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Bee genome reconstruction through the sequencing of hive products

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► **To cite this version:**

Kamila Canale Tabet, Elisa Simon, Emmanuelle Labarthe, Emilien Rottier, Benjamin B. Basso, et al.. Bee genome reconstruction through the sequencing of hive products. Apimondia 2023, Sep 2023, Santiago, Chile. hal-04338919

HAL Id: hal-04338919

<https://hal.science/hal-04338919>

Submitted on 12 Dec 2023

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BACKGROUND

Our research team located at INRAE in Toulouse, France specializes in the **description of the genetic diversity** 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 diversity in Europe (Fig. 1).

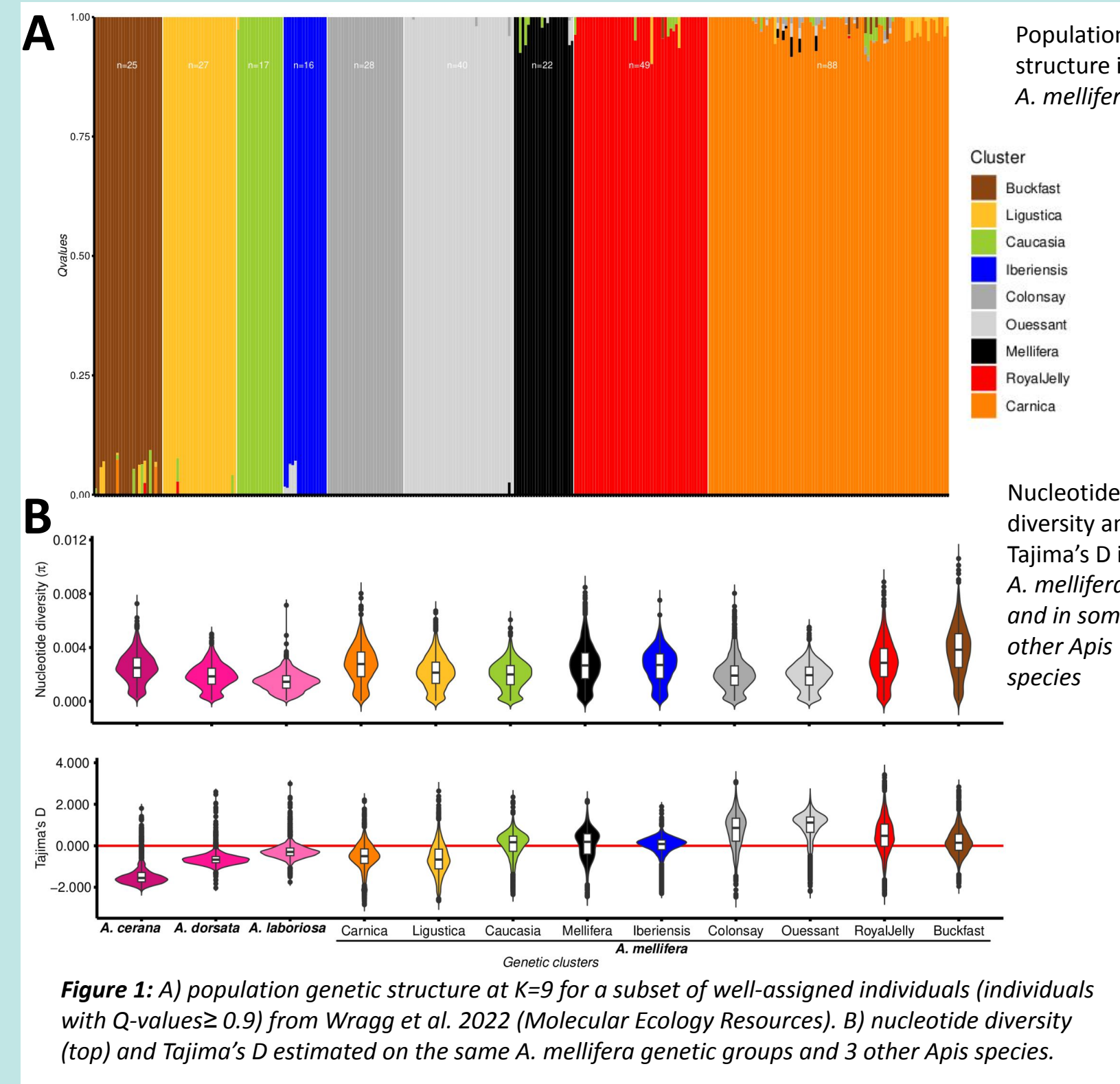


Figure 1: A) population genetic structure at K=9 for a subset of well-assigned individuals (individuals with Q-values ≥ 0.9) from Wragg et al. 2022 (Molecular Ecology Resources). B) nucleotide diversity (top) and Tajima's D estimated on the same *A. mellifera* genetic groups and 3 other *Apis* species.

Recently Bovo et al (Scientific Reports, 2020) sequenced **three honey samples** at high coverage 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**.

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, some campaigns could be difficult or **impossible to organize** for practical and/or geopolitical issues.

MAIN QUESTION

Does direct sequencing of honeys represent a good strategy for future large-scale population genomic investigations?

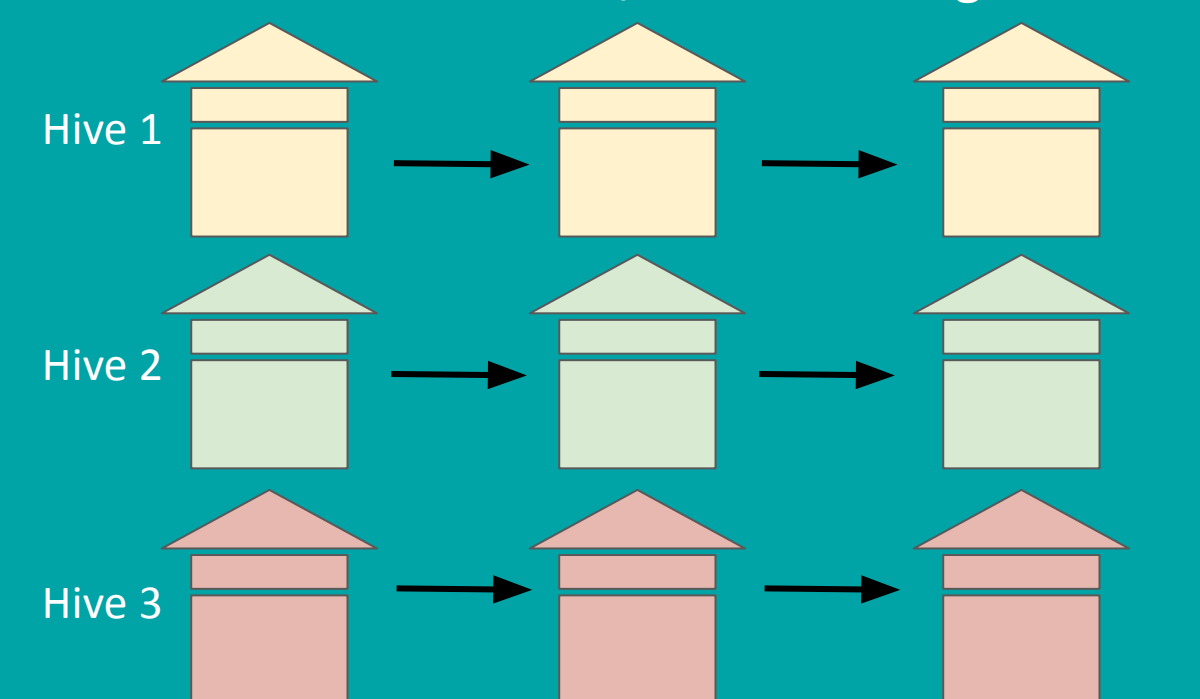
ADVANTAGES

- The direct sequencing of honey allows to:
- **Reduce the carbon footprints of our research** (especially associated with the sampling)
 - Easy access to many samples given the importance of the honey market across the globe
 - **Non-lethal sampling**
 - **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
 Lavander (*Lavandula*) August 2023



Comb honey

DNA extraction for each comb honey & QC

- Anonymizing the samples (full-blind study)
- Fine tune a DNA extraction protocol to yield high quality DNA from comb honey and also additional honeypot samples
- Overall DNA quantification using Nanodrop & Picogreen
- Bee DNA (specifically): PCR amplification of two mitochondrial and a nuclear DNA loci

High-throughput DNA sequencing

- First round of sequencing on a MiSeq instrument to estimate the proportion of bee DNA in honey
- Second round of sequencing on a NovaSeq instrument to obtain honey bee whole-genome sequences, after adjusting the sequencing effort to the estimated proportion of bee DNA in the sample (first round)

Data analysis

- Plant DNA: can we recover the origin of the honey using the sequencing reads from the plants?
- Varroa DNA: can we observe an increase of the proportion of varroa parasite sequencing reads during the season?
- Bee DNA: can we precisely discriminate the genetic makeup of the colonies using the reads from the bees present in honey?

PROSPECTS

Our pilot study is part of a larger project (called *Mallaurie*) including 100+ samples, with a larger honey diversity (different geographical origins and plant species, different *A. mellifera* subspecies, Fig. 2). Thanks to this larger diversity, we will perform a **metagenomic analysis of all DNAs present in honey**. This will allow us to propose a potential geographical origin of the honeys by comparing the sequencing results (plants and bees) from honey samples with the genetic structure described above (Fig. 1).



Fig. 2: Diversity of the honey samples to be included in our future investigations for the Mallaurie project (on this picture: honeys from Algeria, India, Nepal, Sweden, ...)

Interested to contribute to our study?

Do not hesitate to provide us a jar of your honey!