No sign of Species-Genetic Diversity Trade-off in Pseudotsuga menziesii (Mirb.) Franco in UK Continuous Cover Forests

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Abstract

Context:

Increasing the structural and species diversity of planted forests may improve their ability to face ongoing environmental shifts. However, converting planted forests to Continuous Cover Forestry (CCF) may reduce genetic diversity of these stands, potentially hindering their ability to cope with environmental changes.

Aims:

To assess the genetic diversity of planted trees and their offspring in six stands of *Pseudotsuga menziesii* at different stages of conversion to Continuous Cover Forestry (CCF).

Methods:

We conducted species diversity surveys and determined the genotypes of 513 individuals in adult and juvenile stand strata.

Results:

The observed heterozygosity was consistently high (0.48 on average); however, the effective population size (Ne) was variable with Ne < 500 at five of six sites. Overall, we did not observe any loss of heterozygosity or statistically significant differences in Ne among generations, i.e., between the adult trees and juvenile seedlings.

Conclusion

Our findings suggest that stand conversion to CCF does not cause a short-term trade-off between species diversity and genetic diversity. Our study highlights the key aspects associated with the maintenance of standing genetic diversity in conjunction with CCF in the face of ongoing environmental changes.

Key message

Transitioning plantations into Continuous Cover Forestry (CCF) effectively increases species diversity without compromising the standing genetic diversity. This ensures that high levels of heterozygosity are maintained across generations, from adult canopy trees to juvenile seedlings. Such diversity is essential for ensuring the long-term potential of planted forests relying on natural regeneration to face ongoing environmental changes.

1. Introduction

Continuous Cover Forestry (CCF) is a forest management approach that avoids large-scale clearfelling, thus maintaining a permanent tree cover. CCF uses silviculture based on ecological and environmental principles, maximising species and structural heterogeneity in the woodland (Mason et al., 1999). Essentially, this approach provides the tools to manage an irregular forest of mixed species considering both the site potential and the multi-use purpose of woodlands (Kirby & Watkins, 2015). This approach has been predicted to be more effective in curbing the impact of climate change than other traditional forestry practices (Helliwell & Wilson, 2012; Mason et al., 1999).

Forest management must also consider the risk that rapid environmental changes will outpace the ability of tree species to adapt to new conditions (Rosenzweig et al., 2008; St. Clair & Howe 2007; Taylor et al., 2015, Tew et al., 2023). To anticipate the potential effects of environmental change on forests, it is vital to understand both the phenotypic plasticity and adaptation ability of forest species to cope with observed and predicted conditions (Kremer et al., 2014; Peters, 1990). Researchers have outlined the potential benefits of the CCF approach for increasing woodland resilience, have

defined silvicultural practises to transform even-aged plantations to an irregular woodland structure (Kerr et al., 2010; Mason & Kerr, 2000), and described methods to monitor the transition (Bennett et al., 2020; Kerr et al., 2017). Currently, CCF is being deployed in some UK woodlands to increase biodiversity, increase resilience, and mitigate climate change while maintaining timber production. However, there is a paucity of studies looking at genetic effects of applying CCF in woodlands.

Finkeldey (2002) discussed the genetic impact of forests being transformed into CCF associated with a change in the population density due to selective cutting. A decrease in the number of individuals that could contribute genetically to the offspring (effective population size (Ne)) leads to an increase in inbreeding and selfing, limiting the gene pool of future natural generations. Similarly, Smith et al. (2025) suggest that there may be a trade-off between the increase of species diversity and the levels of genetic diversity due to the reduction of individuals per species. As a result, future stands may have reduced standing genetic diversity and be less able to adapt to emerging climate stressors (Finkeldey 2002). Additionally, when primary trees hold a reduced gene pool as may be observed in planted woodlands, the vigour of the future forest may be further compromised. To address this issue, several genetic diversity indexes are used, such as heterozygosity or nucleotide diversity; however, Ne has become an important parameter for monitoring and conserving genetic diversity (Capblancq et al., 2020; Hoban et al. 2020; Slavov et al., 2012; Wojacki et al., 2019).

Pseudotsuga menziesii (Mirb.) Franco has become a predominant introduced species in UK CCF-managed forests. Several studies have investigated the genetic diversity of planted *P. menziesii* stands in central Europe (Eckhart et al., 2017; Pakull et al., 2021; van Loo et al., 2019; Wojacki et al., 2019), including a comparison between adults and juveniles (Neophytou et al., 2019) but none have included woodlands in the UK nor woodlands under CCF. Essential genetic resources are available for *P. menziesii*, including a genome sequence (Neale et al., 2017), comprehensive transcriptome (Howe et al., 2013), and informative Single Nucleotide Polymorphism (SNP) datasets (Howe et al., 2013, 2020; Krutovsky & Neale, 2005; Müller et al., 2012).

Trade-offs in genetic diversity may result from managing to increase species diversity, such as in the context of CCF (Smith et al. 2025). Specifically, we hypothesized that CCF may reduce genetic diversity from one generation to the next because CCF may rely primarily on natural regeneration. To test this hypothesis, we analysed planted *P. menziesii* trees and their naturally regenerated offspring using PCR-based genotyping methods. The main goal of this study was to assess genetic diversity differences among the six sites and between adults and their juvenile offspring from UK woodlands in transition to CCF. Specific objectives were to: 1) characterise the level of transformation of the CCF stands based on species diversity and stand structure; 2) design and validate a reliable SNP genotyping assay; and 3) compare the genetic diversity in planted adults and naturally regenerated juveniles within each stand and among stands.

2. Material and methods

2.1. Study sites: structure and species diversity

We sampled six sites at different stages of CCF development located in England (UK) that included *P. menziesii* as part of the canopy. These sites, which are identified as Ringwood (R), Mortimer (M), Stourhead1 (S1), Stourhead2 (S2), Longleat1 (L1), and Longleat2 (L2) (Fig. 1), were composed of at least 100 adult trees as recommended by Wojacki et al. (2019).

We characterised the stand structure and diversity by performing a forest survey (Northwest Natural Resource Group, 2014). We established up to 25 randomly located plots at each site using QGIS (QGIS, 2023). We used 8 m and 3 m radius plots for the adults and juvenile offspring, respectively. We measured each tree over 10 cm in diameter inside the plot. We measured diameter at breast height (DBH) using a DBH tape, and height using the Bitterlich Relascope method (Bitterlich, 1984). We estimated the overall canopy density of each plot by using a densiometer and the basal area using the Bitterlich Relascope (Bitterlich, 1984).

Species diversities of adult canopy trees and juvenile offspring were estimated using the Shannon diversity index, H', (Shannon, 1948). To do so, we counted the number of species and their frequencies in each plot, and then applied the following formula (1) where S = species richness (i.e., number of species) and pi = relative abundance of species i.

$$H\prime = -\sum_{i=1}^{S} pi \cdot \ln(pi)$$

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2.2. Overview of genetic analyses

We designed two SNP assays that offer cost-effective genotyping of informative SNPs. SNPs were genotyped by using the 1) Standard Biotools microfluidics SNP genotyping platform (Std Biotools, San Francisco, CA, USA) (Std Biotools assay) and 2) MassARRAY® iPLEX GOLD technology (Agena Bioscience, San Diego, California, USA) (MA assay). The initial objective was to develop a Std Biotools assay for use in our laboratory; however, we were able to validate and to extend the dataset by using an independent external platform, i.e., MA assay, which was available for *P. menziesii* and has been used in other conifers (Godbout et al., 2017; Trembizki et al., 2014; Vidal et al., 2017).

To develop the Std Biotools assay, we selected 96 SNPs from available resources of 28,904 SNPs (Howe et al., 2020) to design the panel based on desirable SNP characteristics (Fig. 2). The MA assay includes two 40 sets of SNPs. The first set was previously developed and tested by Howe et al (unpublished), and the other set was designed through a collaboration between the Conifer Breeding Co-operative, the University of Oxford, Forest Research, and the UK Centre for Hydrology and Ecology. These MA assays allowed us to increase the number of markers to provide better statistical power in our population genetics analysis.

2.2.1. Sampling and DNA isolation

Foliage was collected from canopy trees following Youngentob et al. (2016) using an arborist slingshot. We collected foliage from naturally regenerated seedlings near every adult that was sampled. We collected leaf material from around 50 adults and 50 juveniles to estimate Ne (Manunza et al., 2025) at each site, for a total of 513 sampled trees. DNA was isolated using the DNeasy Plants mini kit (Qiagen, USA, Valencia, CA) following Guillardin et al. 2023.

2.2.2. SNP assay design & genotyping

The Std Biotools assay (Fig. 2) was developed by selecting 96 SNPs from the SNPs discovered by Howe et al., (2020). We filtered by minor allele frequency (MAF) > 0.4 because SNPs with high MAFs may represent common variants that have higher likelihoods of being polymorphic in our study populations. We aligned the SNP sequences to the *P. menziesii* genome assembly (Neale et al., 2017) using BLAST (Camacho et al., 2009), and kept those with a complete alignment (Fig. 2). We retained only one SNP per scaffold to maximise the genome coverage, and then we used the efetch e-utility tool from NCBI (Kans, 2023) to identify the SNP genome location and corresponding scaffold. This allowed us to extend the flanking sequence to 100 bp to enable primer design. We submitted the SNP sequences to the D3 assay primer designer (Std Biotools) and ordered 156 primers with 40–60% GC content to ensure stable binding to the template DNA. We tested them using FlexSix IFC plates and used the call rate and level of polymorphism on ten samples to select the final 96 SNPs (Fig. 2).

To genotype the samples, we used the 192.24 ICF plates on the Std Biotools microfluidics SNP genotyping platform. We used the Juno system to load and combine the microfluidics and the Biomark (Std Biotools) to perform the PCR. We followed a modified preparation protocol (Std Biotools, 2015a) using the Fast Probe Master Mix (Biotium Inc. Fremont, CA) and avoided the pre-amplification step, which improved the assay performance. The results were visualised using the FluidiGM SNP genotyping software (Std Biotools) (confidence threshold: 50). We removed SNPs that had > 50% missing data.

The MA assay included two multiplexes each containing 40 SNPs which showed high levels of polymorphisms in previous tests using materials from North America (Howe, unpublished) (Fig. 2). The samples were genotyped at the Genome Transcriptome Facility of Bordeaux (INRAE, University of Bordeaux) using the Sequenom MassARRAY® iPLEX GOLD technology (Agena Bioscience).

2.2.3. Genetic diversity analysis

We estimated genetic diversity indicators, including observed heterozygosity (Ho), unbiassed expected heterozygosity (uHe), inbreeding coefficient (Fis), and effective population size (Ne) using both SNP genotyping datasets (assays) individually and combined. We used the gl.report.heterozygosity function from the dartR package (Mijangos et al., 2022) implemented in R studio (R Core Team, 2018; RStudio Team, 2020) to estimate Ho and uHe. We then used the gl.test.heterozygosity function to test differences among age classes and sites. The gl.test.heterozygosity function implements a pairwise re-randomised approach to test heterozygosity differences between defined groups. We used the R package diveRsity (Keenan et al., 2013) to estimate mean Fis and 95% confidence intervals based on 1000 bootstraps. We estimated the contemporary Ne of adults and juveniles using the linkage disequilibrium method implemented in NeEstimator V2.1 software (Do et al., 2014), and used the recommended Jackknife method to estimate confidence intervals (Jones et al., 2016).

3. Results

3.1. Structure and species diversity of study sites

We assessed stand structure and vegetation diversity for each site individually and across all sites. S1 and S2 were the largest sites at > 13 ha, whereas M, R, L1, and L2 had surface areas between 5 and 9 ha. On average, L1 had the tallest *P. menziesii* trees, although the tallest tree (55 m) was found at L2 (Table 1). Canopy cover across all species ranged from 63.5% at L2 to 85.9% at M (Table 1). The basal area was similar across sites, with lower basal areas found at S2 (35.2 m2/ha) and L2 (35.8 m2/ha), and the highest basal area was at M (43.2 m2/ha) (Table 1).

Table 1
Stand characteristics at six forest sites. See methods for details.

Site	Year planted	Site area (ha)	Canopy cover (%)	Basal area (m^2/ha)	Avg. Height (m)	Max. Height (m)
M	1980	5.3	85.9	43.2	27.9	48.0
R	1961	5.8	75.5	40.5	25.1	38.5
S1	1957	13.3	69.6	37.1	25.9	48.0
S2	1939	18.6	73.5	35.2	29.4	44.5
L1	1950	6.3	80.4	38.2	34.1	42.0
L2	1925	8.8	63.5	35.8	26.3	55.0

Species diversity (H') of the juvenile trees was similar across sites, ranging from 1.25 to 1.75, whereas the diversity of the adult trees ranged more widely from 0.25 to 1.75. L2 and S2 had high species diversity (H' > 1.5) in adults and juveniles, M and L1 had very low species diversity in adults (H' < 0.5), whereas S1 and R had intermediate diversity in adults and juveniles (Fig. 3 panel A). S2 had a reverse-j diameter distribution, indicating a late stage of CCF development, but had few trees in the smallest diameter class. L2 and S1 seemed to be transitioning towards a reverse-j diameter distribution but showed a rotated sigmoidal curve, indicating some type of intervention may have occurred. M and L1 had a distribution

profile indicative of a plantation, whereas R had two peaks, which suggests an intermediate stage of transition into a CCF (Fig. 3 panel B) according to Pommerening & Murphy, (2004).

3.2. Genotyping assay

We designed the Std Biotools assay using the 96 SNPs that were most polymorphic, had the highest call rates, and had the lowest levels of missing data (see methods for details). We genotyped 513 individuals across six study sites using these 96 SNPs and then visualised the results using FluidiGM SNP genotyping software. We retained data from 72 SNPs that had < 50% missing data across all sites. The resulting dataset had an overall call rate of 91.2% and contained no private or non-polymorphic SNPs at any of the sites (Fig. 2).

He ranged from 0.47 to 0.48 across sites using the MA platform, compared to 0.48 to 0.49 using the Std Biotools platform. Thus, both platforms gave the same pattern of genetic diversity. Therefore, we combined datasets, resulting in a total of 152 analysed SNPs (Fig. 2).

3.3. Genetic diversity

Each study site had high levels of observed and expected heterozygosity, ranging from Ho = 0.437 in S1 to Ho = 0.467 in S2, and uHe = 0.483 in M to uHe = 0.486 in S2 and R (Table 2). The only significant difference found among sites was between M and R (Table 2). Regarding the genetic diversity within each site, we found no significant differences between adults and juveniles across sites (Table 3). Overall, the inbreeding coefficient (Fis) was relatively low across all sites, ranging from 0.034 to 0.095 (Table 2). However, Fis was significantly lower in S2, L2, and M than in S1 and L1. We found no significant differences in Fis between adults and juveniles within sites (Table 3). Estimates of Ne were first obtained for all trees within a site, both adults and juveniles, and these ranged widely among sites and generally showed large confident intervals. Only L1 had Ne > 500 (Ne = 840), whereas three sites had Ne < 300 (L2, M, and S1), (Table 2). Ne was also estimated separately for adults and juveniles across sites. Although Ne varied substantially, the only statistically significant difference was in M, in which juveniles showed higher Ne than the adult trees (Table 3).

Table 2

Genetic diversity indicators among sites. Asterisks indicate that differences among sites were significant at a p-value < 0.05. Ns: number of samples collected, Ne: effective population size, C.I: confidence intervals for Ne or Fis, Ho: observed heterozygosity, uHe: unbiased expected heterozygosity, S.D: standard deviation, Fis: inbreeding coefficient.

Ns	**S2**	**L2**	**M**	**R**	**S1**	**L1**
	80	**87**	**80**	**89**	**90**	**87**
Ne	425	284.5	210.7	347.5	259	840
lower C.I	242.6	182.9	139	220.8	156.4	345.9
upper C.I	1444.1	583.1	399.5	747.6	635.3	Inf
Ho	0.467	0.464	0.462	0.451	0.437	0.443
Ho S.D	0.073	0.078	0.085	0.068	0.074	0.094
uHe	0.486	0.485	0.483^{-}	0.486^{+}	0.484	0.484
uHe S.D	0.023	0.019	0.025	0.021	0.025	0.026
Fis	0.034^{-}	0.039^{-}	0.038^{-}	0.067	0.092^{+}	0.095^{+}
lower C.I	0.015	0.021	0.019	0.050	0.071	0.068
upper C.I	0.051	0.057	0.057	0.083	0.115	0.120

Table 3
Genetic diversity indicators among sites and age classes. Asterisks indicate that differences between adults (A) and juveniles (J) within each site were significant at a p-value < 0.05. Ns: number of samples collected, Ne: effective population size, C.I: confidence intervals for Ne or Fis, Ho: observed heterozygosity, uHe: unbiased expected heterozygosity, S.D: standard deviation, and Fis: inbreeding coefficient.

	S2		L2		M		R		S1		L1	
	A	J	A	J	A	J	A	J	A	J	A	J
Ns	37	43	42	45	39	41	42	47	43	47	40	47
Ne	479.2	427.5	299.6	266.8	137.1	228.5	1650.2	352.8	348.8	264.3	566.5	410.4
lower C.I	199	205.8	166.8	160.4	96.7	140.1	315.7	192	182.6	161.5	219.6	206.7
upper C.I	Inf	Inf	1212.3	714.7	226.5	565.1	Inf	1693.5	2467	663.6	Inf	5869
Ho	0.468	0.466	0.473	0.452	0.462	0.457	0.452	0.451	0.430	0.438	0.439	0.442
Ho S.D	0.091	0.090	0.090	0.094	0.102	0.098	0.088	0.092	0.095	0.092	0.113	0.101
uHe	0.486	0.484	0.485	0.486	0.481	0.484	0.486	0.486	0.483	0.483	0.483	0.483
uHe S.D	0.027	0.028	0.024	0.024	0.032	0.028	0.026	0.022	0.030	0.030	0.032	0.032
Fis	0.025	0.026	0.013	0.058	0.027	0.045	0.059	0.062	0.099	0.083	0.080	0.074
lower C.I	-9e-04	7e-04	-0.018	0.035	0.002	0.016	0.036	0.039	0.066	0.055	0.045	0.030
upper C.I	0.052	0.049	0.042	0.079	0.048	0.072	0.081	0.086	0.134	0.109	0.113	0.112

4. Discussion

We investigated genetic and species diversity in planted *P. menziesii* woodlands under transformation to CCF in the UK. Species diversity varied among stands with different age class distributions and estimates of genetic diversity varied across genetic diversity indicators. Heterozygosity was high across all sites, both among the planted adults and their naturally regenerated juvenile offspring, and Ne was generally low and highly variable across sites. Overall, our results suggest that there is no trade-off between species diversity and genetic diversity. We discuss how these findings may inform forestry management practices in CCF woodlands, and how our results might contribute to sustainable genetic conservation strategies.

4.1. Site biodiversity and levels of transformation

Based on the criteria described by Pommerening & Murphy (2004), our six *P. menziesii* study sites were at different stages of transformation toward CCF (Fig. 3). Species diversity (H') varied across sites, and higher levels of diversity were found at more structurally complex sites (L2 and S2), which is consistent with other studies (Bravo-Oviedo et al., 2014; Gärtner & Reif, 2004; Kerr et al., 2010, 2017). At three sites, the adult canopy had a Shannon diversity index between 1 and 2, but at one site (M, age 40), it was much lower (H' = 0.17). Pommerening, (2002) reported H' = 0.42 in a 24-year-old *P. menziesii* plantation under transformation to CCF in Germany. The differences between studies suggest that the number of species in a stand often depends on factors other than age, such as silvicultural practices or the ability of new species to regenerate on the site. In two *P. menziesii* plantations on Vancouver Island (Canada), species diversity was H' = 0.77 to

1.01 before commercial thinning (El-Kassaby & Benowicz, 2000). Together, these observations indicate the relative variability of species diversity, which is a main indicator of change in the transition to CCF.

Higher levels of species diversity were reported in pine dominated forests in Northern Europe (H' = 2.9) across three sites classified as near-natural, selected-logged, and managed (De Quesada & Kuuluvainen, 2020). The near-natural pine wood had a reverse-j profile stand structure, similar to S2, the stand that was closest to complete CCF transformation in our study. The managed pine forest had a rotated sigmoidal curve, similar to L2, but its largest diameter class was 50 cm (De Quesada & Kuuluvainen, 2020) whereas L2 had trees over 100 cm.

Overall, the adult trees at L2 had more species diversity and a diameter distribution that indicates a complete transition to CCF. In contrast, S2 had both high species diversity of adult trees and juvenile offspring (H’ > 1.5), which may lead to a more diverse canopy in the future. Both R and S1 had diameter distribution profiles and H' levels that suggested a transition to CCF, whereas M and L1 had a plantation diameter distribution profile and low canopy species diversity (H' < 0.5). This variability indicates that the existing complexity and pathway to CCF forest differ between sites.

4.2. Genetic diversity analysis

Heterozygosity was high across sites (Ho > 0.44 and uHe > 0.48) and no differences among adults and juveniles were found, suggesting no loss of genetic diversity across generations. Other studies found high He rates in European plantations (using microsatellites markers), with no reduction compared to stands in the species natural range (van Loo et al., 2019; Neophytou et al., 2019). These studies suggest that the reproductive material imported to Europe was selected to maintain similar levels of genetic diversity than the original stands. However, contrasting our results, Neophytou et al. (2019) found a significant reduction of He and allelic richness in the offspring of these imported plantations, which may indicate a population bottleneck caused by a limited number of reproducing trees.

Ne is predictive of evolutionary change resulting from genetic drift (Waples & Do, 2010) and it may decrease more rapidly between generations than does heterozygosity (He). Overall, Ne may be a more suitable indicator of genetic adaptability of populations faced with rapid environmental change (Hoban et al., 2022) and to detect bottlenecks. Ne varied from 137.2 to 1650.2 in adults and from 228.6 to 427.5 in juveniles. Only one of the sites (L1) had a Ne > 500. which has been recently accepted as a target threshold to monitor losses of genetic diversity and an indicator to monitor genetic conservation by the Convention on Biological Diversity (CBD) (CBD, 2022; O’Brien et al., 2022). The Ne > 500 target may be too conservative to be applied in our scenario as the study stands were not fully isolated from other *P. menziesii* populations (Mergeay et al., 2026). However, we estimated Ne in each site to detect loss of genetic diversity due to genetic drift between adults and juveniles. Our results showed no significant reduction of Ne in any of the study sites. Only M had non-overlapping confidence intervals between the two groups, (adults = 137.2, and juveniles = 228.6). Here, the higher diversity in juveniles may be driven by external gene flow into the woodland.

5. Conclusion

It is significant that the estimated genetic diversity indicators (heterozygosity and Ne) showed no significant reduction across generations, suggesting no trade-off between genetic diversity and species diversity when transitioning single-species plantations into CCF woodlands. These results contrast with the results of Finkeldey (2002) and Smith et al. (2025), and suggest that reducing the number of trees per species within a site may not affect genetic diversity substantially. Additionally, gene flow from neighbour stands may compensate the reduction of reproductive individuals within the sites. Worldwide, plantations are being transformed into CCF woodlands to better support forest service benefits, such as increasing CO2 sequestration, water quality, and soil protection, among others. CCF introduced woodlands that hold enough genetic diversity from the start may not need an influx of gene flow to increase their potential adaptability. On the other hand, five of our six study sites had a Ne < 500 and though a conservative target, these sites may experience inbreeding depression and genetic drift in the future, which could compromise their adaptability to rapid

environmental change. Assisted gene flow into these sites is recommended to avoid these effects as part of a resilience strategy.

Declarations

Author Contribution

Conceptualization: John MacKay, Laura Guillardin; Methodology: Laura Guillardin, Ella Glover; Formal analysis and investigation: Laura Guillardin; Writing - original draft preparation: Laura Guillardin; Writing - review and editing: John MacKay, Glenn Howe, Laura Guillardin; Funding acquisition: John MacKay, Laura Guillardin; Resources: Glenn Howe; Supervision: John MacKay.The authors read and approved the final manuscript.

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Data Availability

The datasets generated and analysed during the current study are publicly available in the GitHub repository, https://github.com/LauGuillardin/Pmenziesii_supplementary_information

References

1. Bennett G, Hardy A, Bunting P, Morgan P, Fricker A (2020) A Transferable and Effective Method for Monitoring Continuous Cover Forestry at the Individual Tree Level Using UAVs. Remote Sensing 2020, Vol. 12, Page 2115, 12(13), 2115. https://doi.org/10.3390/rs12132115
2. Bravo-Oviedo A, Pretzsch H, Ammer C, Andenmatten E, Barbati A, Barreiro S, Brang P, Bravo F, Coll L, Corona P, Den Ouden J, Ducey MJ, Forrester DI, Giergiczny M, Jacobsen JB, Lesinski J, Löf M, Mason B, Matovic B, Metslaid M, Morneau F, Motiejunaite J, O'Reilly C, Pach M, Ponette Q, del Rio M, Short I, Skovsgaard JP, Soliño M, Spathelf P, Sterba H, Stojanovic D, Strelcova K, Svoboda M, Verheyen K, von Lüpke N, Zlatanov T (2014) European mixed forests: Definition and research perspectives. For Syst 23(3):518–533. https://doi.org/10.5424/fs/2014233-06256
3. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: Architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421
4. Capblancq T, Butnor JR, Deyoung S, Thibault E, Munson H, Nelson DM, Fitzpatrick MC, Keller SR (2020) Whole-exome sequencing reveals a long-term decline in effective population size of red spruce (*Picea rubens*). Evolutionary Applications, eva.12985. https://doi.org/10.1111/eva.12985
5. CBD. (2022) Decision adopted by the conference of the parties to the Convention on Biological Diversity. 15/15 Monitoring framework for the Kunming-Montreal Global Biodiversity Framework. In Convention of Biological Diversity. https://www.cbd.int/doc/decisions/cop-15/cop-15-dec-05-en.pdf
6. De Quesada G, Kuuluvainen T (2020) Tree diametric-species diversity is affected by human impact in old Scots pine dominated forest in boreal Fennoscandia. https://doi.org/10.1186/s40663-020-0219-6
7. Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol Ecol Resour 14(1):209–214.

https://doi.org/10.1111/1755-0998.12157

8. Eckhart T, Walcher S, Hasenauer H, van Loo M (2017) Genetic diversity and adaptive traits of European versus American Douglas-fir seedlings. Eur J For Res 136(5–6):811–825. https://doi.org/10.1007/s10342-017-1072-1
9. El-Kassaby YA, Benowicz A (2000) Effects of commercial thinning on genetic, plant species and structural diversity in second growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands. For Genet 7(3):193–203
10. Finkeldey R (2002) Reproduction in Continuous Cover Forests—The Geneticist's Perspective. In Continuous Cover Forestry (pp. 67–79). https://doi.org/10.1007/978-94-015-9886-6_6
11. Gärtner S, Reif A (2004) The impact of forest transformation on stand structure and ground vegetation in the southern Black Forest, Germany. Plant Soil 264(1–2):35–51. https://doi.org/10.1023/B:PLSO.0000047751.25915.77
12. Godbout J, Tremblay L, Levasseur C, Lavigne P, Rainville A, Mackay J, Bousquet J, Isabel N (2017) Development of a traceability system based on a SNP array for large-scale production of high-value white spruce (*Picea glauca*). Front Plant Sci 8(July):1–13. https://doi.org/10.3389/fpls.2017.01264
13. Guillardín L, MacKay JJ (2023) Comparing DNA isolation methods for forest trees: quality, plastic footprint, and time-efficiency. Plant Methods 19:111. https://doi.org/10.1186/s13007-023-01086-y
14. Helliwell R, Wilson ER (2012) Continuous Cover Forestry in Britain. Quarterly Journal of Forestry
15. Hoban S, Bruford M, D'Urban Jackson J, Lopes-Fernandes M, Heuertz M, Hohenlohe PA, Sjogren-Gulve P, Segelbacher G, Vernesi C, Aitken S, Bertola LD, Bloomer P, Breed M, Rodriguez-Correa H, Funk W, Grueber CE, Hunter M, Jaffe R, Liggins L, Mergeay J, Moharrek F, O'Brien D, Ogden R, Palma-Silva C, Paz-Vinas I, Pierson J, Ramakrishnan U, Simo-Droissart M, Tani N, Waits L, Laikre L (2020) Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. Biol Conserv 248:108654. https://doi.org/10.1016/j.biocon.2020.108654
16. Hoban S, Archer FI, Bertola LD, Bragg JG, Breed MF, Bruford MW, Coleman MA, Ekblom R, Funk WC, Grueber CE, Hand BK, Jaffé R, Jensen E, Johnson JS, Kershaw F, Liggins L, MacDonald AJ, Mergeay J, Miller JM, Muller-Karger F, O'Brien D, Paz-Vinas I, Potter KM, Razgour O, Vernesi C, Hunter ME (2022) Global genetic diversity status and trends: Towards a suite of Essential Biodiversity Variables (EBVs) for genetic composition. Biol Rev 97(4):1511–1538. https://doi.org/10.1111/brv.12852
17. Howe GT, Yu J, Knaus B, Cronn R, Kolpak S, Dolan P, Lorenz WW, Dean JFD (2013) A SNP resource for Douglas-fir: De novo transcriptome assembly and SNP detection and validation. BMC Genomics 14(1). https://doi.org/10.1186/1471-2164-14-137
18. Howe GT, Jayawickrama K, Kolpak SE, Kling J, Trappe M, Hipkins V, Ye T, Guida S, Cronn R, Cushman SA, McEvoy S (2020) An Axiom SNP genotyping array for Douglas-fir. BMC Genomics 21(1):9. https://doi.org/10.1186/s12864-019-6383-9
19. Hoban AT, Ovenden JR, Wang YG (2016) Improved confidence intervals for the linkage disequilibrium method for estimating effective population size. Heredity 117(4):217–223. https://doi.org/10.1038/hdy.2016.19
20. Kans J (2023) Entrez Direct: E-utilities on the Unix Command Line. In Entrez Programming Utilities Help [Internet]. National Center for Biotechnology Information (US). https://www.ncbi.nlm.nih.gov/books/NBK179288/
21. Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA (2013) diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol Evol 4(8):782–788. https://doi.org/10.1111/2041-210X.12067
22. Kerr G, Morgan G, Blyth J, Stokes V (2010) Transformation from even-aged plantations to an irregular forest: The world's longest running trial area at Glentress. Scotl Forestry 83(3):329–344. https://doi.org/10.1093/forestry/cpq015
23. Kerr G, Snellgrove M, Hale S, Stokes V (2017) The Bradford-Hutt system for transforming young even-aged stands to continuous cover management. Forestry 90(4):581–593. https://doi.org/10.1093/forestry/cpx009

24. Kirby KJ, Watkins C (eds) (2015) Europe's changing woods and forests: From wildwood to managed landscapes. CABI
25. Kremer A, Potts BM, Delzon S (2014) Genetic divergence in forest trees: Understanding the consequences of climate change. Funct Ecol 28(1):22–36. https://doi.org/10.1111/1365-2435.12169
26. Krutovsky KV, Neale DB (2005) Nucleotide diversity and linkage disequilibrium in cold-hardiness- and wood quality-related candidate genes in Douglas-fir. Genetics 171(4):2029–2041. https://doi.org/10.1534/genetics.105.044420
27. Manunza A, Cozzi P, Boettcher P, Curik I, Looft C, Colli L, Sölkner J, Mészáros G, Stella A (2025) Estimating the optimal number of samples to determine the effective population size in livestock. Front Genet 16:1588986. https://doi.org/10.3389/fgene.2025.1588986
28. Mason B, Kerr G, Simpson J (1999) What is Continuous Cover Forestry? Forestry Commission, 1–8
29. Mason B, Kerr G (2000) Transforming Even-aged Conifer Stands to Continuous Cover Management. 40:1–8
30. Mergeay J, Gargiulo R, Moodley Y, Russo IR (2026) The Importance of Effective Population Size in Conservation and Biodiversity Monitoring. Evol Appl 19(1):e70196. https://doi.org/10.1111/eva.70196
31. Mijangos JL, Gruber B, Berry O, Pacioni C, Georges A (2022) dartR v2: An accessible genetic analysis platform for conservation, ecology and agriculture. Methods Ecol Evol 13(10):2150–2158. https://doi.org/10.1111/2041-210X.13918
32. Müller T, Ensminger I, Schmid KJ (2012) A catalogue of putative unique transcripts from Douglas-fir (*Pseudotsuga menziesii*) based on 454 transcriptome sequencing of genetically diverse, drought stressed seedlings. BMC Genomics 13(1). https://doi.org/10.1186/1471-2164-13-673
33. Neale DB, McGuire PE, Wheeler NC, Stevens KA, Crepeau MW, Cardeno C, Zimin AV, Puiu D, Pertea GM, Sezen UU, Casola C, Koralewski TE, Paul R, Gonzalez-Ibeas D, Zaman S, Cronn R, Yandell M, Holt C, Langley CH, Yorke JA, Salzberg SL, Wegrzyn JL (2017) The Douglas-fir genome sequence reveals specialization of the photosynthetic apparatus in Pinaceae. G3: Genes|Genomes|Genetics. 7(9):3157–3167. https://doi.org/10.1534/g3.117.300078
34. Neophytou C, van Loo M, Hasenauer H (2019) Genetic diversity in introduced Douglas-fir and its natural regeneration in Central Europe. Forestry: Int J For Res 1–10. https://doi.org/10.1093/forestry/cpz055
35. Northwest Natural Resource Group (2014) Forest Inventory and Monitoring Guidelines. Northwest Natural Resource Group
36. O'Brien D, Laikre L, Hoban S, Bruford MW, Ekblom R, Fischer MC, Hall J, Hvilsom C, Hollingsworth PM, Kershaw F, Mittan CS, Mukassabi TA, Ogden R, Segelbacher G, Shaw RE, Vernesi C, MacDonald AJ (2022) Bringing together approaches to reporting on within species genetic diversity. J Appl Ecol 59(9):2227–2233. https://doi.org/10.1111/1365-2664.14225
37. Pakull B, Eusemann P, Wojacki J, Ahnert D, Liesebach H (2021) Genetic diversity of seeds from four German Douglas fir (*Pseudotsuga menziesii*) seed orchards. Eur J For Res 140(6):1543–1557. https://doi.org/10.1007/s10342-021-01419-3
38. Peters RL (1990) Effects of global warming on forests. For Ecol Manag 35(1–2):13–33. https://doi.org/10.1016/0378-1127(90)90229-5
39. Pommerening A (2002) Approaches to quantifying forest structures. Forestry: Int J For Res 75(3):305–324. https://doi.org/10.1093/forestry/75.3.305
40. Pommerening A, Murphy ST (2004) A review of the history, definitions and methods of continuous cover forestry with special attention to afforestation and restocking. Forestry 77(1):27–44. https://doi.org/10.1093/forestry/77.1.27
41. QGIS (2023) QGIS Geographic Information System. Open Source Geospatial Foundation Project. (3.32.3) [Computer software]. http://qgis.org
42. R Core Team (2018) R: A language and environment for statistical computing (4.3.0) [Computer software]. R Foundation for Statistical Computing. https://www.R-project.org/

43. Rosenzweig C, Karoly D, Vicarelli M, Neofotis P, Wu Q, Casassa G, Menzel A, Root TL, Estrella N, Seguin B, Tryjanowski P, Liu C, Rawlins S, Imeson A (2008) Attributing physical and biological impacts to anthropogenic climate change. Nature 453(7193):353–357. https://doi.org/10.1038/nature06937
44. RStudio Team (2020) RStudio: Integrated development for R (2023.06.0) [Computer software]. Posit. http://www.rstudio.com/
45. Slavov GT, DiFazio SP, Martin J, Schackwitz W, Muchero W, Rodgers-Melnick E, Lipphardt F, Gunter LE, Ranjan P, Tuskan GA (2012) Genome resequencing reveals multiscale geographic structure and extensive linkage disequilibrium in the forest tree *Populus trichocarpa*. New Phytol 196(3):713–725. https://doi.org/10.1111/j.1469-8137.2012.04258.x
46. Smith SW, Cavers S, Luna E, Edwards D, Cottrell J, Kyle C, Nichols C, Stokes V, Mitchell RJ (2025) Using tree species and genetic diversity and epigenetic effects in UK woodland and forests to increase resilience. UKRI Future of UK Treescapes programme research note
47. Standard Biotools (2015a) Genotyping with the 192.24 IFC Using SNP Type Assays. PN:100–3913C1
48. St. Clair JB, Howe GT (2007) Genetic maladaptation of coastal Douglas-fir seedlings to future climates. Glob Change Biol 13:1441–1454. https://doi.org/10.1111/j.1365-2486.2007.01385.x
49. Taylor SA, Larson EL, Harrison RG (2015) Hybrid zones: Windows on climate change. Trends Ecol Evol 30(7):398–406. https://doi.org/10.1016/j.tree.2015.04.010
50. Tew ER, Ambrose-Oji B, Beatty M, Büntgen U, Butterworth H, Clover G, Cook D, Daniels ET, Doick KJ, Doughty LA, Edwards-Jones A, Hemery G, Hogan GP, Jenkins LAR, Lawrence AC, Maclean MC, Moore R, O'Brien L, Pasalodos-Fornas M, Reed K, Simmonds V, Sutherland WJ (2023) A horizon scan of issues affecting UK forest management within 50 years. Forestry: Int J For Res cpad047. https://doi.org/10.1093/forestry/cpad047
51. Trembizki E, Smith H, Lahra MM, Chen M, Donovan B, Fairley CK, Guy R, Kaldor J, Regan D, Ward J, Nissen MD, Sloots TP, Whiley DM (2014) High-throughput informative single nucleotide polymorphism-based typing of *Neisseria gonorrhoeae* using the Sequenom MassARRAY iPLEX platform. J Antimicrob Chemother 69(6):1526–1532. https://doi.org/10.1093/JAC/DKT544
52. van Loo M, Lazic D, Chakraborty D, Hasenauer H, Schüler S (2019) North American Douglas-fir (*P. menziesii*) in Europe: Establishment and reproduction within new geographic space without consequences for its genetic diversity. Biol Invasions 21(11):3249–3267. https://doi.org/10.1007/s10530-019-02045-2
53. Vidal M, Plomion C, Raffin A, Harvengt L, Bouffier L (2017) Forward selection in a maritime pine polycross progeny trial using pedigree reconstruction. Ann For Sci 74(1). https://doi.org/10.1007/s13595-016-0596-8
54. Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evol Appl 3(3):244–262. https://doi.org/10.1111/j.1752-4571.2009.00104.x
55. Wojacki J, Eusemann P, Ahnert D, Pakull B, Liesebach H (2019) Genetic diversity in seeds produced in artificial Douglas-fir (*Pseudotsuga menziesii*) stands of different size. For Ecol Manag 438(February):18–24. https://doi.org/10.1016/j.foreco.2019.02.012
56. Youngentob KN, Zdenek C, van Gorsel E (2016) A simple and effective method to collect leaves and seeds from tall trees. Methods Ecol Evol 7(9):1119–1123. https://doi.org/10.1111/2041-210X.12554

Figures

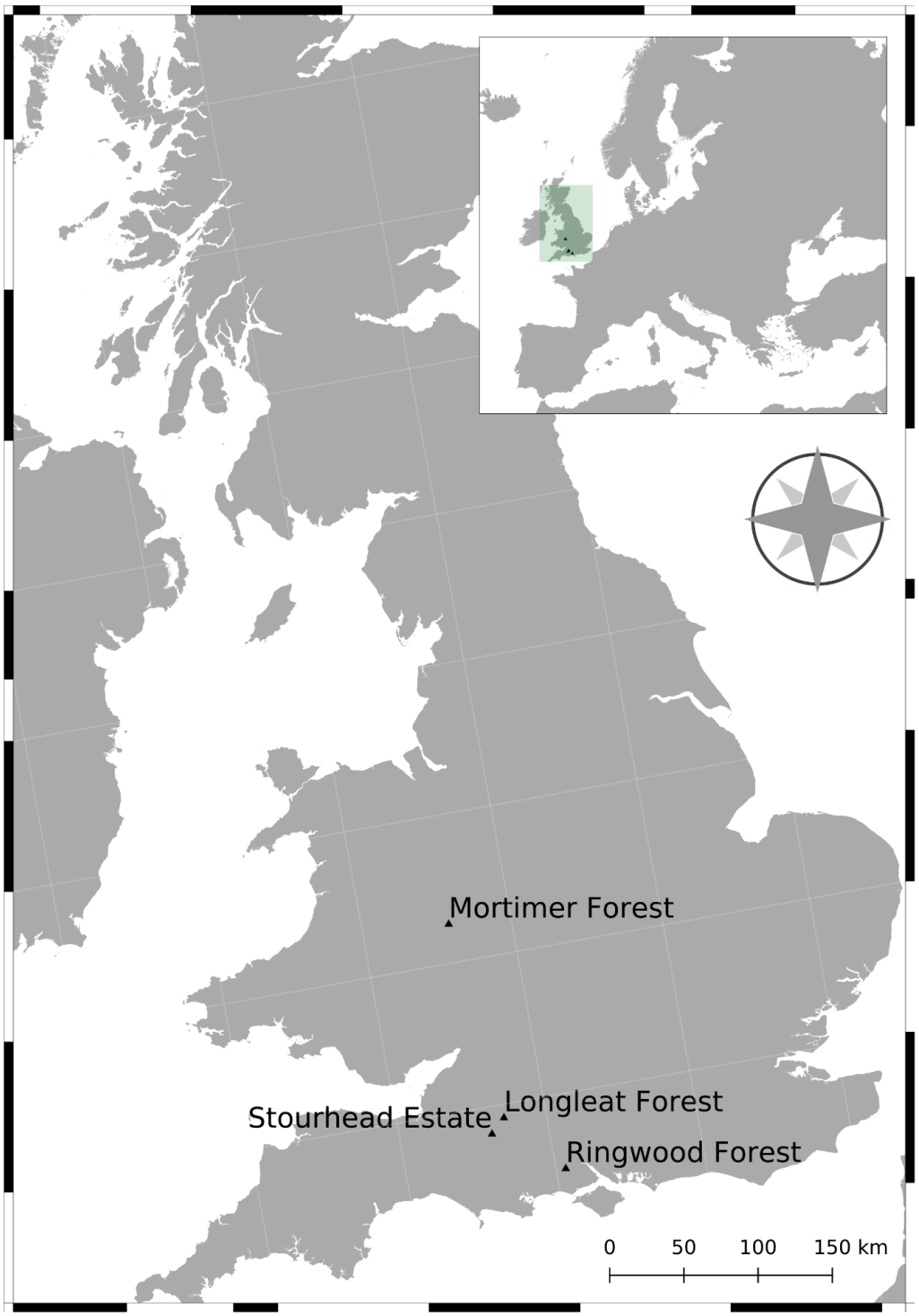

Figure 1

Study site locations. Stand R occurs in Ringwood Forest, M occurs in Mortimer Forest, S1 and S2 are found in Stourhead Estate, and L1 and L2 are in Longleat forest.

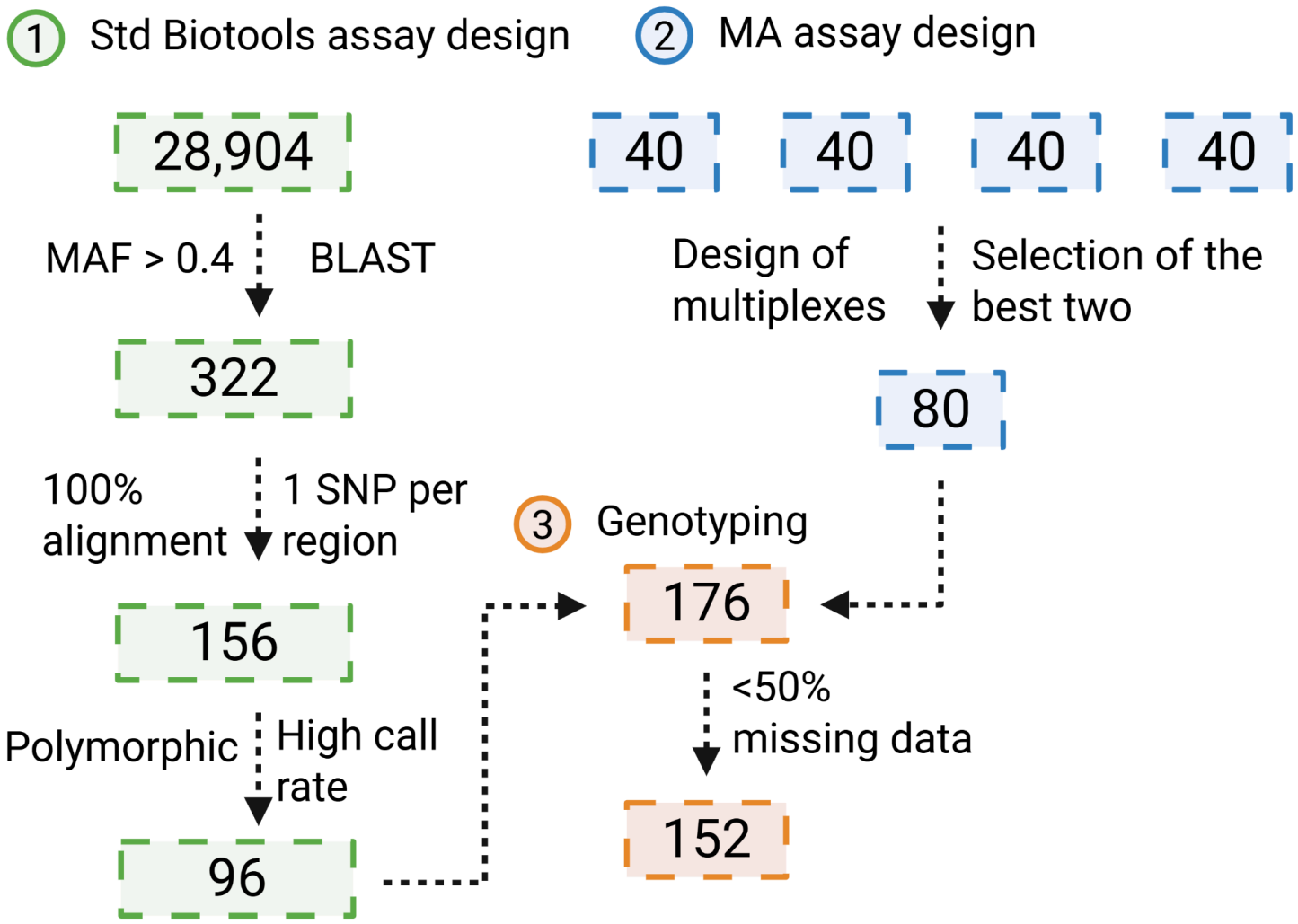

Figure 2

Genetic analysis workflow. Design of two SNP assays: (1) Std Biotools and (2) MassArray (MA), and (3) the total SNPs used in the genotyping step. MAF = minor allele frequency.

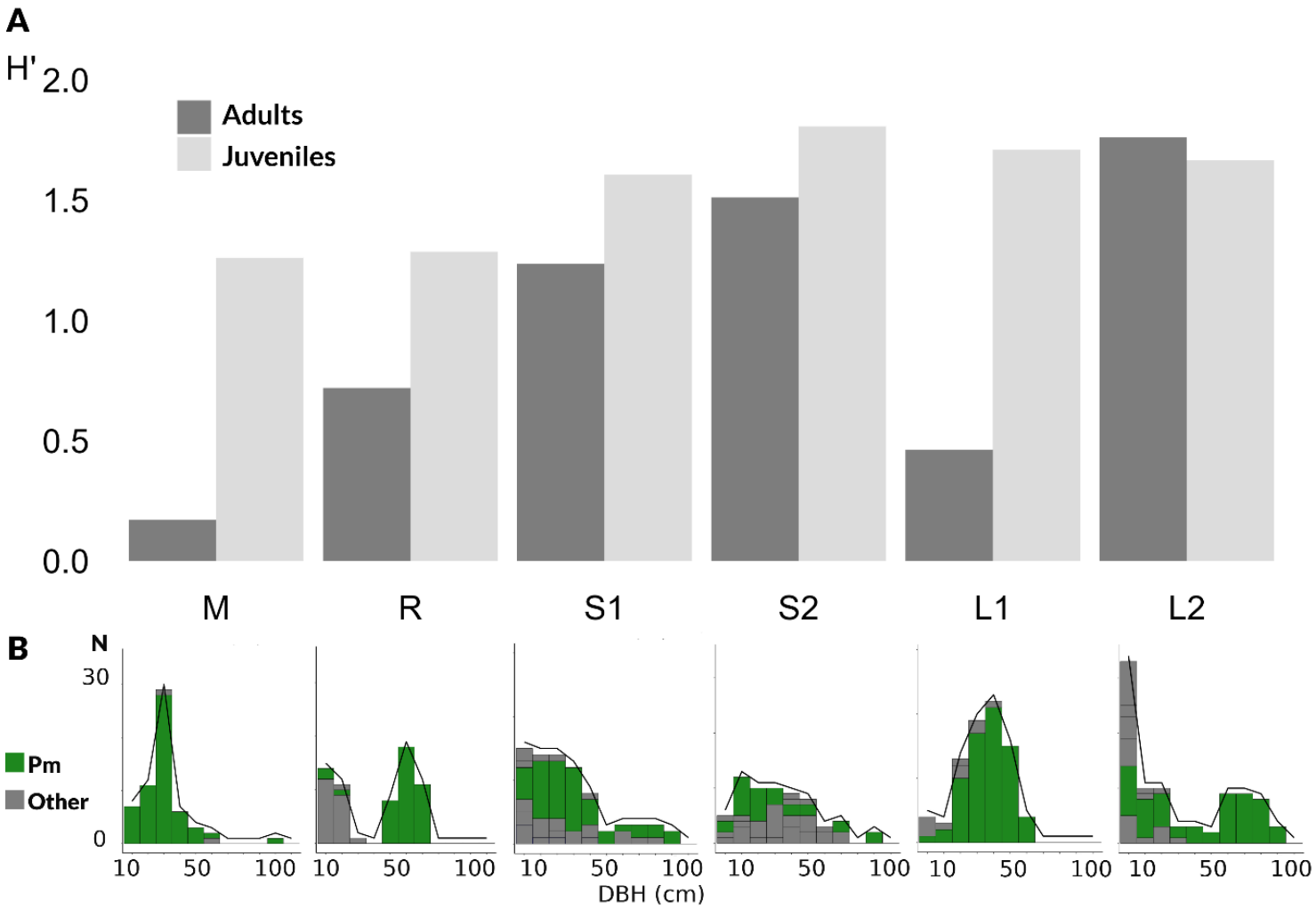

Figure 3

Structural and Species diversity. A) Shannon diversity index (H') of adult and juvenile trees at each site, B) Structural diversity of adult trees at each site shown as the number of trees per diameter class. N: number of trees, DBH: diameter at breast height. Pm: *P. menziesii* individuals are coloured in green.