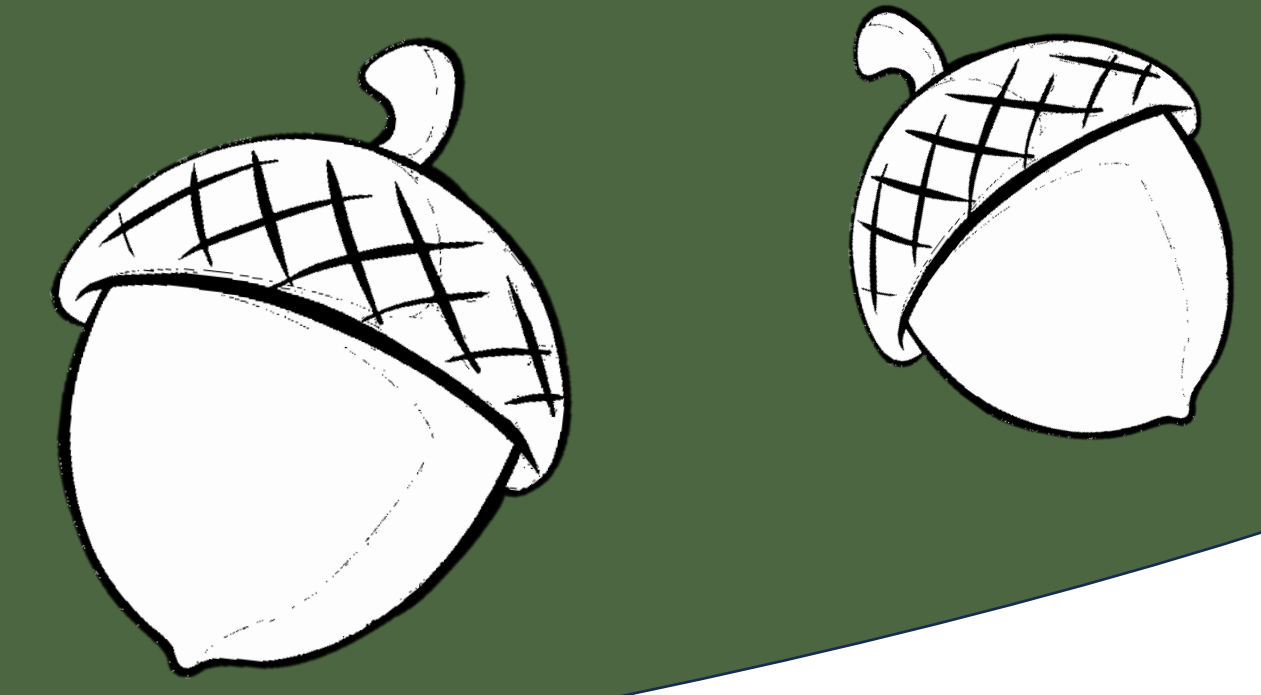


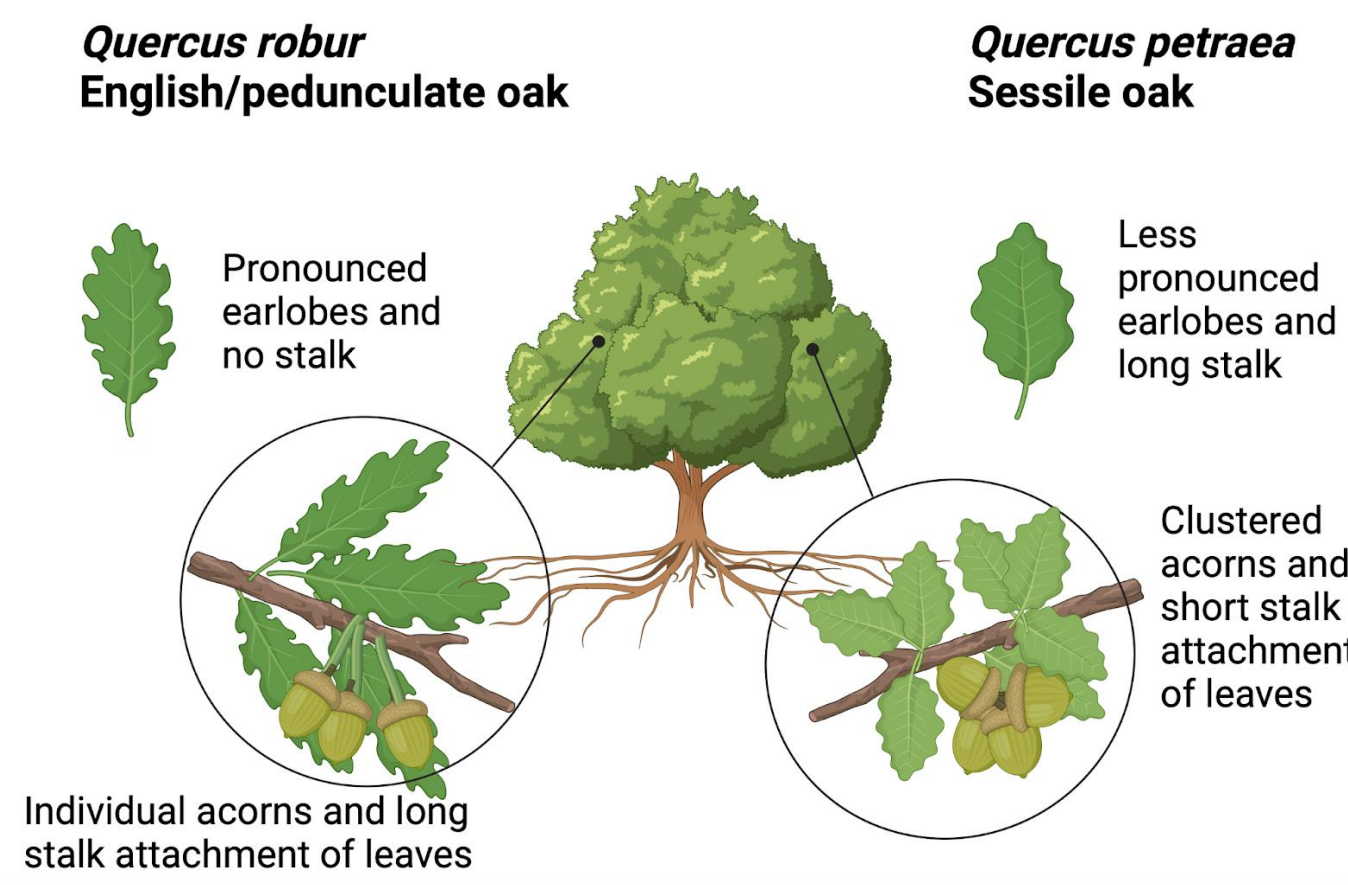
Genomic Similarities and Relationships of Oak Populations in Hainault Forest

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Background

- Quercus robur* (English oak) and *Quercus petraea* (sessile oak) are significant tree species in the UK.
- These oaks are vital to their ecosystems, providing habitat and food for various species, thus enhancing forest biodiversity and health [1].
- Hainault Forest contains oaks of different ages - ancient trees (900 years old) and replanted ones (250 years old). [2]
- Genetic diversity of the oak population in Hainault Forest reflects their resilience as keystone species.
- Oaks usually reproduce sexually and have hybridization potential between two different species [2].
- They could also exchange genes through chloroplast and mitochondrial genomes [2,3,4].
- Various chloroplast genotypes are shared from survivors of ice age refugia [5].



Research Questions

- This research aims to analyze the genetic diversity of both species of oaks in Hainault Forest in terms of the nuclear, chloroplast and mitochondria genomes. How diverse are the trees from the online reference genome and the ancient Squires oak?
- Besides, we also aim to identify any evidence of hybridization between the two oak species? Is the "line" between the two species strictly drawn or do they exhibit mixed phenotypes? Does this increase the diversity in the oak species?
- By having this information, how does this reflect the health status of the oak population in Hainault Forest? Are they resistant to climate change and rising global temperature?

Methods

The data collection

- 97 oak leaf samples from Hainault Forest - 22 *Q. petraea*, 75 *Q. robur*.
- Followed by DNA extraction, the whole genomes were sequenced via low-coverage Illumina sequencing and the reads were assembled using reference genomes provided by the European Nucleotide Archive.

Using reference allele

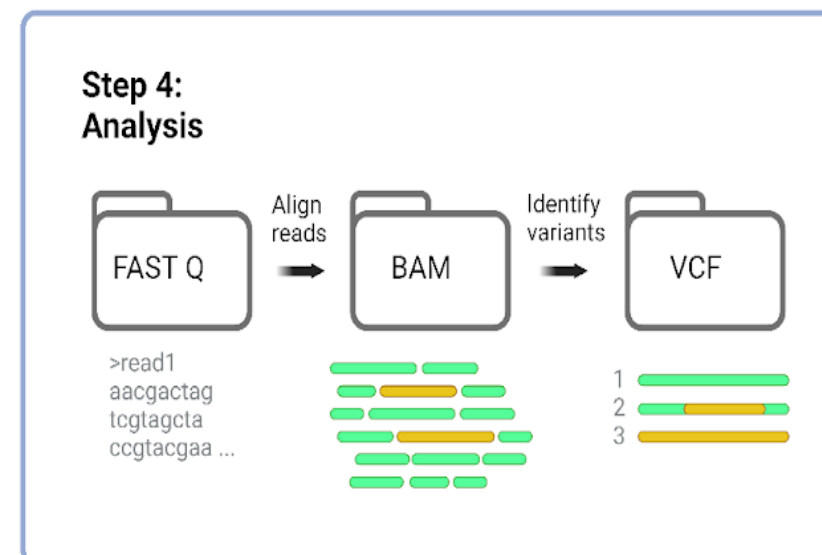
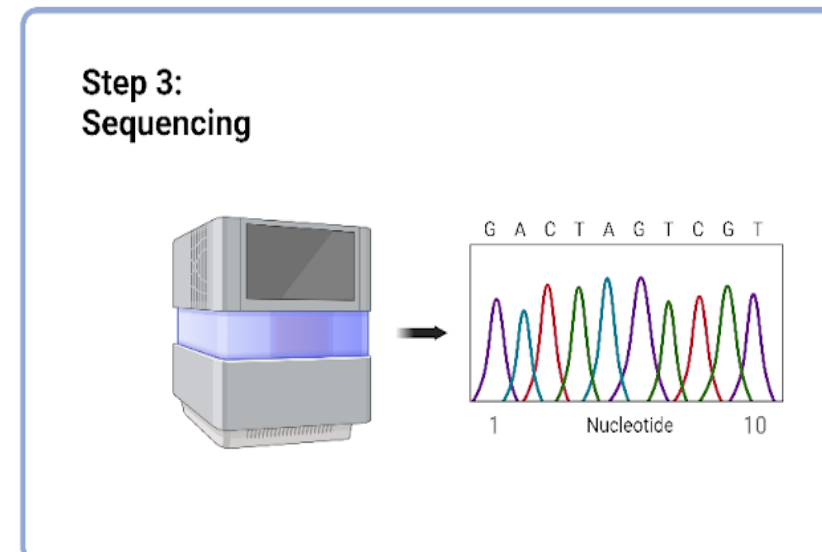
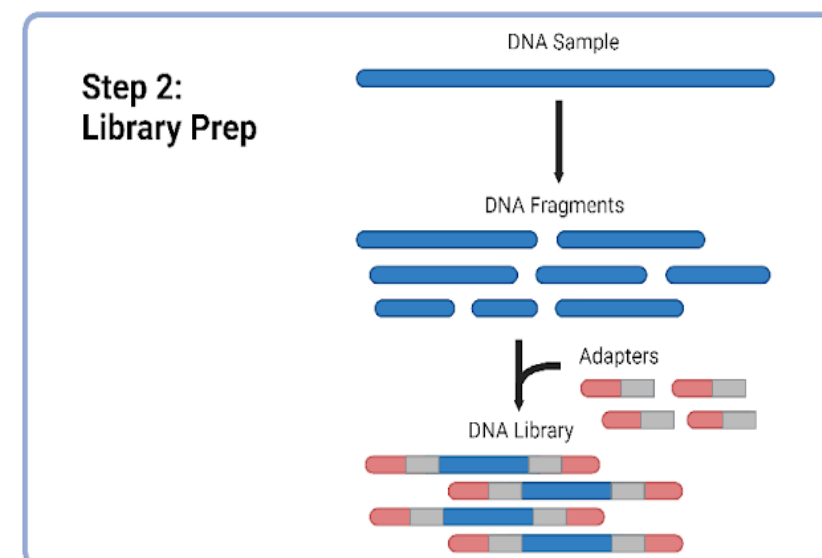
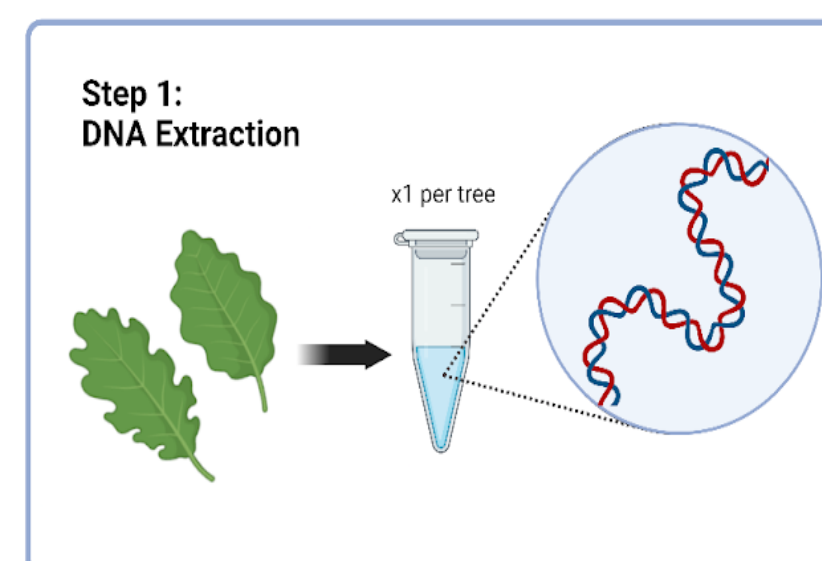
- SNP score is assessed by comparing the sample alleles from the reference alleles: 0 for similar homozygosity; 1 for heterozygous; 2 for different homozygosity.
- Averaging method:
 - $\sum(\text{Sum for SNP scores in each chromosome} \div \text{Total number of SNPs in each chromosome})_n$
 - $n = 12$ (12 chromosomes)
- Mapping method:
 - gg-plo*t with colour gradient to indicate the level of similarity

PCA plot

- Using the SNP scores of each sample, PCA was then performed to illustrate clusters that represent two species and their hybrid.

Heatmap

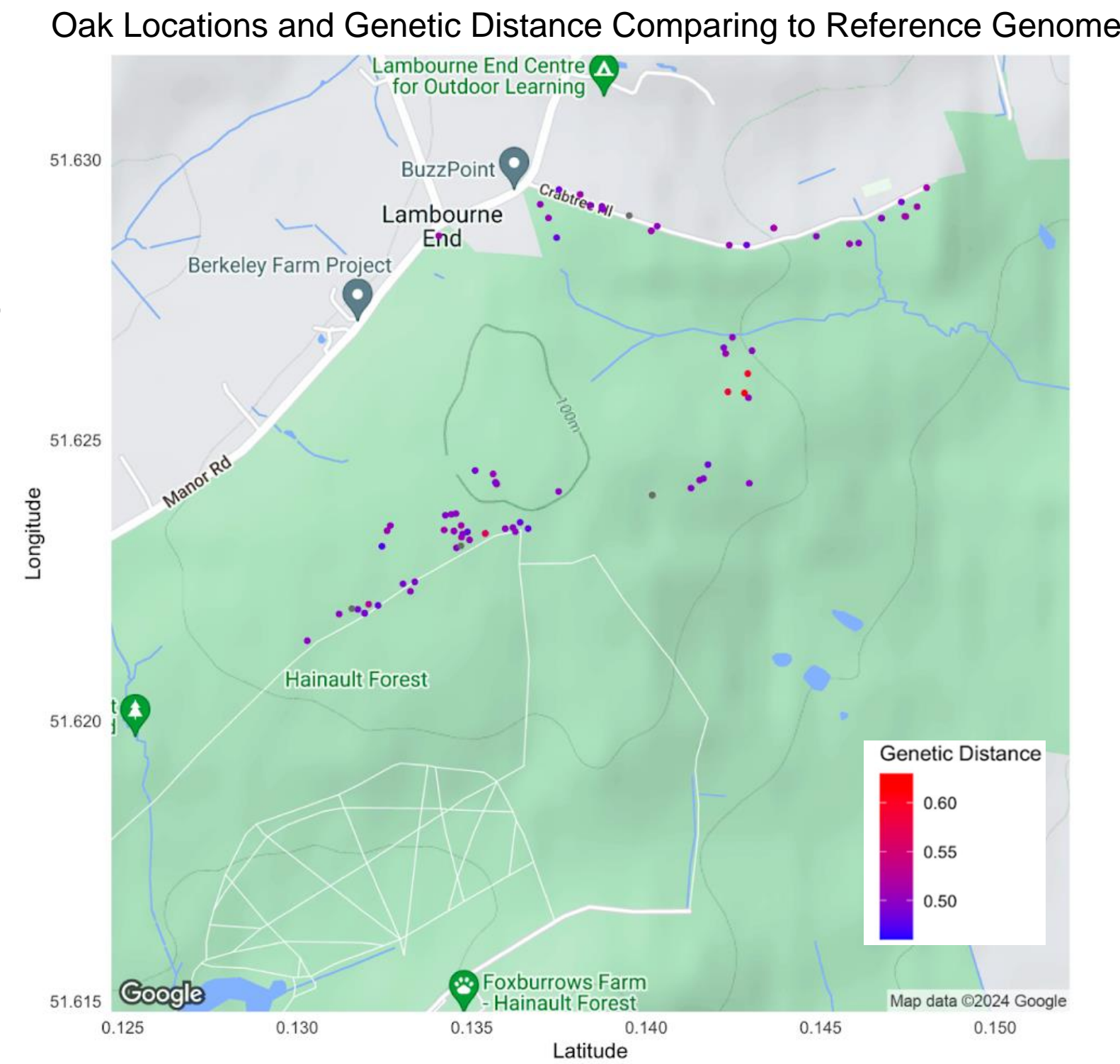
- Using the correlation matrices, heat maps were constructed using *pheatmap()* function
- Squires Oak (the ancient tree): The column of the correlation matrix was extracted and converted into a colour gradient for GPS points.
- A separate map was produced showing the location of each species (pedunculate or sessile oak).



Results

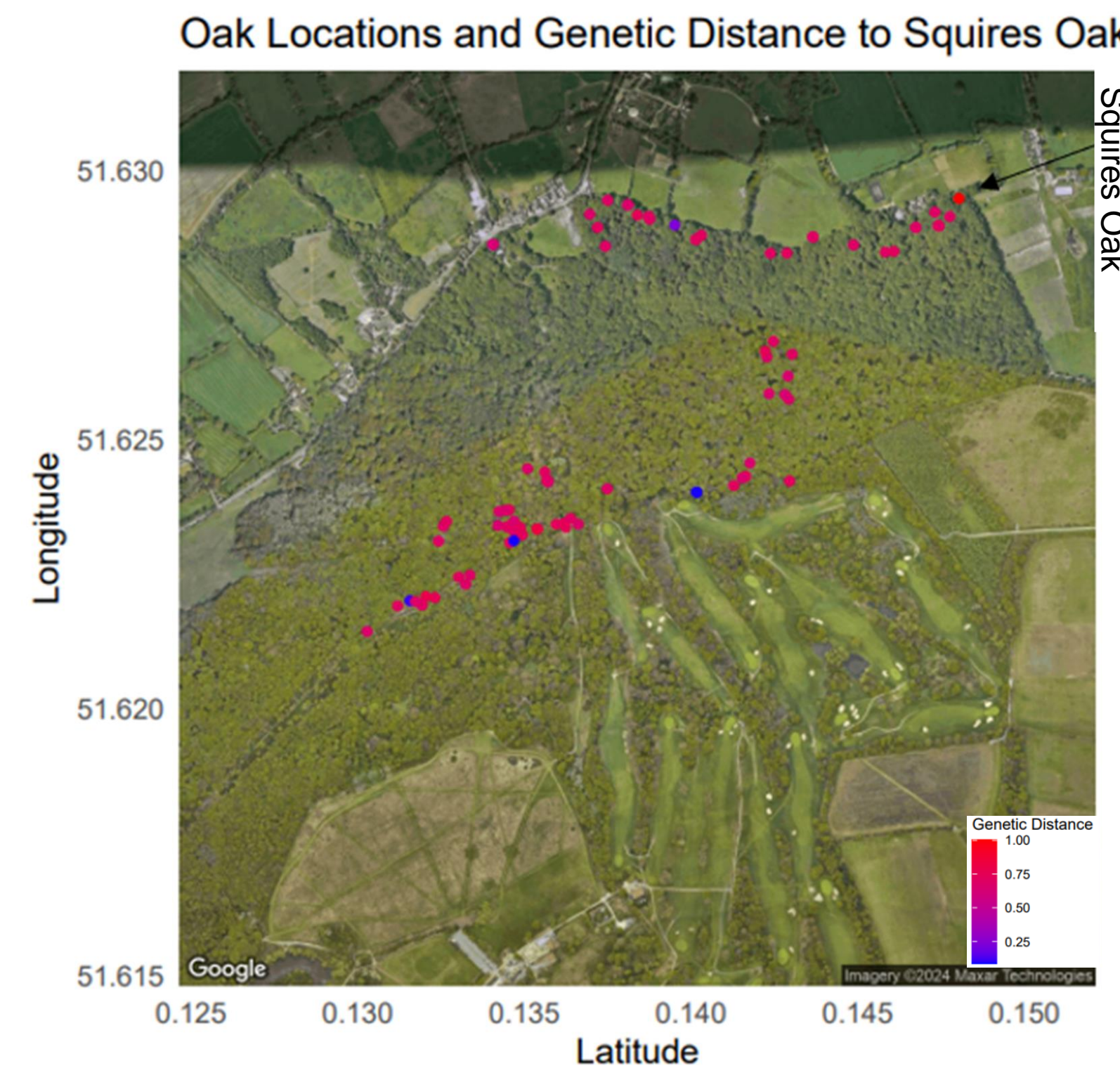
Assessing diversity using reference genome as comparison

- Purple: Most trees have high relatedness to the reference genome - distributed both in clusters and parallel to the trail. This suggests evidence of cutting-based propagation.
- Red: Only a total of four trees that have lower relatedness - three of them are spatially clustered. This indicates a localized-based sexual reproduction.
- Grey: Some were found to have significantly lower relatedness compared to the reference genome and are randomly dispersed with other red- and purple-coded trees. This postulates sexual reproduction or replantation from foreign sources in a random manner.



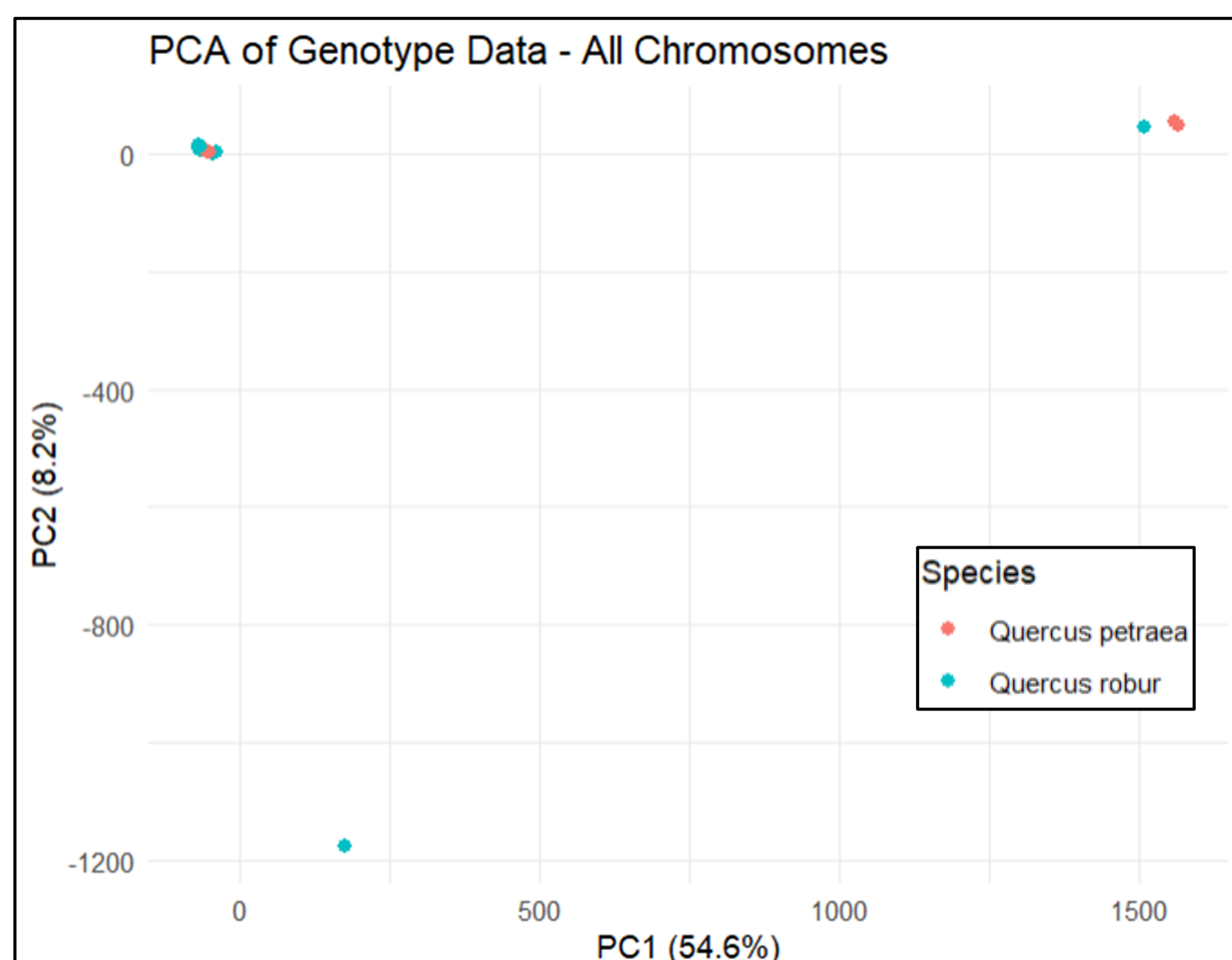
Assessing diversity using ancient tree as comparison

- No gradient of spatial relatedness (relatedness-distance function) from the Squires oak.
- This absence is independent to the species of the oak.
- Possible inference: High pollen dispersion distance from the Squires oak & an extensive hybridization and interfertility between the oak species [2].

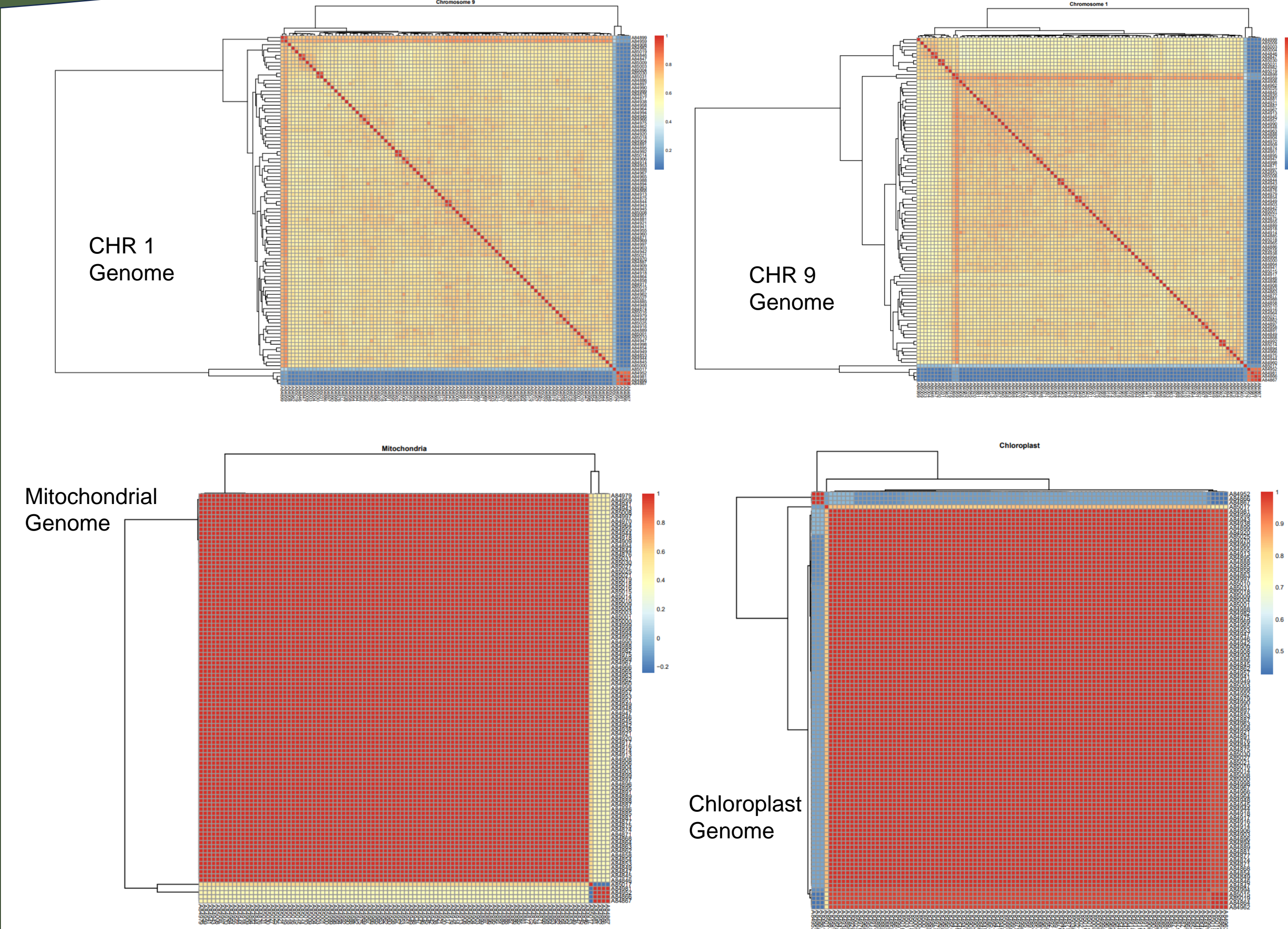


Assessing hybridization using PCA plot

- Top: Two distinct clusters indicate that the genetic elements from *Q. petraea* and *Q. robur* are different.
- Some trees are in the cluster of another species
- Bottom: The only anomaly, possible evidence of hybridization.
- This potentially suggests that introgression may be the mechanism of hybridization - interspecies genome incorporation through backcrossing.



Heatmap Analyses



The heatmaps show high similarity between the mitochondrial and chloroplast genomes of *Q. robur* and *Q. petraea*, with minimal and no differences in mitochondrial and chloroplast haplotypes respectively, indicating extensive genome sharing. Nuclear genome heatmaps from CHR 1 and CHR 9 reveal familial relationships between the two species.

Conclusion

- The significant genetic relatedness among individual oaks reflect a high familial relationship between them, suggesting they are planted through cuttings (asexual reproduction).
- Spatial genetic relatedness is not strongly influenced by geographical distance.
- No evidence of hybridization between *Q. robur* and *Q. petraea*, where their genetic signatures are reflective of significant variations within their DNA sequences
- If hybridization is present, it could be mechanized by introgression, which is the exchange of genetic materials between species through back-crossing.

Future Implications

- Identifying clusters with high genetic distance to reveal factors driving diversity.
- Enhancing propagation practices and vetting replanting efforts can preserve genetic health.
- Conservation strategies should prioritize habitat protection and diversity enhancement.
- Identifying hybrid variants of *Q. robur* and *Q. petraea* using fine-scale analyses, e.g., STRUCTURE and ADMIXTURE [6] to study the natural mechanisms of hybridization for the resilience and longevity of oak population.
- Identifying chloroplast haplotype to suggest the strategy deployed by oaks to survive and spread since the last ice age [1], to provide insights for the efforts to preserve oak in the midst of global temperature change.

References

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