

WHY?

Climate Change

Multi-purpose Forests

Increase resilience

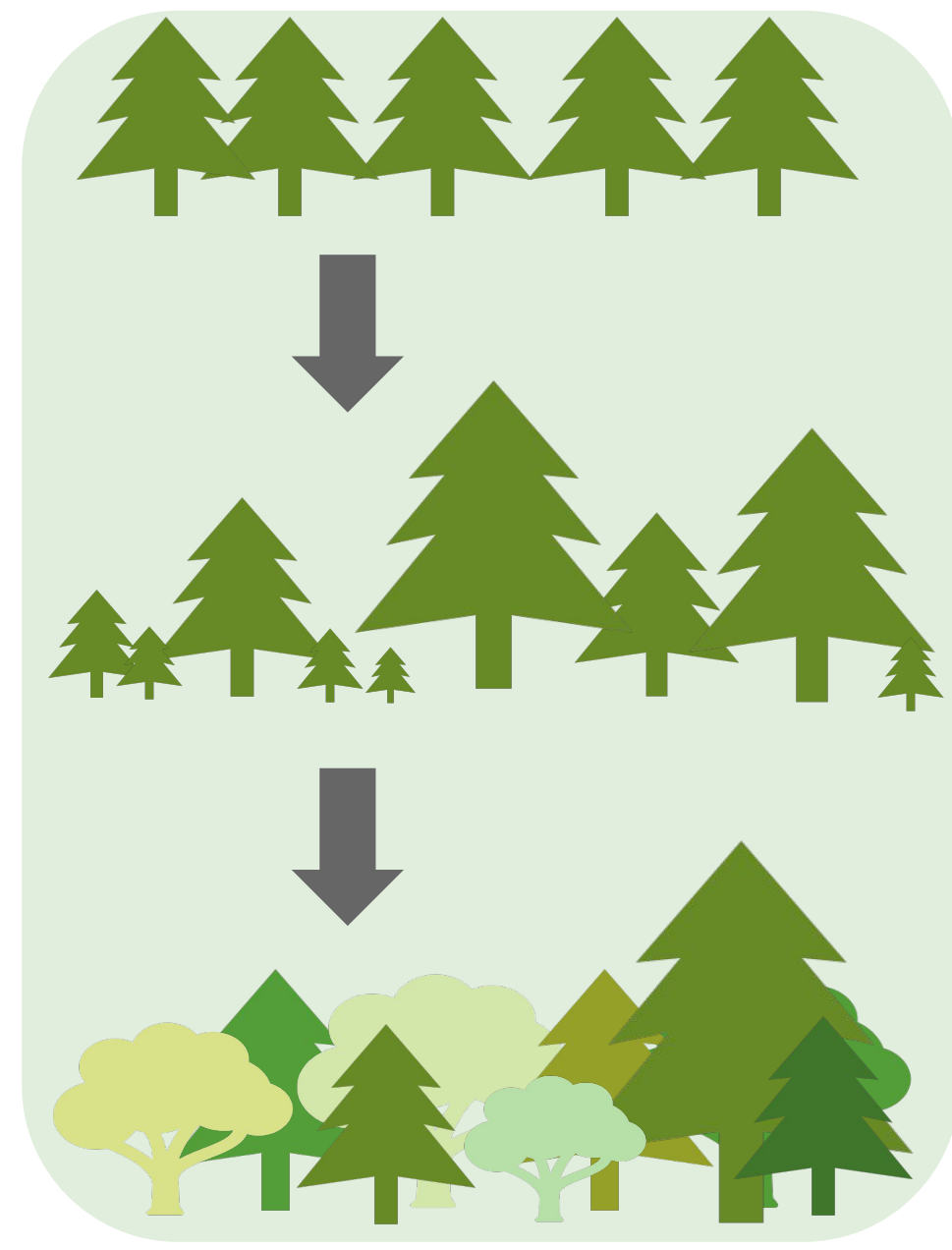
CCF approach:

Ecosystem management

Rely on **natural regeneration** and disturbances

Work with site limitations

Irregular stand structure and a mixture of ages and species



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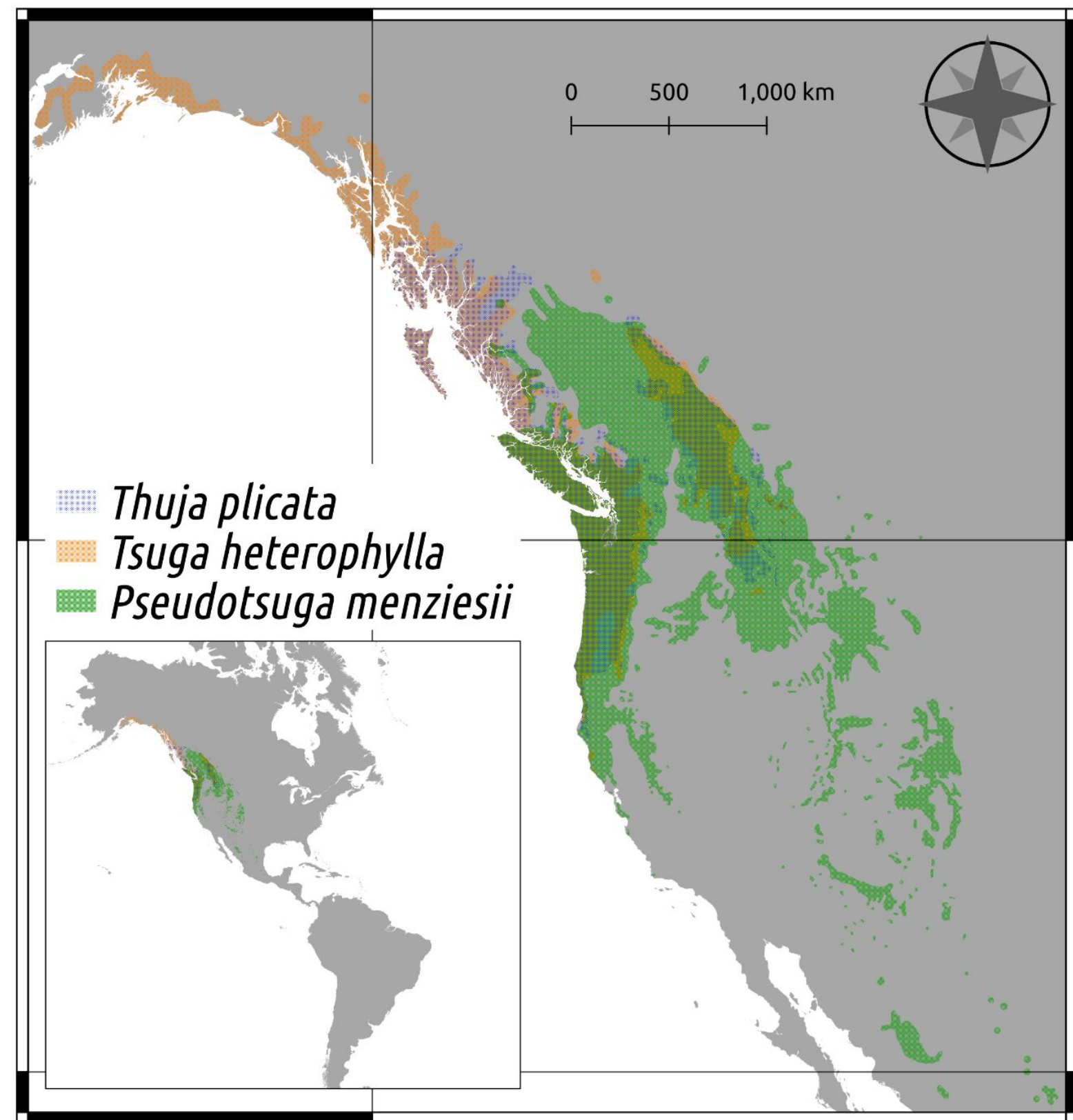
Hypothesis

The planted trees that composed the UK forests may not hold enough genetic diversity to face the current and future disturbances.

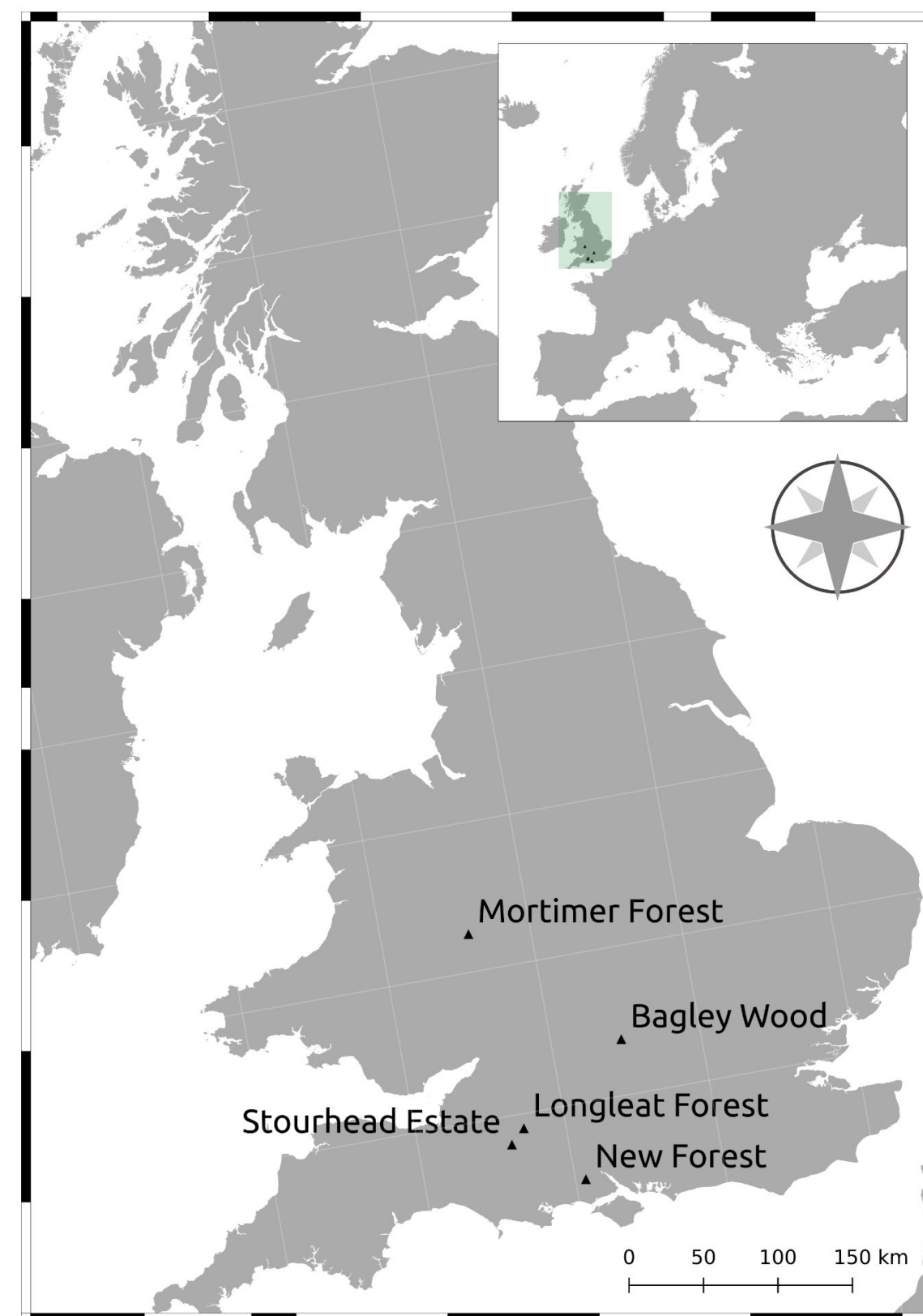
So, how the gene pool is being transmitted to the offspring?

Study Species and UK sites

Species Natural distribution:



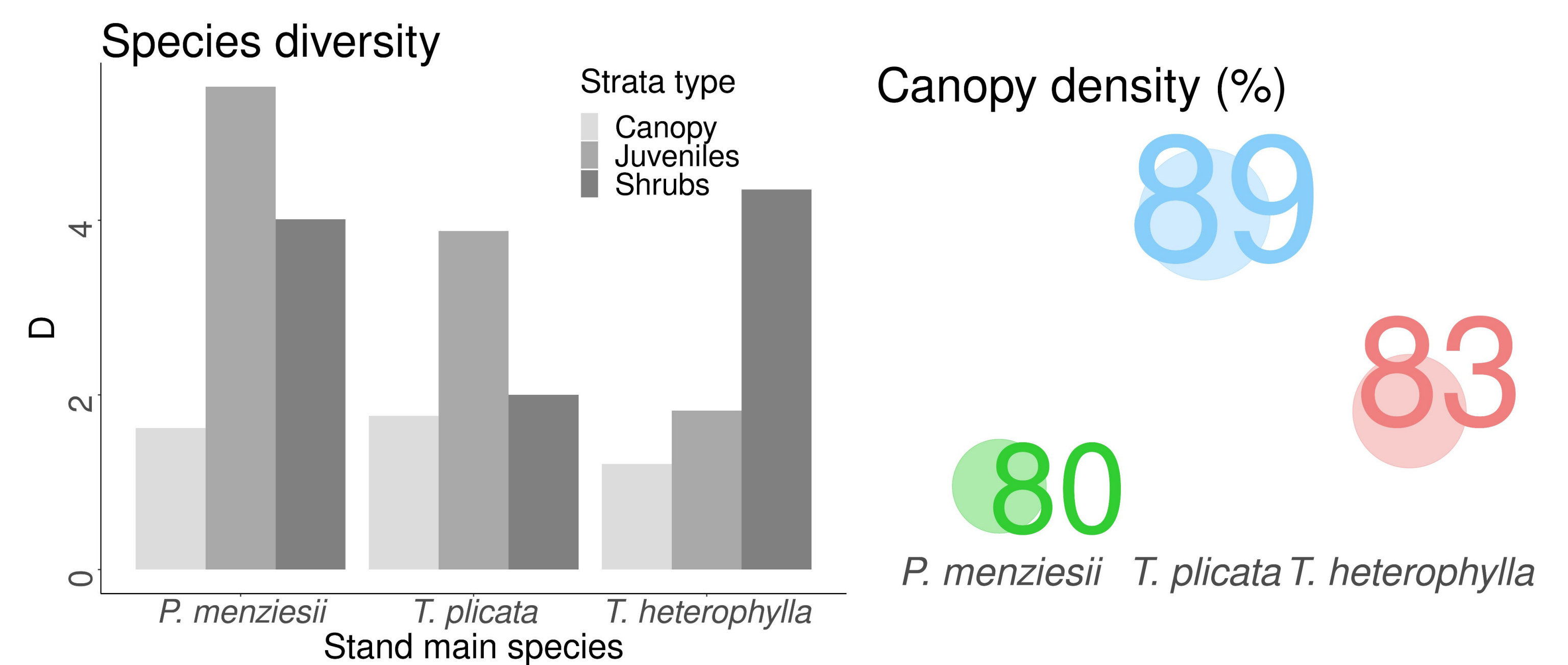
Study sites:



Thuja plicata (*Tp*), *Tsuga heterophylla* (*Th*) and *Pseudotsuga menziesii* (*Pm*) are all evergreen conifers used for timber production both in N. am. and EU. The UK sites are planted forests managed under CCF approach.

Sites characterization

Aim: To investigate the stands CCF stage of transformation



Results (Longleat):

Species diversity in the canopy does not vary among stands.

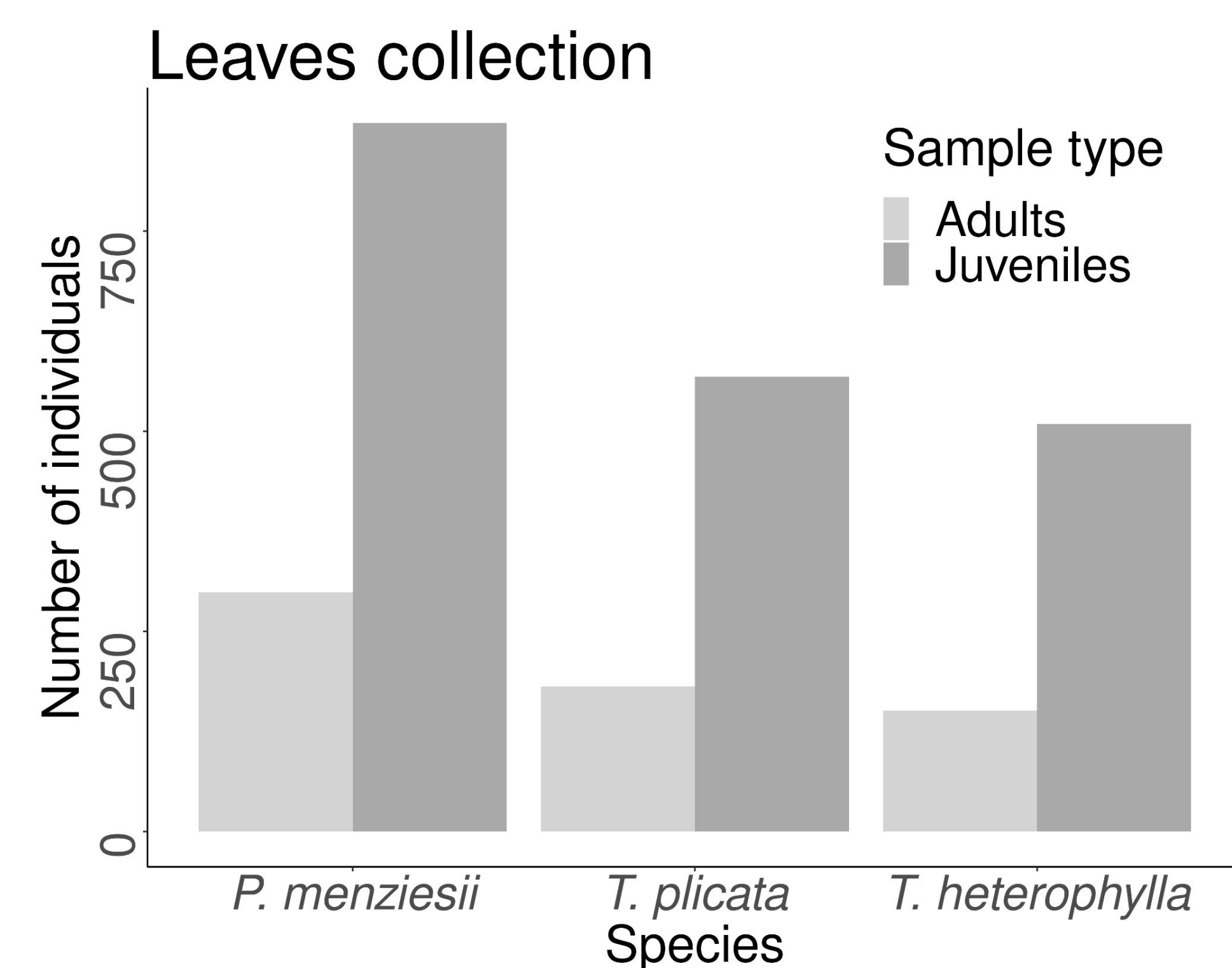
Under *Pm* canopy the diversity of understory is high.

Under *Th* the tree regenerated diversity is low but shows a high shrub species diversity.

Canopy density is higher in *Tp* than in the other stands.

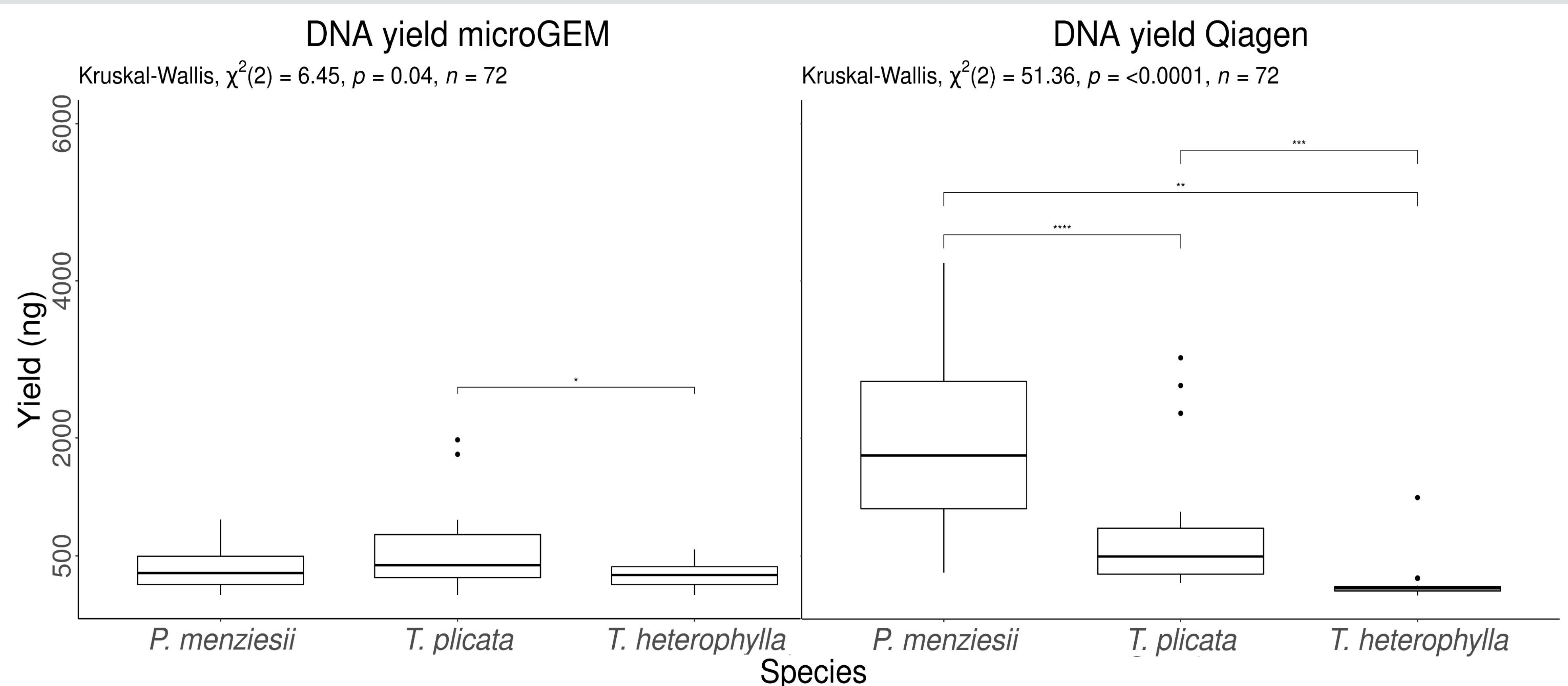
Tissue sampling

Aim: To collect leaves from 50 adults and 150 juveniles per stand and species (when present). Use of an arborist slingshot and dried by storing them with silica gel.



DNA extraction

Aim: To compare the performance in each species of two DNA extraction protocols (microGEM and QIAGEN).



Results: microGEM recovers lower amount of DNA and shows steady results. Qiagen protocol results varied among species.

Genotyping

Aim: To estimate and compare levels of genetic diversity among different groups (adults and juveniles) by studying Single Nucleotide Polymorphisms (SNPs).

To look into the patterns of genetic variation in *Pm* we used available SNPs resources (Howe et al. 2020). To select candidate SNPs to test the genotyping technology (Fluidigm) we performed various filters over the SNPs database. For the other two species we will perform a SNPs discovery extra step.

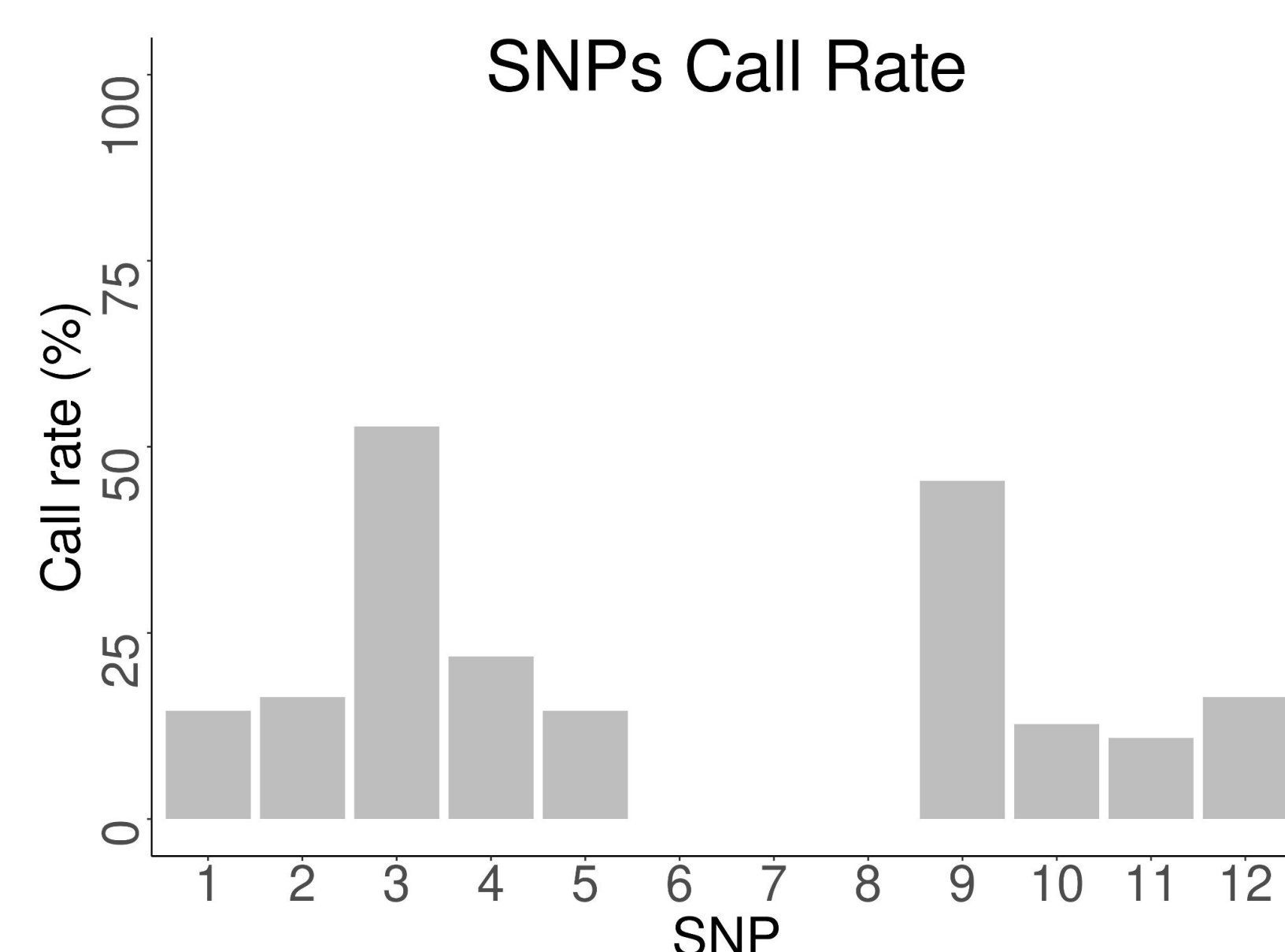
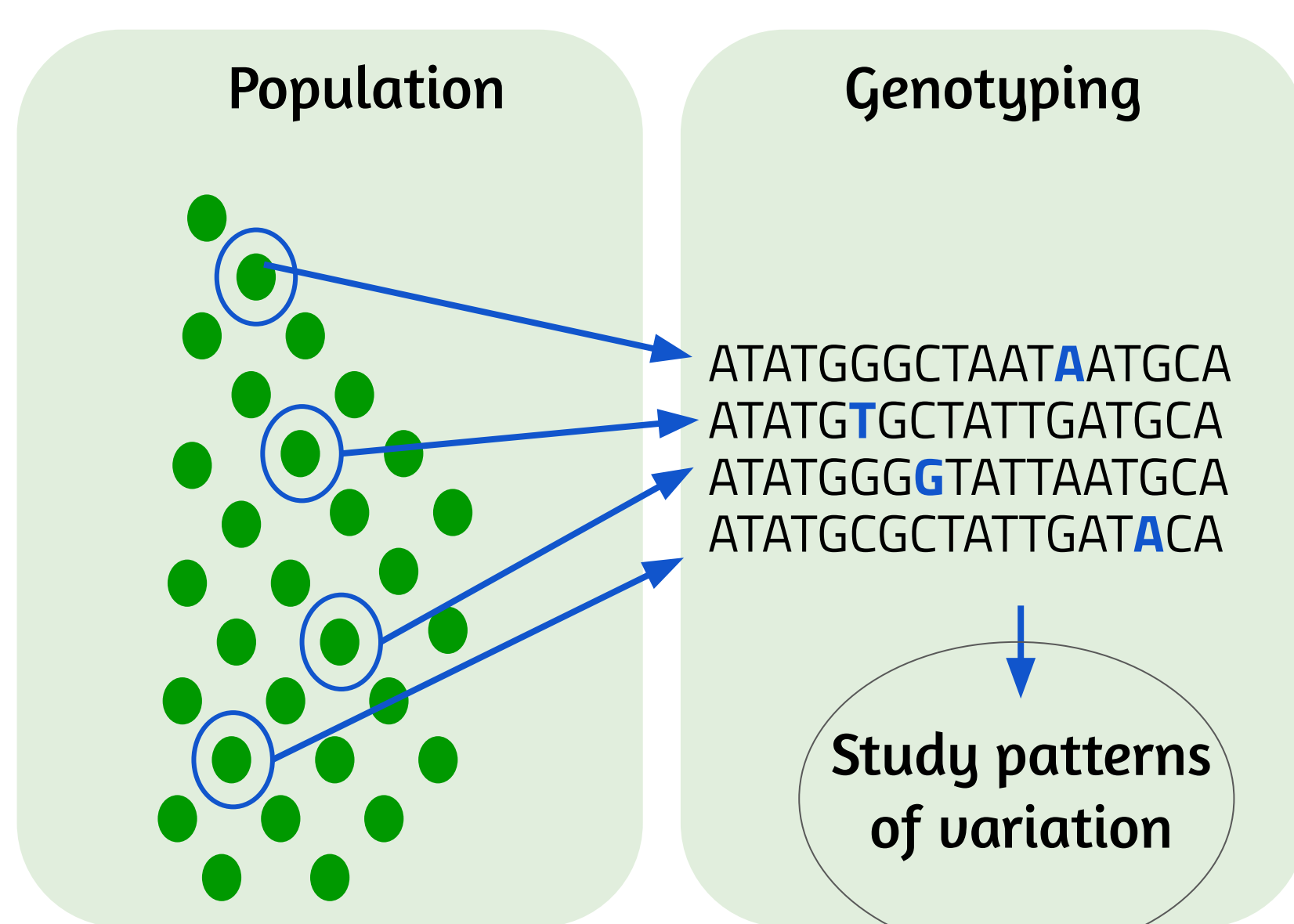
Fluidigm genotyping platform steps:

D3 software to design assays (from SNP sequences)

Genomic DNA pre-amplification

Allele-specific PCR (Juno)

Detect SNPs intensity (Biomark)



Preliminary results: We tested 12 assays on 66 samples. Only two SNPs (3,9) showed a call rate > 45%. Three candidate SNPs did not make any call (6,7,8), while the call rate of the rest is about 20% in average. Further assays optimization is needed.

