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Mitochondrial genome analysis of *Apis mellifera intermissa* and *sahariensis* reveals alignment biases and genetic variability

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Introduction

→ Characterizing the genetics of *Apis mellifera* subspecies plays a key role in understanding biodiversity, tracing population evolution, and guiding conservation efforts [1].

→ During the last decades, many mitochondrial DNA–based studies have explored the genetic diversity of honeybee populations across the world. Mitochondrial genomes, inherited maternally and highly conserved, provide valuable insights into honeybee evolution and diversity [2].

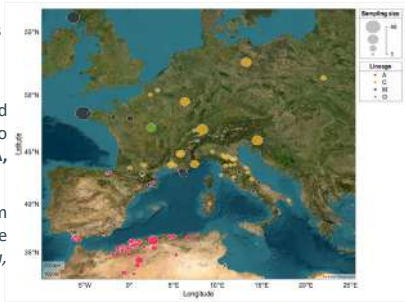
→ Despite extensive research on *Apis mellifera*, the genetics of some African subspecies remains poorly described. In Algeria, *A. m. intermissa* and *A. m. sahariensis* show distinct ranges and morphologies.

→ **What can mitochondrial genome analysis of *A. m. intermissa* and *A. m. sahariensis* reveal about the diversity and genetic composition of Algerian honey bee subspecies?**

Methods

Dataset: Illumina sequencing

- **151 Algerian drone samples**
  A. m. intermissa (n = 108)
  A. m. sahariensis (n = 43).
- **344 samples** described previously as belonging to the evolutionary lineages **A, M, C** and **O** [3, 4].
- **18 sequences** retrieved from NCBI, representing the African lineage **A** (*scutellata, monticola, capensis*).



Algerian and European honey bees geographical locations.

SNP detection and genotyping performed as in Wragg et al., 2022 [4].
Our analysis focused only on **SNPs** within the **13 protein-coding** and **2 rRNA genes**, to avoid potential biases from false-positive SNPs arising due to read mapping in repetitive genomic regions.

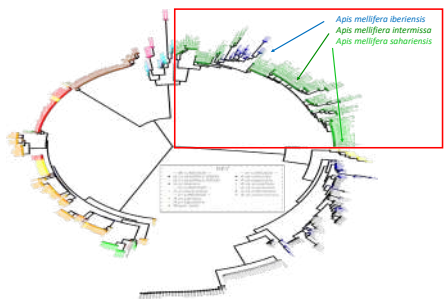
Genetic diversity and phylogenetic analyses were conducted on a final VCF containing **464 sequences and 207 SNPs**. To determine the phylogenetic relationship, median-joining network and Maximum Likelihood Tree were constructed.

Conclusions

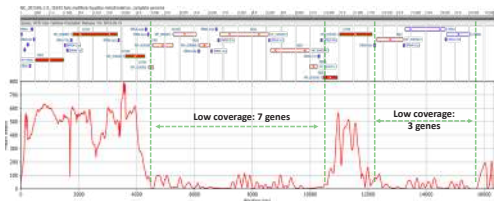
- Our analysis revealed **no significant genetic admixture** (not shown here) in Algerian honey bees, confirming their **belonging to African lineage A**. It also showed **no genetic differentiation between the two subspecies**, as no distinct mitochondrial haplotypes were detected.
- The analysis highlighted the **genetic relatedness of Algerian subspecies to *A. m. iberiensis*** from the south of the Iberian Peninsula.
- The **reference genome used for read mapping influences the results**, potentially creating coverage gaps or misalignments.
- **Pangenome approaches will solve the problems** of using a single reference genome.
- Careful bioinformatics analyses are essential for mitochondrial sequences analyses.

Results

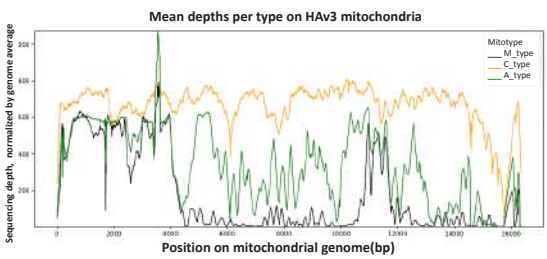
1 - Absence of distinct mitochondrial haplotypes between *A. m. intermissa* and *A. m. sahariensis*.

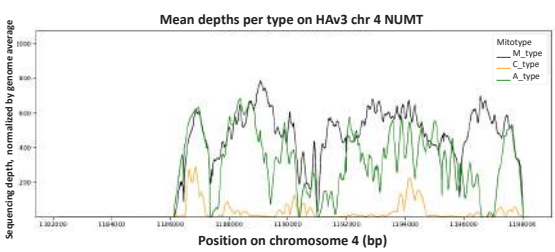


2- Mapping reads from Type A and M samples to the HAV3 reference genome (Type C) showed low coverage in some mitochondrial regions.



3 – These reads map instead to a large NUMT (Nuclear Mitochondrial insertion) on chromosome 4.





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Genetic characterisation of Apis mellifera subspecies is crucial for the understanding of biodiversity, population evolution and conservation strategies. Mitochondrial DNA (mtDNA), inherited maternally and highly conserved, is a valuable marker for studying population genetics and phylogeography. While extensive research has been conducted to explore the genetic diversity of Apis mellifera, the genetic makeup of certain African subspecies remains insufficiently described. In Algeria, two subspecies, Apis mellifera intermissa and Apis mellifera sahariensis, exhibit distinct geographical range and morphological characteristics. We aimed to investigate their mitogenome in order to provide key insights into their population structure and their evolutionary history as part of the partnership PHC Tassili 2021-2023 and the INRAE TSARA initiative. In this study, we analyzed the complete mitogenome of 151 haploid drones from A. m. intermissa and A. m. sahariensis, obtained through whole-genome sequencing using Illumina technology, to assess genetic diversity. Comprehensive analyses using a dataset of drone sequences from European and African reference populations revealed no significant genetic admixture in Algerian honey bees. The results highlighted the genetic proximity between the Algerian subspecies and the Iberian Peninsula subspecies, A. m. iberiensis. We also confirmed the absence of genetic differentiation between the two subspecies as no distinct mitochondrial haplotypes were found. Additionally, our analysis identified alignment biases that may affect the accuracy of mitochondrial genome studies. The presence of Nuclear Mitochondrial DNA segments (NUMTs) causes reads misalignment, leading to errors in variant calling. These biases, potentially due to sequence complexity and reference genome selection, could lead to misinterpretations of genetic variation. Overall, this study enhances our understanding of the genetic diversity of A. m. intermissa and A. m. sahariensis, and highlights the need for conservation efforts. These findings emphasize the importance of applying rigorous bioinformatic approaches when analysing mitochondrial sequences, particularly in highly diverse or structurally complex genomes.