

Introduction

Tilia cordata and *T. platyphyllos* are historically important trees. These species naturally hybridise producing *T. x europaea* (Common Lime). Since the 17th century Common Limes have been planted, forming iconic ancient lime avenues in areas such as Ascot park, Bushy Park, Sussex, Westonbirt, Silverdale and Shipton Court. Hatfield Tall and Pallida are common types of hybrid species. In order to conserve the genetic integrity of lime avenues, a genotype database is required for accurate species identification, as morphological identification alone is sometimes insufficient. To identify cultivar species, size differences in microsatellite regions can be analysed. The variation in microsatellites and allele frequency can be used to identify species. Microsatellites are simple sequence repeats usually in noncoding regions of DNA. The variation rate in microsatellites is often high due to mutations. During DNA replication of meiosis the strands can bind incorrectly leading to slipped strand mispairing. These mutations change the nucleotide sequence and therefore the size of microsatellite alleles.

Aims

- To establish a genotype database of cultivars from ancient lime avenues using microsatellite alleles. Cultivar samples Ascot park, Bushy Park, Sussex, Westonbirt, Silverdale and Shipton Court, will be analysed and the species
- Identify whether 22 cultivar samples from a clonal patch in the National Trust area Westonbirt originate from one genome of a 2000 year old *T. cordata*.

Methods

DNA was extracted using the CTAB method(Doyle and Doyle 1987):

- 1cm² cutting of leaf material was ground to a 'green soup solution' with CTAB buffer and PVP (fig.1)
- The solution is heated to 60°C for 25 minutes (fig.2)
- A series of buffer washes is carried out until a pellet forms (fig.3) and the tube placed in the freezer for the precipitation step to occur
- Gel electrophoresis was used to quantify the presence of DNA, by running samples on agarose gel, stain with ethidium bromide, and visualising under UV light (fig.4)

After DNA extraction polymerase chain reaction (PCR) was used to amplify the 14 microsatellite regions(Phuekvilai and Wolff 2013). Forward and reverse primers, which anneal to regions before and after the microsatellites, were used to amplify microsatellites. The PCR product was analysed using a genetic analyser, and Genemapper was used to score microsatellites. GenALEX 6.5 (Peakall and Smouse 2012)software was used to create a PCA.

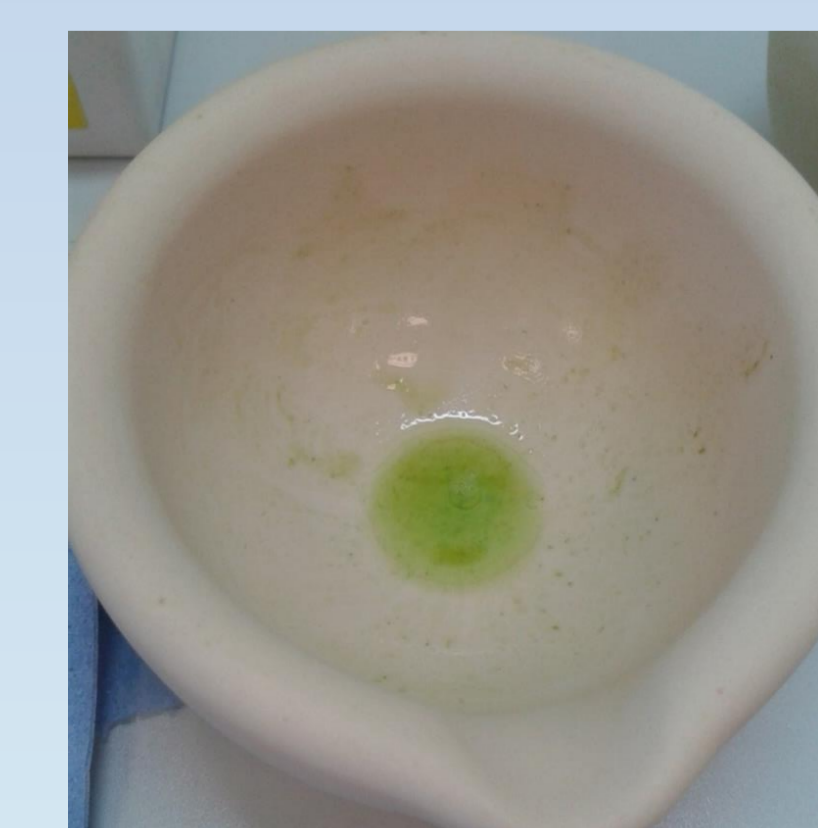


Figure 1. 'green soup solution' of ground leaf material with CTAB buffer and PVP



Figure 2. 'green soup solution' in an eppendorf tube heated to 60°C for 25 minutes



Figure 3. white pellet formation of DNA from *Tilia* tree ready to be dissolved and diluted

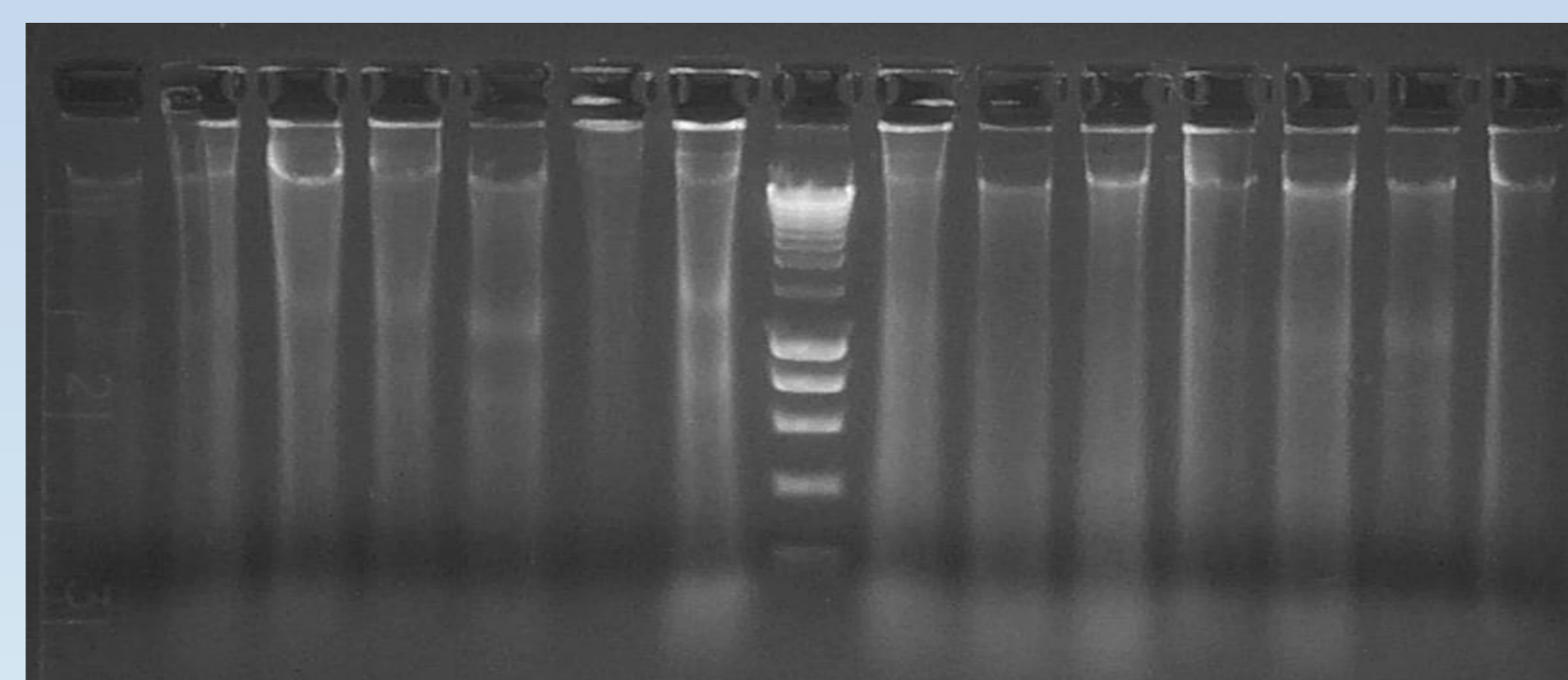


Figure 4. Gel electrophoresis image of 14 samples of the 22 cultivars from Westonbirt. A ladder was loaded into lane 8 and samples in all other lanes. Image shows white bands quantifying the presence of DNA

Results and Discussion

Overall 42 cultivar samples were extracted and analysed to create a database. As expected samples from Bushy Park, Ascot Park and Shipton Court are hybrids, these samples are most likely Pallida hybrids, as the genotype is closely related to a known Pallida genotype (fig.5). None of the samples, from the 9 populations, were identified as Hatfield Tall hybrids. However, this finding may be due to the small population size sampled. Samples from CULBA (a commercial grower) and Germany(natural sample) are most likely *T. platyphyllos*, due to close relatedness to a known genotype (fig.5). The variation between genotypes of the same species, such as the known *T. cordata* and the Westonbirt samples, is due to population differences.

The Westonbirt samples are very likely identical and may have originated from one *T. cordata* tree (fig.5). Figure 5 shows 3 different genotypes for the 22 cultivar samples, however, the differences between these genotypes is due to analytic interpretation of data.

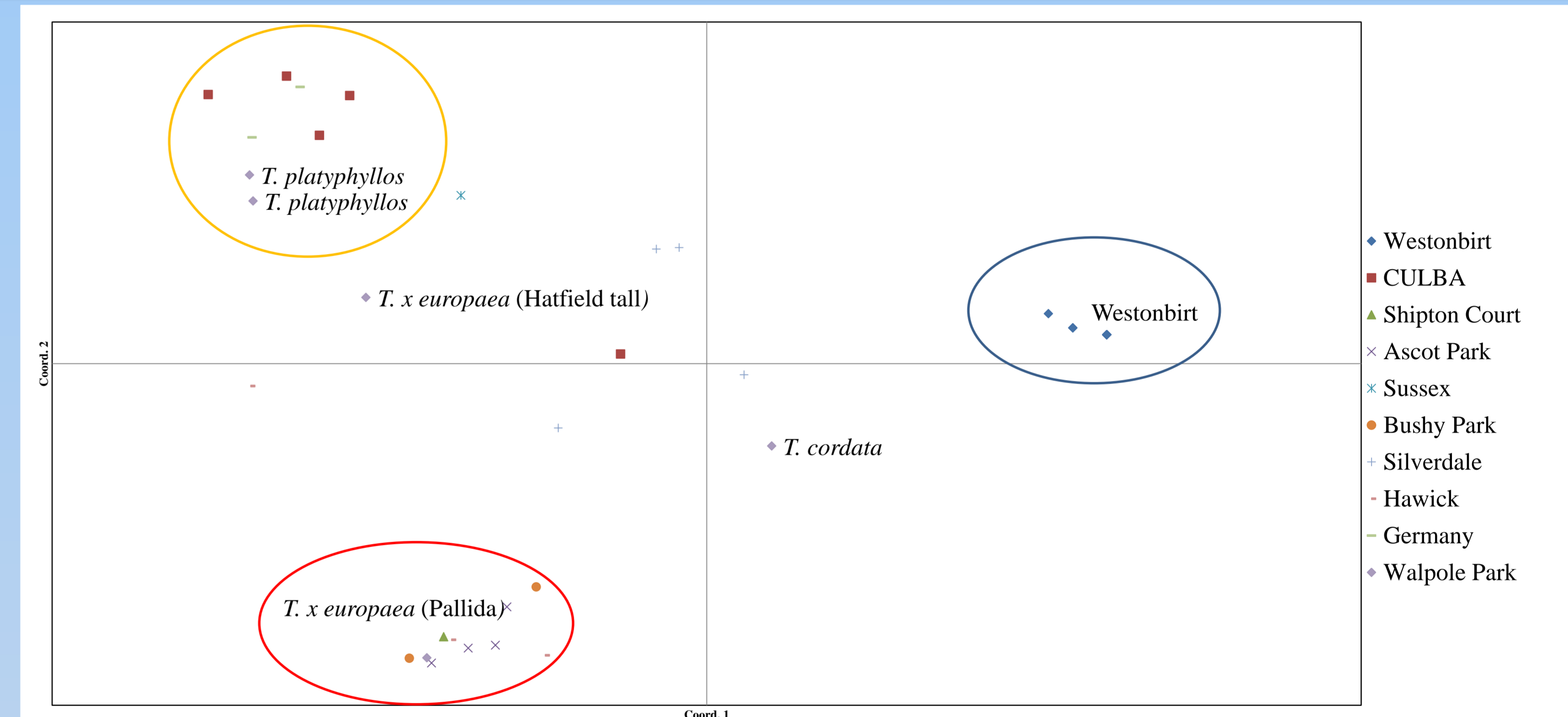


Figure.5 Principal Coordinate Analysis (PCA) showing relatedness of species of 42 cultivar samples, from Ascot park, Bushy Park, Sussex, Westonbirt, Silverdale and Shipton Court, compared to known *Tilia* species.

Conclusion and further studies

- The database set up, for the 42 cultivar samples from various populations, will be used to inform commercial growers, The National Trust and the forestry commission of the hybrid type populating an area. This information can be used to conserve and restore *Tilia* trees.
- The data suggests that the 22 cultivar samples from National Trust Westonbirt are identical, when analysed using 14 microsatellite regions. However, it is possible mutations could have occurred on other microsatellite regions, therefore further studies could analyse different regions. In addition studies could use a different analysis technique to investigate whether these samples are a clonal patch, techniques such as rDNA-ITS.

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References:

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