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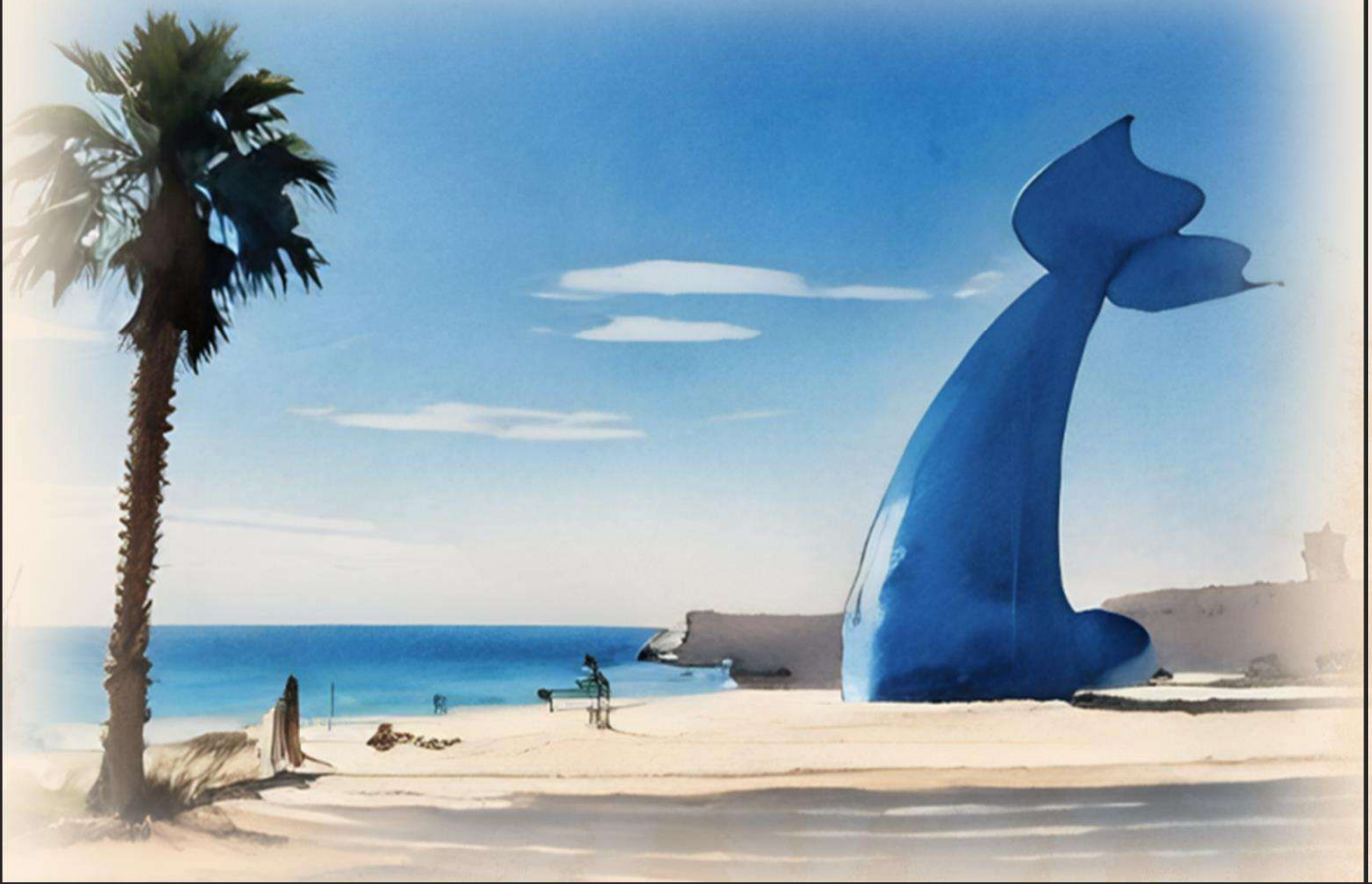


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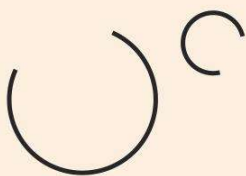
**Instituto de Tecnologia  
Química e Biológica**  
Universidade NOVA de Lisboa

**SPG Conference**

# **International Conference** of the **Portuguese Society of Genetics**



**5-6 June 2025, Oeiras**



### Large Scale Mitochondrial Evidence of C-Lineage Dominance in European Honey Bees

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In Europe, distribution of the several endemic honey bee (*Apis mellifera*) subspecies has suffered a considerable shift in the last century. In particular, beekeepers tend to favour subspecies of Eastern European ancestry (C-lineage), such as the Italian honey bee (*A. m. ligustica*), due to their perceived docility and high honey production. As a result, large scale migratory beekeeping and trade of C-lineage queens have exposed the native European honey bees to introgression and replacement, jeopardizing their genetic integrity and locally adapted traits.

The maternally-inherited and highly polymorphic mitochondrial intergenic region tRNA<sup>leu</sup>-cox2 is routinely used for the assessment of honey bee diversity and introgression at large geographical scales. In this study, we conducted a survey on tRNA<sup>leu</sup>-cox2 variation in more than 1300 colonies from 33 European countries to assess current status of mitochondrial diversity patterns in Europe. Total genomic DNA was extracted from the bee thorax followed by PCR amplification of the tRNA<sup>leu</sup>-cox2 region and sequencing. The haplotypes were identified using alignments in MEGA 11. A clear dominance of C-lineage haplotypes was found (65%). The most prevalent C-lineage haplotype was C2 (54%) followed by C3 (23%) and C1 (22%). Contrary to this trend, in Portugal, Spain and Ireland, less than 10% of the colonies exhibit the C-haplotype. Furthermore, the analysed apiaries in isolated protected areas (n=7) revealed a high proportion of colonies of western European (M-lineage) ancestry (80%) with M4 as the most frequent haplotype. African haplotypes (A lineage) were also found, albeit at lower frequencies, and were mainly concentrated in the Iberian Peninsula (9%).

Intensive queen breeding and migratory beekeeping is homogenizing the gene pool of European bee populations. This survey of honey bee maternal diversity across Europe highlights the alarming dominance of C-lineage haplotypes and underscores the importance of conservation apiaries, as they have effectively preserved the autochthonous M-lineage subspecies in different countries.