does direct sequencing of hive products represent a good strategy for future population genomic and metagenomic investigations?**

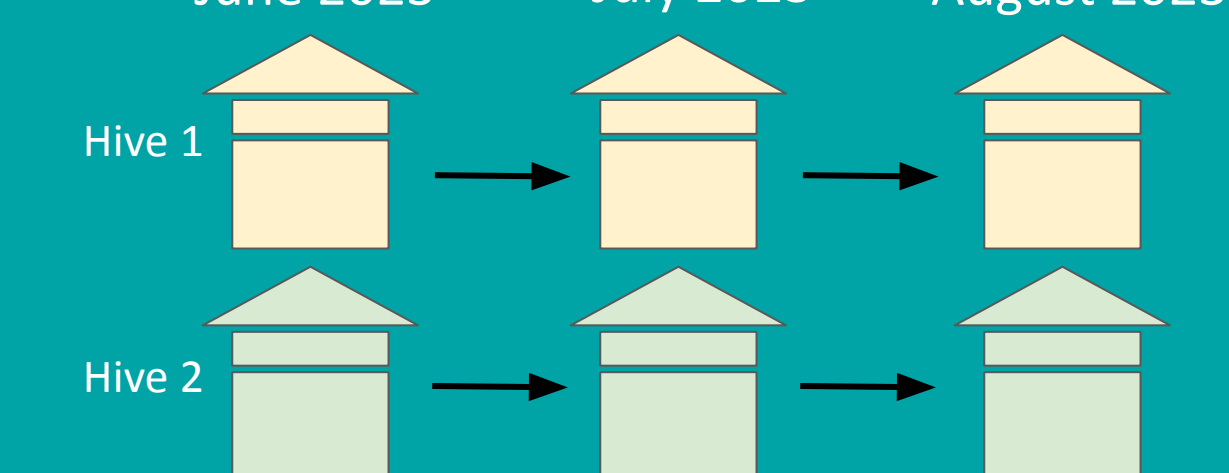
## ADVANTAGES

- The direct sequencing of honey allows to:
- **Perform non-lethal sampling**
  - **Reduce the carbon footprints of our research** (especially associated with the sampling)
  - Have easy access to many samples given the importance of the market across the globe
  - **Access to some other genetic information** (pollinated plant species, microbiome, Varroa, ...)

## PILOT STUDY

### Transhumance of beehives

Black locust (Robinia) June 2023  
 Sweet chestnut (Castanea) July 2023  
 Lavender (Lavandula) August 2023



### Worldwide collection of samples



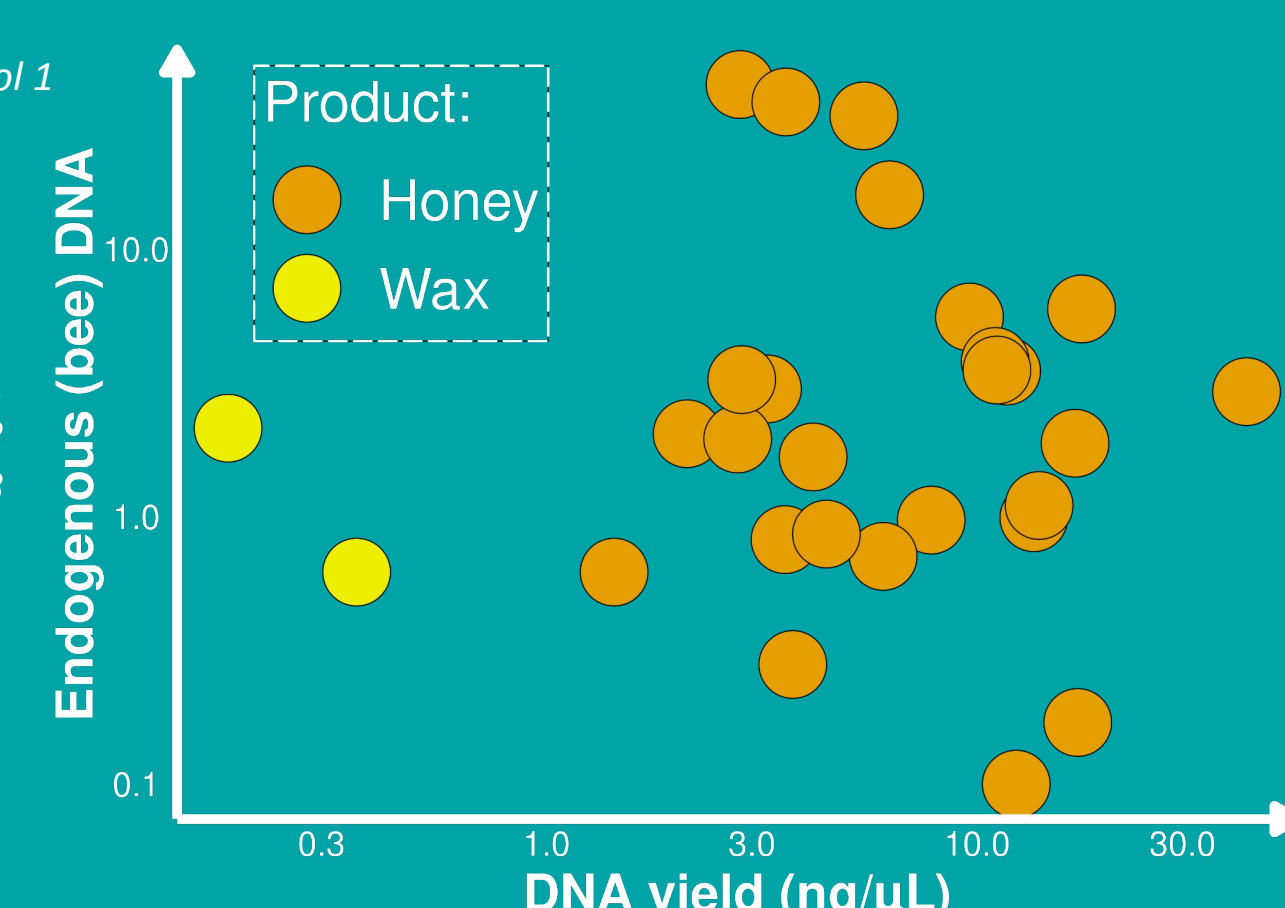
### DNA extraction for each beehive sample & QC

- Fine tune a DNA extraction protocol to yield high quality DNA from comb honey and also additional honeypot samples
- Develop a specific protocol for DNA extraction from beeswax
- Overall DNA quantification using Nanodrop & Picogreen
- Specific amplification of honey bee DNA using mitochondrial and nuclear loci

### Bee DNA amplification & shotgun sequencing



We successfully extracted and dosed DNA, and we were able to amplify mtDNA from several honeys and beeswaxes sample.

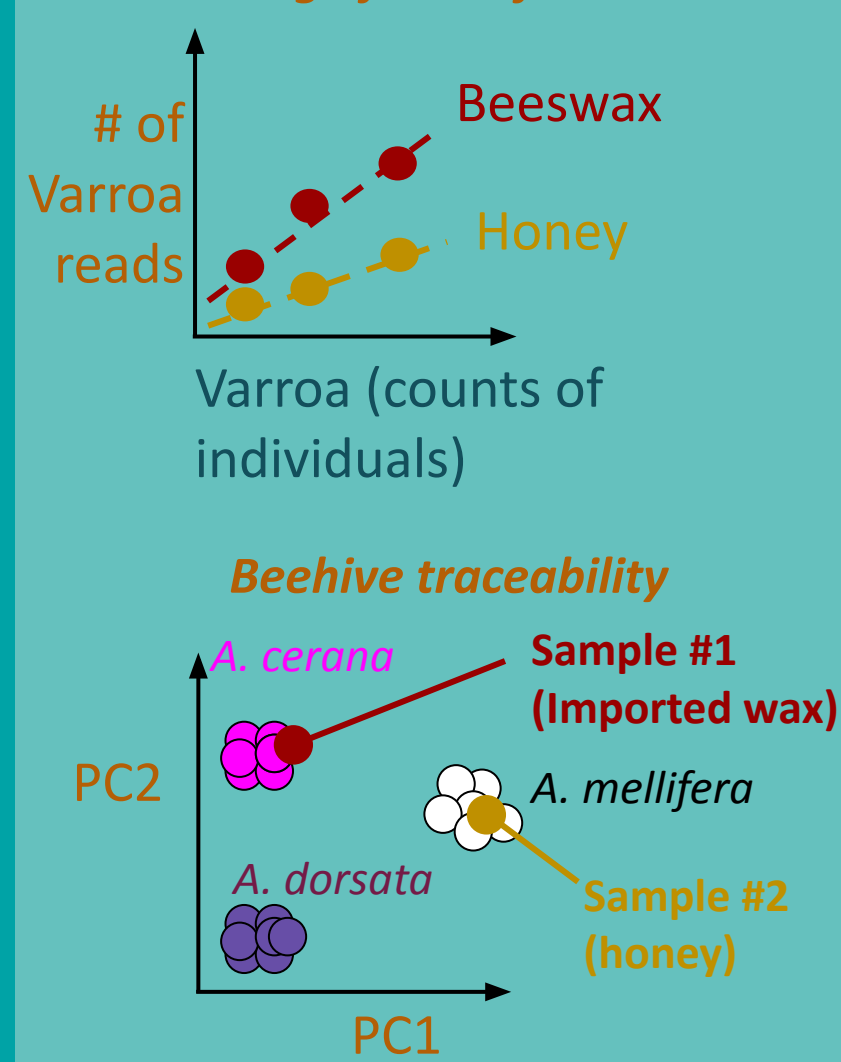


We also successfully identified bee DNA among the Illumina sequencing reads, including from wax samples, a world premiere to the best of our knowledge.

## PROSPECTS

These results are promising, pointing to a number of possible avenues. Thanks to the larger diversity of the project samples, we will perform a **metagenomic analysis of all DNAs present in honey and wax**. Reads associated with plant and Varroa DNA will be especially studied. We will especially try to monitor colony health by studying the **infestation level of Varroa** along a beekeeping season.

### Monitoring of the infestation level



We also plan to analyse in detail the population structure of the samples, and to compare them to those already described (Fig. 1) in order to **trace the geographical origin of the honey bees**. This topic is crucial given that trade channels remain poorly traced, especially for beeswax. Chemical testing of wax is indeed not resolute enough to allow this tracing. Consequently, **direct shotgun sequencing of beeswax could be a promising solution**.