

Native honeybee conservation genomics: status of the European dark bee in the Netherlands and beyond

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Introduction

- The European dark bee (*Apis mellifera mellifera*) is the only honeybee subspecies indigenous to northern Europe
- Introgression from non-native subspecies threatens the genetic integrity of *A. m. mellifera*, while small population size may constrain adaptive potential and long-term viability
- In the Netherlands it persists almost exclusively on the island of Texel, where fewer than 200 relatively pure colonies remain, but breeding of other species and subspecies is prohibited by law

Methods

- In collaboration with the black bee breeding association on Texel we sampled the remaining colonies across the island. Whole genome resequencing (10x) was performed for 133 haploid males
- We assessed population structure, genetic diversity and purity of Texel *A. m. mellifera* colonies, and used published WGS data for 10 related subspecies across 12 different European countries to contextualise our results

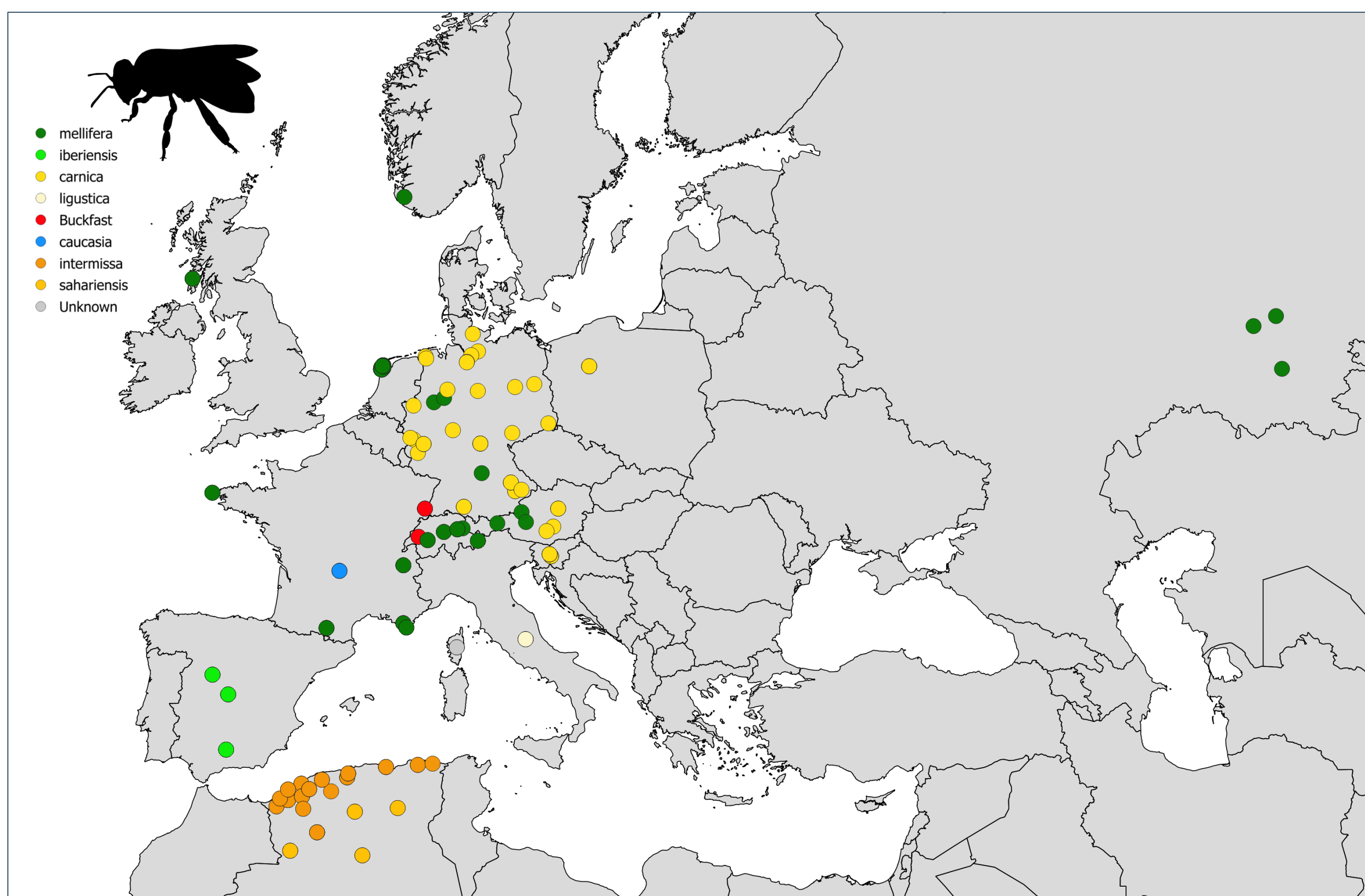


Figure 1. Sample map of all WGS bee data used for this study, including 133 individuals from Texel (NL) and integration with 376 individuals from 12 neighbouring countries representing most major European subspecies and breeding lines

- Genomic data (and phenotypic wing measurement data) were used to select Texel colonies for drone semen cryopreservation using optimized protocols to establish a secure genetic resource for future breeding and conservation

Results

- After alignment with the Amel_HAv3.1 reference genome and filtering for strand bias, indels, missing genotypes, linkage and quality scores, 6,385,553 SNPs remained for further analysis
- Texel bees clustered in the expected Western European M-lineage and were unique from bees in adjacent European countries

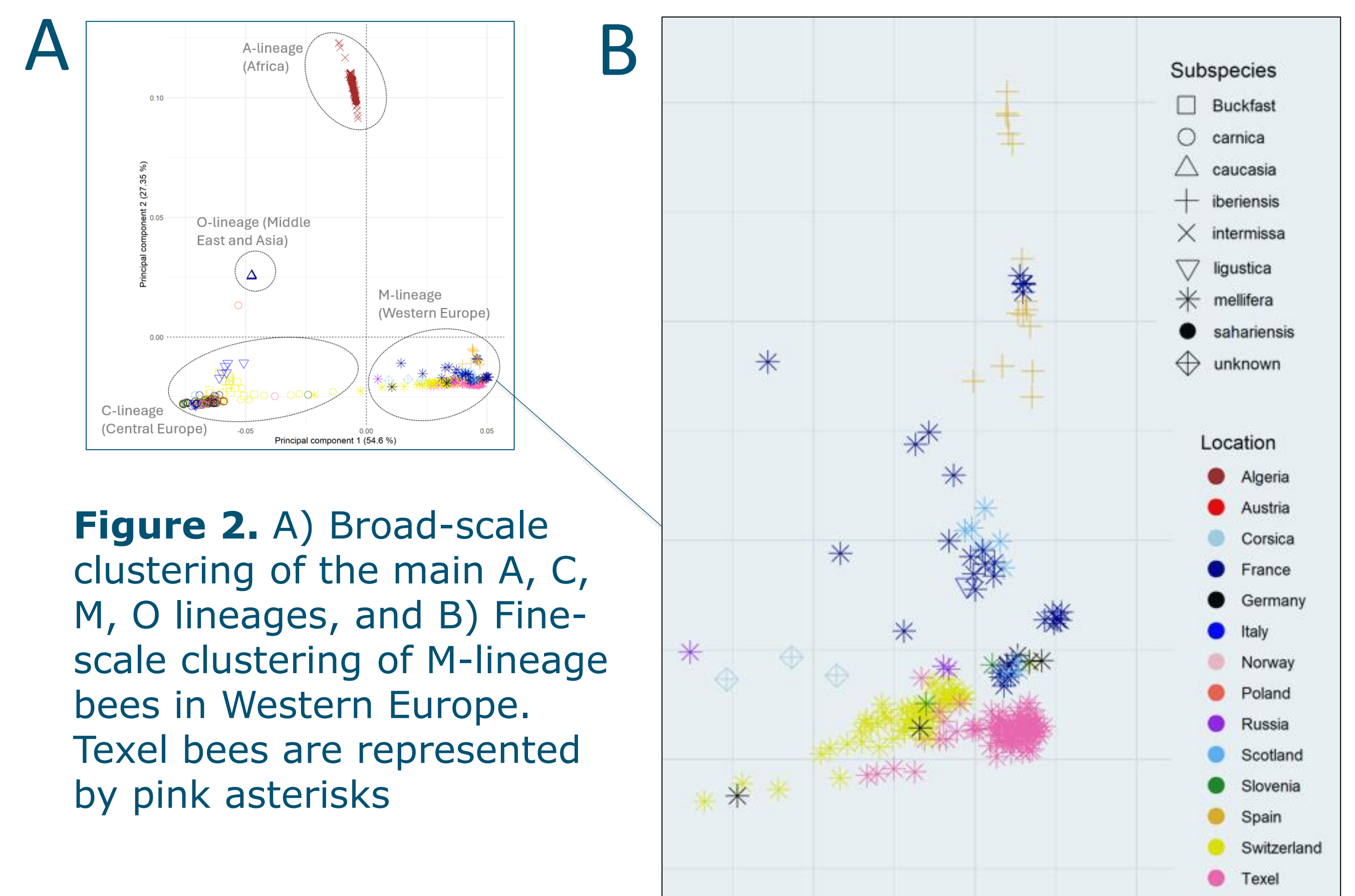


Figure 2. A) Broad-scale clustering of the main A, C, M, O lineages, and B) Fine-scale clustering of M-lineage bees in Western Europe. Texel bees are represented by pink asterisks

- Genetic diversity was relatively low but comparable to other European *A. m. mellifera* populations

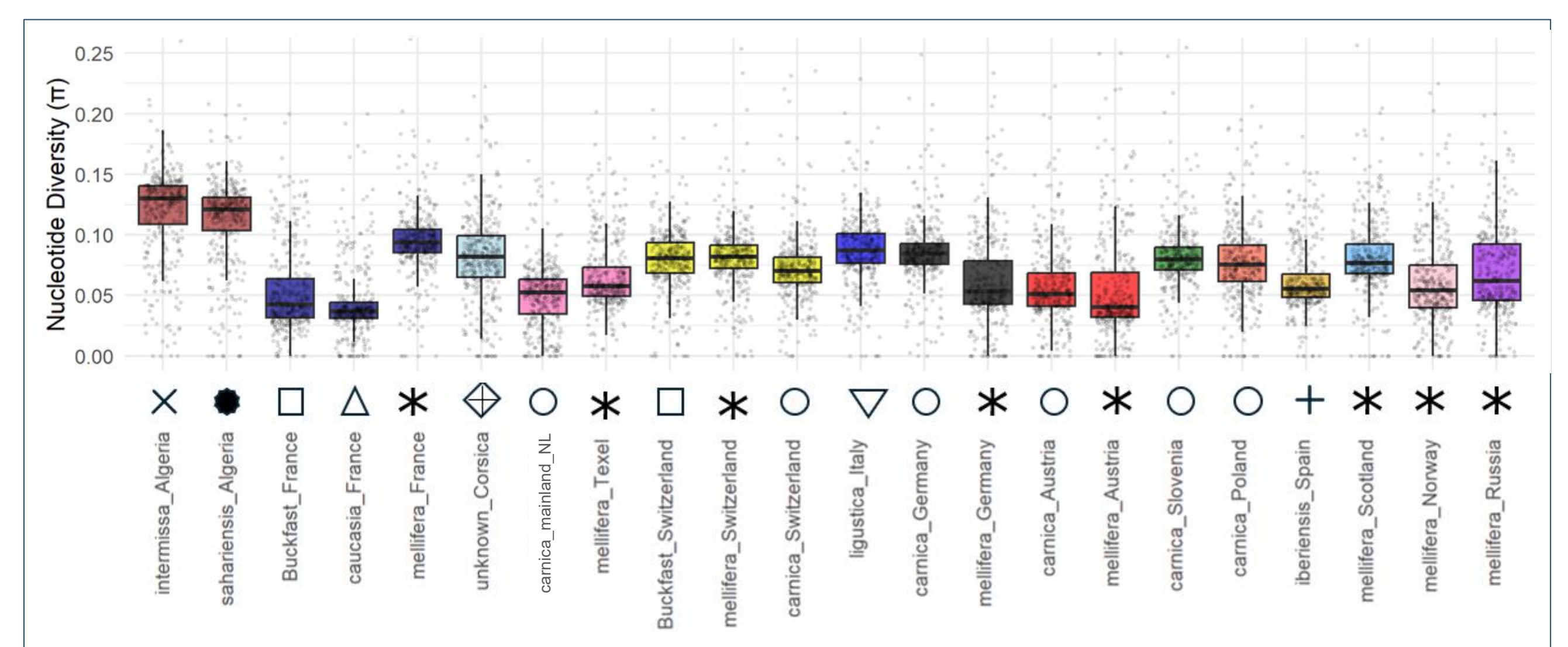


Figure 3. Nucleotide diversity (calculated in 1Mbp windows across the genome) for all bees, clustered by subspecies and country. Colours and symbols follow legend of Figure 2.

Conclusions

- *Apis mellifera mellifera* from Texel represent a unique population that is small and under threat
- Our cryopreservation efforts are the first steps to safeguard this unique diversity for the future
- Publicly available and well-documented whole genome resequencing datasets are an invaluable tool for interpreting novel datasets, providing a very useful broader context

