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Verifying the origin of forest reproductive material using genetic fingerprinting: development of an operational framework

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Abstract

Ensuring the traceability of forest reproductive material (FRM) is a crucial step for establishing resilient forests under accelerating climate change. To address this need, we developed and implemented a standardized genetic fingerprinting workflow for nine important forest tree species enabling the verification of the origin of commercial seed lots and nursery plantlets. The method is based on reference samples (cones or seeds) collected from each individual tree during seed harvesting, as mandated by the Austrian Act and Regulation on FRM. For each species, eight to nine highly polymorphic nuclear microsatellite loci were selected, and laboratory protocols were optimized and validated on various types of plant tissue from mother trees including cone scales, testa, and pericarp as well as on offspring samples with known maternal origin. Subsequently parentage analyses of seed lots and nursery plants demonstrated that 72 to 86% of samples could be successfully assigned to their respective mother trees. Assignment success was strongly influenced by the quality of maternal tissue and completeness of the reference collection. The workflow also enabled the detection of duplicate submissions among reference mother samples, providing an independent verification of compliance with minimum number of tree harvesting requirements. Collectively, these results establish a robust and operational framework for DNA-based verification of FRM origin and highlight its potential to enhance transparency and quality assurance throughout FRM supply chains.

Key words: genotyping; parentage analysis; provenance; traceability

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1. Introduction

In the context of climate change, it is becoming increasingly important to carefully select suitable forest reproductive material (FRM) to enhance local adaptation and climate resilience of afforestation efforts (Konnert et al. 2015). The performance and adaptability of plants within the same tree species but of different provenance can differ enormously. This is also well known to the field practitioners who encounter susceptibility to disease, low vitality, slow growth and undesirable growth habit by trees of unsuitable origin (Memišević Hodžić et al. 2020). For example, spruce trees from high altitudes are better adapted to snow breakage and frost than spruce trees from low altitudes (Caré et al. 2018). Only adapted and adaptable material will enhance the resistance, resilience and stability of future forest stands (Fady et al. 2020; Vinceti et al. 2020; EUFORGEN 2023).

Based on a recent survey on European forest nurseries, current annual production capacity lies between 2.5 and 3 billion plants (Konrad et al. 2025). Under the increasing pressure of global warming, countries will search for climate-adapted and adaptable FRMs in regions that already experience our future climate (Chakraborty et al. 2024). The strategy of 'assisted migration', i.e., facilitated movement of species and populations within and across their natural range, will exacerbate the challenges of trade and traceability of FRM across Europe (Konnert et al. 2015). This presents a significant challenge.

In Austria, ca. 21.5 million forest trees are sold annually for afforestation (Federal Forest Office, personal communication, September 2025). Thereof 1.3 million conifers (7.5%) and 1.5 Mio (37.1%) deciduous plants originate from outside Austria, including EU and third countries. The country is typographically very diverse and is divided into 22 provenance regions for forest reproductive material

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(FRM) and further delineated by seven altitudinal zones. These provenance zones were based on climatic, soil and topographic characteristics and serve as the foundation for afforestation planning to optimize local adaptation. They also form the basis for the allocation of afforestation subsidies, as the use of FRM from an appropriate source is generally required to qualify for such funding.

Forest owners need to be assured that the material supplied to them is indeed the one they selected for their specific site. Despite the utmost care during harvesting, seed cleaning and cultivation, incorrect declarations can occur along the trade chain. Seed harvesters, seed processors, forest nurseries and traders have to ensure that the plant production process runs smoothly and that the reproductive material supplied corresponds with the label of origin. Only in this way, damage caused by FRM of the wrong identity with potentially poor properties or lacking adaptation to the respective location which manifests in the future can be avoided at an early stage.

The EU Council Directive 1999/105/EC and the associated national legislation, such as the Austrian Act and Regulation of Forest Reproductive Material, set very high standards regarding the labelling and traceability of FRM (European Union 2000; Republic of Austria 2023). Nevertheless, paper-based master certificates, which are literally the "birth certificate" and "passport" of any FRM batch, are currently the only proof of identity.

An effective strategy to improve traceability and transparency of FRM is through seamless administration of the seed harvest operations. Different countries have developed their own systems with varying levels of digitalisation supporting their implementation. In Germany e.g., a digital portal with geo data was put into place to process all steps from seed harvest notifications to issuing master certificates (https://www.nw-fva.de/EZR-HE/; https://www.awg.bayern.de/074362/index.php). They are divided into a public and a non-public area and provide an end-to-end digital administration. Such systems reduce sources of errors; are time saving and allow the efficient analysis of data. Nevertheless, genetic-based systems for verifying the declared origin of plantlets or seed are highly desirable as a complementary approach.

The genetic fingerprinting system outlined in this paper for verifying the origin of forest reproductive material is based on reference samples, collected from mother trees during harvest. When verification is required, genetic fingerprints can be generated from seeds available on the market or from plant material in forest nurseries and subsequently compared with the stored reference materials (Fig. 1). By matching their characteristic fingerprinting profiles, offspring can be reliably assigned to their respective mother trees through parentage analysis. A similar concept is applied in the German certification systems (Konnert & Hussendörfer 2002; see also below).

Austria holds a unique position in Europe, as the collection of reference samples from forest seed stands or seed orchards has been regulated by national law since 2003 (Republic of Austria 2023). Under this regulation, seed harvesters are required to collect samples from each individual harvested mother tree (single-tree sample, in German referred to as 'Einzelbaumprobe') during harvest. These samples of cones or fruits must be submitted to the Federal Forest Office, when material is harvested in the categories 'selected' (originating from seed stands) or 'qualified' (originating from seed orchards). The category "tested" is currently not available from Austrian sources (Federal Forest Office, Bundesamt für Wald 2025a), thus

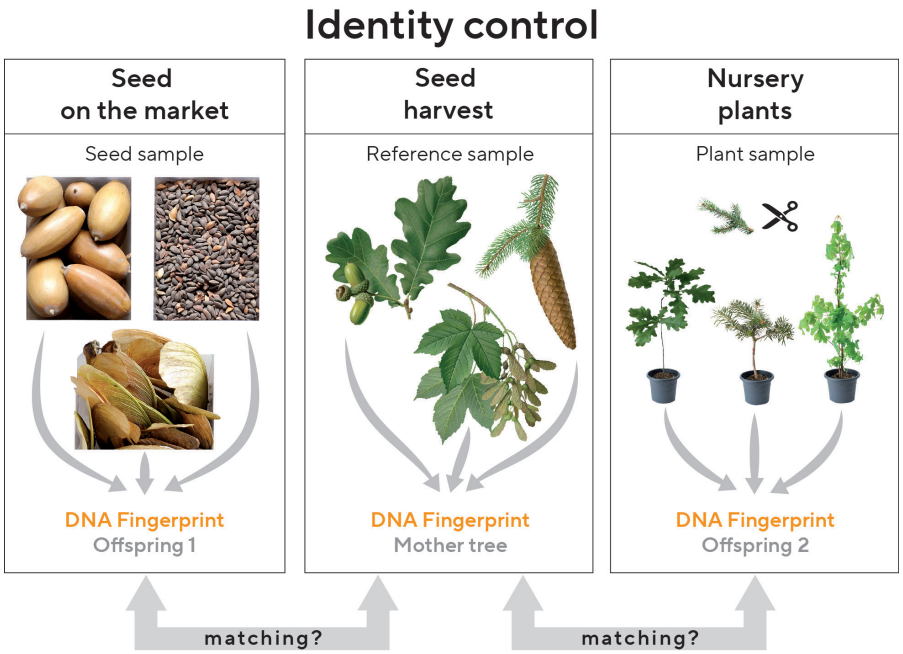


Fig. 1. Verification of forest reproductive material (FRM) using single-tree samples as reference material.

not relevant. These reference samples constitute a robust foundation for genetic control measures.

Maintaining the adaptability of FRM and preserving genetic diversity require harvesting seeds from several mother trees. To ensure this, legislation specifies minimum numbers of mother trees to be harvested, while specific numbers vary by species and country. In Austria, the minimum is 20 for *Abies alba* Mill., *Larix decidua* Mill., *Picea abies* [L.] H. Karst., *Pinus sylvestris* L., *Quercus petraea* [Matt.] Liebl., and *Quercus robur* L., and 10 for *Acer pseudoplatanus* L., *Prunus avium* L., and *Pseudotsuga menziesii* [Mirbel] Franco (Federal Forest Office, Bundesamt für Wald 2025b). The proposed genetic analysis of reference samples additionally allows for independent verification of compliance with these minimum tree-harvesting requirements.

Around the time when single-tree sampling became mandatory in Austria, two certification systems were established in Germany to enhance the traceability of FRM beyond the requirements of national forest regulations (Konnert & Hosius 2008). The ZüF (Zertifizierungsring für überprüfbare forstliche Herkunft Süddeutschland (ZüF 2023) was founded in Bavaria in 2002, the FfV (Forum forstliches Vermehrungsgut) based in Göttingen (Lower Saxony) followed. Both systems are organised as associations and validate the origin of FRM through reference samples, genetic analysis and documentation. While participation in the German certification systems are voluntary, certain buyers, like the Bavarian State Forests, purchase only certified forest reproductive material (Bayerische Staatsforsten 2023). The report by ZüF (Konnert et al. 2018) revealed that the number of wrongly declared tested samples ranged from 0 to 15% in the years 2008 till 2016.

Several laboratory-based molecular marker systems have been developed to verify the identity and origin of FRM, including isozymes, PCR-RFLP, cpDNA haplotypes, AFLPs, SNPs, and microsatellites (Finkeldey et al. 2010). These methods have been successfully used across a wide range of European tree species, such as *Fagus sylvatica* L. (Konnert & Hussendörfer 2002), *Populus alba* L. (Fussi et al. 2012), *Quercus* spp. (Coelho et al. 2006; Gailing et al. 2007; Degen et al. 2010; Cosín-Roldán et al. 2023), *Pinus sylvestris* (Kavaliauskas et al. 2021), and *Pseudotsuga menziesii* (Neophytou et al. 2022). Among these marker systems, microsatellites (SSRs) offer an optimal balance between cost efficiency, high polymorphism, and technical accessibility. Their codominant inheritance and reproducibility make them particularly suitable for individual identification, parentage analysis, and large-scale operational traceability of FRM (Vieira et al. 2016). They can be also used for genetic monitoring of forest stands (Konnert et al. 2011; Fussi et al. 2016; Kavaliauskas et al. 2018).

As optimal material as young leaves, needles or cambium are not always available for tracing of FRM origin other plant tissue has been also investigated (Finkeldey et al. 2010). Previous studies have shown successful DNA extraction from the woody pericarp of acorns in *Quercus* spp. (Ziegenhagen et al. 2003; Grivet et al. 2005), and from dry wings of seeds of *Abies alba* (Ziegenhagen et al. 2003), primarily in the context of seed dispersal research. DNA extraction from hard and woody tissue often presents technical challenges, requiring modifications to standard protocols. The addition of polyvinyl pyrrolidone (PVP) to the lysis buffer has proven effective (Reynolds & Williams 2004; Rachmayanti et al. 2009) or using modified CTAB protocols (Ohyama et al. 2001; Rogers & Kaya 2006).

Unlike previous studies, this work compares different types of maternal material routinely obtained as single-tree samples such as cone scales, testas and seed wings. Parentage analysis was used as the primary method to assign each offspring to its putative mother tree. In addition, we applied STRUCTURE and discriminant analysis comparing samples from two populations. These methods are commonly implemented in the ZüF certification system as briefly described by Cremer et al. 2022 and Konnert & Cremer 2024.

The aims of this study were to a) identify, from the maternal material at harvest, the most suitable starting material for genetic fingerprinting, b) establish a low-cost routine method by using only 8–9 primer pairs per species, c) compare the results of the parentage analysis with those obtained from STRUCTURE and discriminant analysis, d) assess whether the minimum number of trees to maintain high genetic diversity has been harvested, and e) discuss the potential integration of routine genetic testing into field practice.

2. Material and methods

2.1. Sampling of maternal and offspring plant tissue and analysis approach

The optimisation of the fingerprinting technique using reference samples (mother tree material) and their offsprings was carried out on nine forest tree species: *Abies alba*, *Acer pseudoplatanus*, *Larix decidua*, *Picea abies*, *Pinus sylvestris*, *Prunus avium*, *Pseudotsuga menziesii*, *Quercus petraea* and *Q. robur*.

Firstly, to establish the method for the types of plant tissue available, particularly from mother trees for which no fresh material could be obtained, a test with various types of tissue was conducted to assess its suitability for microsatellite analysis. For mother trees, tissue coming from cone scales, testa, pericarp, wings, endosperm and fruit peduncles was available from the reference samples (Table 1). Tissue from the offsprings included embryos, needles, leaves and seedlings.

Secondly, matching samples were tested for optimization of the method, i.e., the maternal part (fruit pedun-

cle, pericarp, scales or testa) were analysed pairwise with embryos or seedlings from the same seed, fruit or cone for one or two provenances each (Table 1). For the offspring, species that are easily germinated (*Larix*, *Picea*, *Pinus*, *Pseudotsuga*, *Quercus*) were first germinated and green leaves were used, while for the other species (*Abies*, *Acer*, *Prunus*) the embryos were extracted. These samples were then subjected to DNA extraction and genotyping.

In the third step, real-world evidence was generated by testing samples obtained from practitioners, with two provenances per species. All samples were provided to us on a voluntary basis. The samples consisted of seed lots from commercial seed harvesters or leaves or needles from nursery plantlets. Offspring material from *Abies*, *Acer* and *Prunus* came from seed lots and thus embryos were analysed. In the other species, freshly harvested twigs from the commercial nurseries were wrapped in wet paper towels, placed in a plastic bag and shipped on the same day to the BFW laboratory. They were stored in a freezer at –20 °C upon arrival and later used for the genotyping. The type of mother tree material that had produced best yield and purity of DNA in the material test was chosen for each studied species (Table 1). Generally, all reference samples from a given population were analysed, together with 24 or 48 samples from the field material. The mother tree materials had been collected during the years 2011 to 2022 and the genetic analyses were performed in 2022.

Reference samples (mother tree materials, single-tree samples) were retrieved from cold storage at BFW. For *Abies alba* and *Picea abies* they consisted of one cone per mother tree, three cones for *Larix decidua*, *Pinus sylvestris* and *Pseudotsuga menziesii*, ten acorns for *Quercus* spp. and 20 seeds for *Acer pseudoplatanus*. In *Prunus avium* 20 seeds are mandated, but the harvesters were asked to additionally send in fruit stalks, which were then used for genotyping. These samples had been processed in the past, sealed in plastic bags and stored at –2 °C, while acorns had been stored at –5 °C.

2.2. DNA extraction

All samples from different types of material (Table 1) were prepared in a collection of microtubes from the Dneasy 96 Plant kit (Qiagen) together with one tungsten carbide bead (3 mm diameter). When isolating embryos, attention was paid, not to have any maternal tissue attached. Cherry stones were cracked open with pliers. Similar amounts of starting material were used for all tissue types. Where necessary, the material was cut into smaller pieces to fit inside the tubes and avoid blocking free movement of the beads. Racks holding the samples were dipped in liquid nitrogen for about 5 min. and then immediately grinded in the TissueLyser II (Qia-

Table 1. Type of plant tissue (Mother tree/Offspring) and origin of 27 populations of nine tree species used the three different tests.

Tree species	ID	Collection year	Material test	Matching test	Field test	Region
Abies alba	362/2020	2020	scales/embryos	scale/embryo	scales/embryo	Lower Austria
	47/2020	2020	scales/embryos	scale/embryo	—	Carinthia
	74/2020	2020	wings, testa/endosperm, embryos	—	—	Lower Austria
	94/2018	2018	wings, testa/needles	—	testa/needles	Lower Austria
Acer pseudoplatanus	59/2020	2020	testa, pericarp, wings/embryos	pericarp/embryo	pericarp/embryo	Carinthia
	60/2020	2020	testa, pericarp, wings/embryos	pericarp/embryo	pericarp/embryo	Carinthia
Larix decidua	347/2018	2018	scales/seedlings	scale/seedling	—	Styria
	404/2018	2018	scales/needles	—	scales/needles	Tyrol
	26/2012*	2012	—	—	scales/needles	Lower Austria
Picea abies	366/2020	2020	scales/seedlings	scales/seedling	—	Styria
	162/2011	2011	testa/needles	—	testa/needles	Lower Austria
	66/2015	2015	testa/needles	—	testa/needles	Lower Austria
Pinus sylvestris	40/2019	2019	testa/seedling	testa/seedling	—	Lower Austria
	42/2019	2019	testa, scales, wings/seedling	—	—	Lower Austria
	178/2015*	2015	testa/needles	—	testa/needles	Lower Austria
	151/2016*	2016	testa/needles	—	testa/needles	Lower Austria
Prunus avium	4/2022*	2022	fruit peduncle/embryo	fruit peduncle/ embryo	fruit peduncle/ embryos	Burgenland
	3/2022*	2022	fruit peduncle/embryo	—	fruit peduncle/ embryos	Upper Austria
Pseudotsuga menziesii	10/2020	2020	scales/seedlings	scale/seedling	—	Lower Austria
	7/2020	2020	scales/seedlings	scale/seedling	—	Carinthia
	88/2018	2018	testa/needles	—	testa/needles	Lower Austria
	79/2018	2018	testa/needles	—	testa/needles	Lower Austria
Quercus petraea	186/2020	2020	testa/seedling, leaves	testa/seedling	testa/leaves	Lower Austria
	239/2020	2020	testa/leaves	—	testa/leaves	Lower Austria
Quercus robur	184/2020	2020	testa/leaves	—	testa/leaves	Carinthia
	121/2020	2020	testa/seedling	testa/seedling	—	Carinthia
	233/2020	2020	testa/leaves	—	testa/leaves	Lower Austria

Note: The last number in the population ID refers to the collection year. *Samples coming from a seed orchard.

gen) at 27 Hz for 5 min. If needed, an additional grinding step was done.

Genomic DNA was extracted from all types of material the same way using the Qiagen Dneasy 96 Plant kit following the manufacturer protocol. Resulting DNA pellets were dissolved in 40 µl of Elution Buffer and DNA concentrations of samples were measured using a Nanodrop 1000 spectrophotometer (Thermo Scientific). In the end, DNA was diluted to 5 ng µl^{-1} prior genotyping. For the samples with DNA concentrations higher than 5 ng µl^{-1} and sufficient purity, PCR and fragment analysis was carried out to check if the PCRs were successful and the quality of allele peaks sufficient.

2.3. Primer selection and genotyping

Microsatellite markers were chosen from a literature search and previous studies (e.g. Trees4Future) where they proved to be polymorphic and suitable for genotyping, parentage and populations analyses. The initial sets of primers, varying from 8 to 22 depending on the genus, were tested on different types of material (Supplementary Table 1, Table 1 column material test). In the end, eight to nine well-scoreable and variable primers were separated and optimised into two PCR multiplexes for each species with WellRED D4 fluorescent dye (Beckman Coulter, Fullerton, CA) bound to forward primers. The description of all tested primers, sequences, size ranges, repeat motifs and their PCR conditions are stated in Supplementary Table 1.

The PCR conditions followed the manufacturer protocol, Qiagen Multiplex PCR kit (Qiagen) with these modifications: 11 µl reaction with 5 µl of 2× Qiagen Multiplex PCR Master Mix, 1 µl of diluted DNA sample, different volumes of the 10nM SSRs primers and by adding distilled water to the final volume. In case of *Q. petrea* and *Q. robur*, 3 µl of Q solution (Qiagen) was also added to a total of 13 µl of PCR mix. The concentration of each primer and multiplexes structure is given in Supplementary Table 1. The PCR was carried out in a T100 Thermal Cycler (Bio-Rad) with the following program: denaturation step for 15 min at 95 °C, 30× cycles of 30 s at 94 °C, primer mixture dependent annealing temperature for 90 s and 1 min at 72 °C, and a final extension for 30 min at 60 °C. 1 µl of PCR product was then mixed with 30 µl of diluted 400 bp ladder (for 96 samples: 50 µl in 3 ml of ddH_2O) and the samples processed by capillary electrophoresis using the CEQ 8000 genetic analysis system (Beckman Coulter) at 90 °C. The sequencing analysis software installed on the CEQ 8000 was used for scoring the microsatellite alleles. Only well-readable alleles were recorded in a matrix of samples and allele sizes. Alleles of the tetraploid species *A. pseudoplatanus* were recorded as dominant (presence/absence) due to the difficulty of reliably determining allele dosage (Wang & Scribner 2014) and for Colony software limitation to diploids as also implemented in (Karfik et al. 2026). We are aware that this could reduce the information per locus and thus parentage resolution compared to codominant scoring, but our combined exclusion probability was high (0.965) which means that the overall marker set still retained high discriminatory power for parentage assignment.

The samples with unclear profiles or showing more than two peaks (in case of *A. pseudoplatanus* samples with more than four peaks) as well as samples with two or more missing primer alleles were excluded from further analyses.

2.4. Genetic data analysis

Parentage analysis

The final matrix of allele sizes for each offspring and mother tree were analysed with Colony ver. 2.0.6.5 (Jones & Wang 2010) to evaluate percentages and number of matches. Initially, matching tests including samples with known mother trees were processed to evaluate accuracy of the method (Table 1, Matching test). Subsequently in the field test, mixed offspring samples from one population were run against the data sets of mother trees from two populations to check if the offsprings were assigned correctly (Table 1, Field test).

The full likelihood (FL) method with long run length was used for parentage assignment. The program's parameters for the mating system were set to 'polygamy' for both male and female, species 'monoecious'. For the first run, the 0.0001 genotyping error rate was set, with differing numbers of loci (8 or 9) to estimate error rate for each primer. The resulting error rates were then used for running the parentage analyses. As we had only mother trees in our data set, probability for mothers of offspring included in candidates was set to 1 and for fathers to 0.001. In addition, non-exclusion probability for each primer set for each species was also calculated in CERVUS v. 3.0.6 (Kalinowski et al. 2007).

If mother tree samples of the same genotype were detected during the analysis (sampling of mother tree duplicated during seed harvesting), one of them was excluded from further analysis.

STRUCTURE analysis

For comparison with the parentage analysis in field material, the two populations of mother trees and offsprings in each tree species were analysed by a Bayesian clustering algorithm implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000). The genetic groups (clusters) were obtained by testing the optimal number of clusters (K) from 1 to 10 with each K repeated ten times. An initial burn-in of 1,000,000 generations and additional 1,000,000 generations of MCMC chains were run after burn-in on the Metacentrum VO infrastructure (https://

metavo.metacentrum.cz) (Falush et al. 2007). The output files were summarized in the program R version 3.6.3 (R Core Team 2019) with the script Structure-sum-2009 (Ehrich et al. 2007) to calculate similarity coefficients between the replicate runs (Nordborg et al. 2005) and ΔK (Evanno et al. 2005). The optimal number of groups (K) was the one with consistent results over ten repeats, high similarity coefficient and highest ΔK. The graphical outputs for selected Ks were generated using the software CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and Distruct 1.1 (Rosenberg 2004) showing percentage of assignment of each sample to the genetic groups.

Discriminant analysis

Both sets of mother trees and offsprings in each species from the field material were compared by discriminant analysis of principal components (DAPC) in the R package adegenet 2.1.11 (Jombart et al. 2010). The output of the analyses showing separation of two populations and the assignment of offspring mixtures are presented by graphs prepared, also in the R package.

3. Results

3.1. Testing different types of starting material

Various materials from the nine tree species were tested to determine its suitability for genetic analyses. The resulting DNA concentrations and purities are presented by boxplots for each tree species (Fig. A1 and Fig. A2) and in Table S2. In conifer mother trees, the testa and scales were found suitable but not the wings. In deciduous mother trees, the pericarp, testa and fruit peduncle worked well. In the offspring, all materials comprising embryos, seedlings, leaves and needles were found suitable (Table 2).

Table 2. Suitability of tested mother tree and offspring material for fingerprinting analysis.

Species	Mother tree	Offspring
	Suitable	Suitable
Abies alba	endosperm, testa, scale	embryo, needles
Acer pseudoplatanus	testa, pericarp	embryo
Larix decidua	scale	needles, seedlings
Picea abies	testa, scale	needles, seedlings
Pinus sylvestris	testa, scale	needles, seedlings
Prunus avium	fruit peduncle	embryo
Pseudotsuga menziesii	testa, scale	needles, seedlings
Quercus spp.	testa	leaves, seedlings

3.2. Primer selection

For genotyping, two PCR multiplexes were established for each species (Table S1.) consisting of eight or nine microsatellite primer pairs in total (Table 3). All the primers were polymorphic and suitable for distinguishing individual trees/genotypes from each other. The resulting alleles were recorded as a separate data matrix for each tree species.

Estimated error rates for each primer pair and tree species from an initial run of parentage analyses are presented in Table S1 as well as their average non-exclusion probability for one candidate parent.

Table 3. Overview of the microsatellite primers selected for the different species.

Tree species	Primers and references
Abies alba	Aag01 (Postolache et al. 2014) NFH15, NFH3 (Hansen et al. 2005) SF1, SF324, SF333, SFb5, SFg6 (Cremer et al. 2006)
Acer pseudoplatanus	Am118 (Kikuchi & Shibata 2008) Aop122 (Segarra-Moragues et al. 2008) MAP2, MAP9, MAP33, MAP40 (Pandey et al. 2004) SM21A, SM60 (Graignic et al. 2013)
Larix decidua	bcLK211, bcLK228 (Isoda & Watanabe 2006) Ld101, Ld31, Ld42, Ld 45, Ld50 and Ld 56 (Wagner et al. 2012)
Picea abies	EATC1D02A, EATC1E03 (Scotti et al. 2002) PAAC3, PAAC23 (Scotti et al. 2000) SpAC1F7, SpAG2, SpAGC2, SpAGD1 (Pfeiffer et al. 1997)
Pinus sylvestris	psyl16, psyl19, psyl25, psyl36, psyl42, psyl44, psyl57 (Sebastiani et al. 2012) PtTX 4001, PtTX 4011 (Auckland et al. 2002)
Prunus avium	EMPaS01, EMPaS05, EMPaS06, EMPaS10, EMPaS12, EMPaS13, EMPaS14 (Vaughan & Russell 2004) PceGA34 (Downey & Iezzoni 2000) UDP98-412 (Testolin et al. 2000)
Pseudotsuga menziesii	PmOSU_5A8, PmOSU_2G12, PmOSU_3D5, PmOSU_3B2, PmOSU_2D9, PmOSU_1F9, PmOSU_4A7, PmOSU_2C2 (van Loo et al. 2015)
Quercus spp.	QpZAG15, QpZAG110, QpZAG104 (Steinkellner et al. 1997) QrZAG7, QrZAG87, QrZAG65, QrZAG5b, QrZAG20 (Kampfer et al. 1998)

3.3. Parentage analysis

Parentage analysis of matching samples

In this test, mother material and matching seed material were compared to evaluate the method. As expected, the parentage analyses resulted in 100% match for all species (Table 4). In *Quercus*, we found one duplicate in the reference samples (population 121/2020) that was removed from the analysis.

Real life scenario

In the final step, seed lots or leaf material were used to test the method in a realistic setting. By comparing their characteristic profiles, relationships could be clarified (Fig. A3). In most species, 72 to 94% of the analysed samples could be assigned to their correct mother tree (for more detail see Table 5). The number of the mother

Table 4. Matching test of offspring samples from the same mother trees.

Species	ID	Type of samples		No of samples	Assigned offsprings %
		Mother trees	Offsprings		
Abies alba	362/2020	Scales	Embryo	17	100
	47/2020			10	100
Acer pseudoplatanus	59/2020	Pericarp	Embryo	11	100
	60/2020			10	100
Larix decidua	347/2018	Scales	Seedling	7	100
Picea abies	366/2020	Scales	Seedling	15	100
Pinus sylvestris	40/2019	Testa	Seedling	12	100
Prunus avium	4/2022	Fruit peduncle	Embryo	15	100
Pseudotsuga menziesii	10/2020	Scales	Seedling	13	100
	7/2020			8	100
Quercus petraea	186/2020	Testa	Seedling	20	100
Quercus robur	121/2020	Testa	Seedling	11	100

tree samples analysed was in some cases lower than the number of samples initially stated by the harvester in the master certificate: in *Abies* and *Larix* two mother tree samples were missing in the material received for population 362/2020 and 404/2018, respectively and in both *Quercus robur* populations four samples were missing from storage. Moreover, in the *Larix* population 26 from the year 2012, low DNA concentration and poor DNA quality, as well as poorly readable microsatellite profiles, were encountered. Consequently, DNA from only nine mother trees was of sufficient quality for PCR amplification and subsequent analyses. However, even with all mother trees represented in the analysis, it was not possible to match 100% of the samples for any species.

The offspring could be traced back to 8 to 17 different mother trees (excluding *Larix* 26/2012). On average 51% of the mother trees were represented in the small samples of FRM, the highest value was in *Acer* with 100% and the lowest in *Prunus* with 24%. There were no problems encountered in duplicates of the reference samples during this analysis, which means all the reference samples were unique and originated from individual mother trees in the field. The STRUCTURE analysis for all the studied species resulted in the best delta K for the division K = 2 (Fig. A4-I). The two compared data sets (provenances) per each species were well separated (Fig. 2A-I). The best division was observed in *Abies* and both *Quercus* spp. On the other hand, low separation was found in *Prunus* samples from the seed orchards. In the discriminant analyses a similar pattern as in STRUCTURE barplots was observed (Fig. 3A-I). All the offspring were correctly assigned to their mother population.

Nevertheless, the species varied in overlap of genetic variation of the offspring with those of the mother trees. The best matches were found in *Abies alba*, *Larix*

Table 5. Parentage analysis of seeds from harvesters or leaf/needle tissue from nurseries and the single-tree samples. The 'Assigned offsprings %' was calculated as a percentage based on the total number of analysed offspring samples and those correctly assigned to the mother trees from the same provenance.

Species	Population	Type of samples		No. of mother trees			No. of offsprings analysed	Assigned offsprings %	No. of mother trees with assigned offsprings
		Mother trees	Offsprings	on the certificate	DNA extraction**	analysed			
Abies alba	94/2018	Testa	Needles	39	39	39	48	94	15
	362/2020	Scale	Embryos	20	18	18	30	80	9
Acer pseudoplatanus	59/2020	Pericarp	Embryos	11	11	11	24	89	9
	60/2020	Pericarp	Embryos	10	10	10	30	87	10
Larix decidua	404/2018	Scale	Needles	21	19	19	48	60	10
	26/2012*	Scale	Needles	52	52	9	48	6	1
Picea abies	162/2011	Testa	Needles	18	18	18	48	76	8
	66/2015	Testa	Needles	22	22	22	48	83	10
Pinus sylvestris	178/2015*	Testa	Needles	48	48	48	48	84	15
	151/2016*	Testa	Needles	18	18	18	48	73	11
Prunus avium	4/2022*	Fruit peduncle	Embryos	26	26	26	48	77	13
	3/2022*	Fruit peduncle	Embryos	46	46	46	47	87	11
Pseudotsuga menziesii	88/2018	Testa	Needles	20	20	20	48	73	12
	79/2018	Testa	Needles	32	32	32	48	79	14
Quercus petraea	186/2020	Testa	Leaves	20	20	20	30	76	10
	239/2020	Testa	Leaves	20	20	20	48	79	13
Quercus robur	184/2020	Testa	Leaves	52	48	48	48	72	14
	233/2020	Testa	Leaves	52	48	48	48	81	17

Notes: The last number in the population ID refers to the collection year. *Material coming from a seed orchard ** corresponds to the number of single-tree samples stored at the BFW.

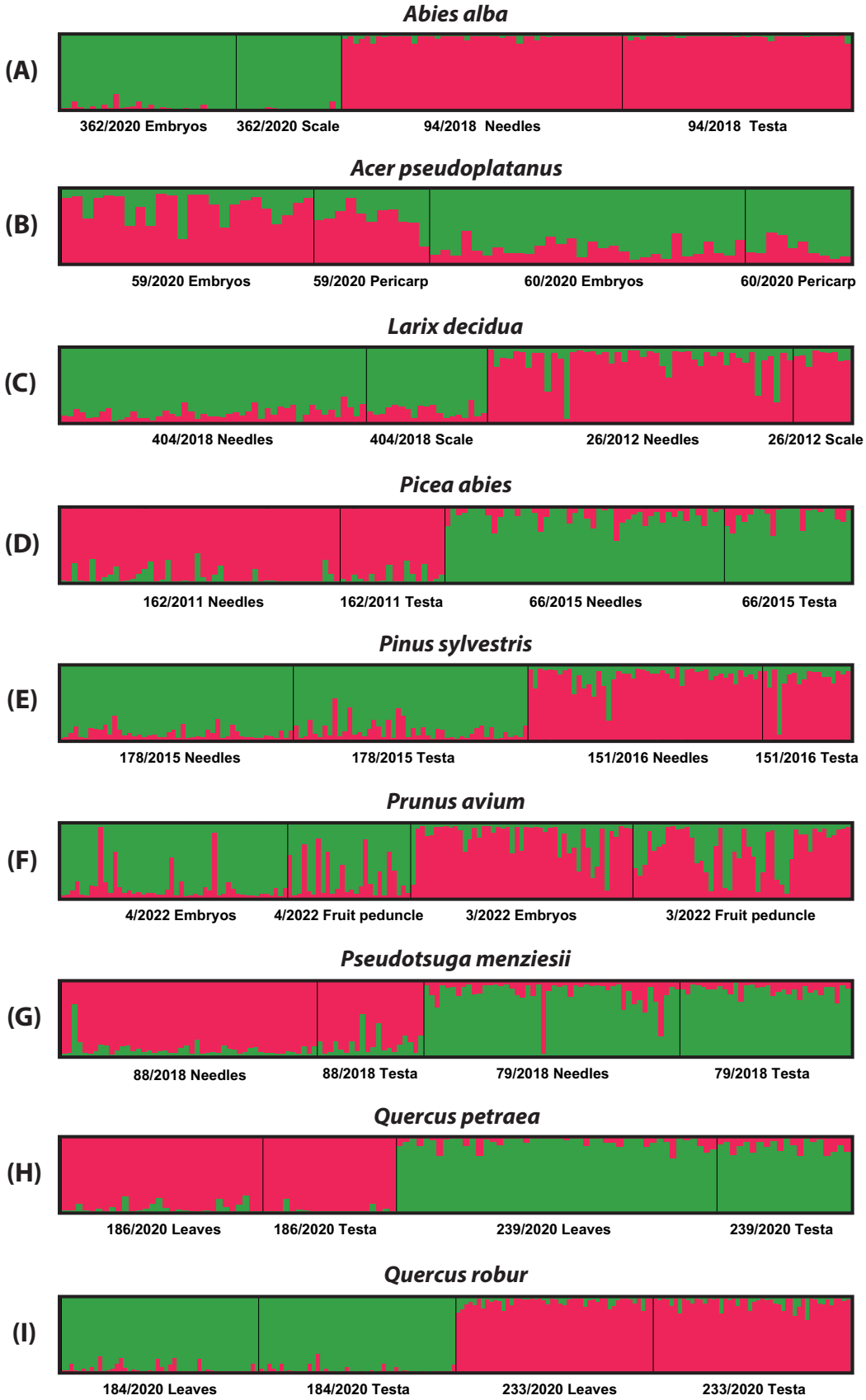


Fig. 2. Barplots from STRUCTURE analysis for division K = 2. (A) 135 *Abies alba* (B) 75 *Acer pseudoplatanus* (C) 124 *Larix decidua* (D) 136 *Picea abies* (E) 162 *Pinus sylvestris* (F) 167 *Prunus avium* (G) 148 *Pseudotsuga menziesii* (H) 118 *Quercus petraea* (I) 192 *Q. robur* samples.

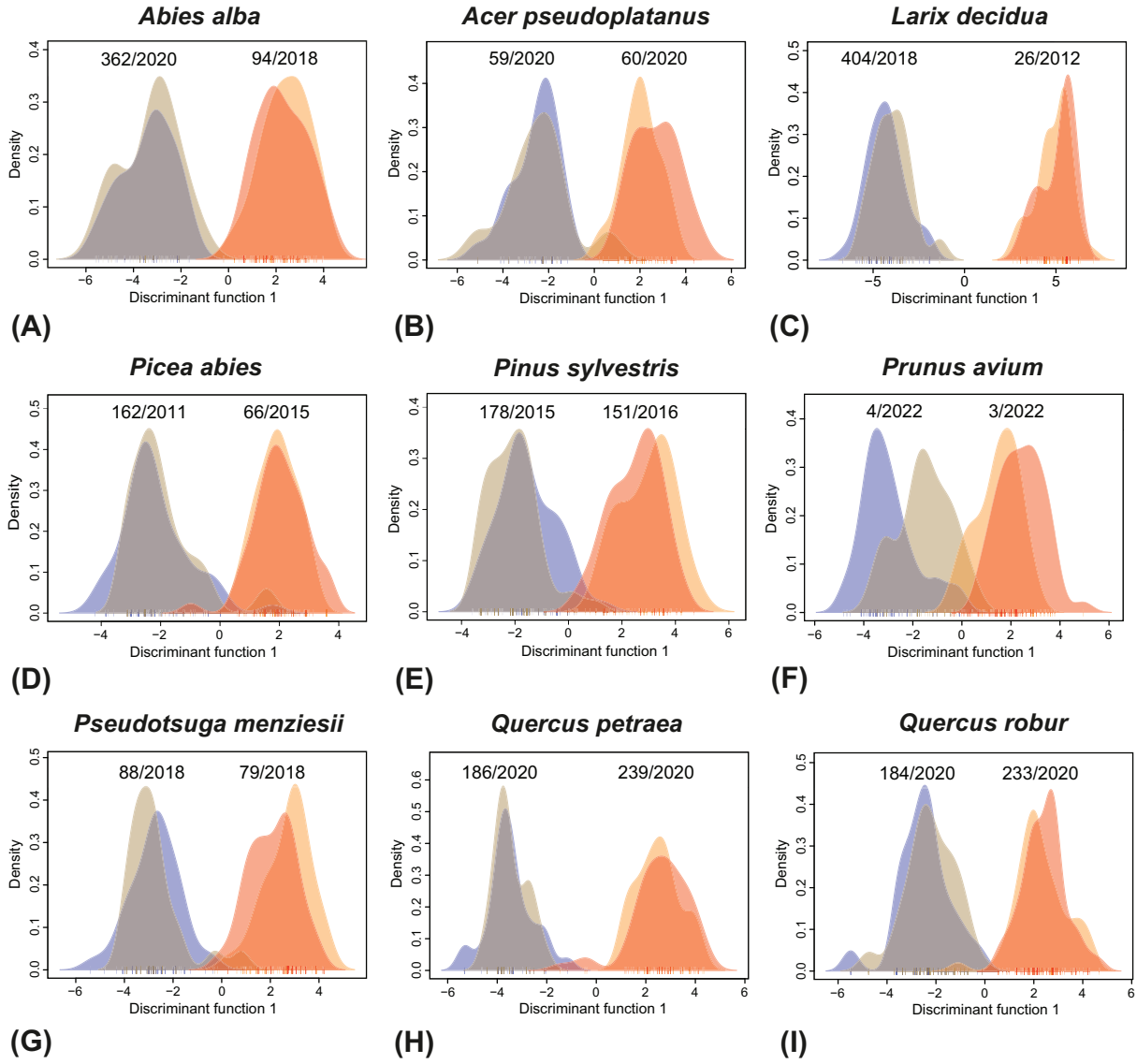


Fig. 3. Discriminant analysis graph of two populations of mother trees and offspring per species. Blue and red color peaks in front represent offsprings, and beige and orange color peaks in the back mother trees. (A) 135 *Abies alba* (B) 75 *Acer pseudoplatanus* (C) 124 *Larix decidua* (D) 136 *Picea abies* (E) 162 *Pinus sylvestris* (F) 167 *Prunus avium*, (G) 148 *Pseudotsuga menziesii* (H) 118 *Quercus petraea* (I) 192 *Q. robur* samples.

decidua, *Picea abies*, *Quercus petraea* and *Q. robur*, where the area of the mother trees and offspring almost completely overlapped. A shift was observed between embryos and fruit peduncles in the *Prunus avium* data set, which originated from seed orchards.

4. Discussion

In this study, a molecular marker-based method was successfully developed to verify the origin of forest reproductive material (FRM) in nine forest tree species. The approach is based on genetic fingerprinting using microsatellites and reference samples collected from individual mother trees (Fig. 1). In contrast to Germany, where allele frequencies of tested samples are compared with reference samples to assess genetic similarity on a population level (Züf 2023; FfV 2026), we applied parentage analysis to assign each offspring to its mother tree. To our knowledge, this is the first application of parentage analysis in this specific context. By matching characteristic microsatellite profiles, offspring can be reliably attributed to their respective mother trees. The method provides a robust basis for more stringent controls of the origin of FRM. The implementation requires the collection and storage of reference material, genetic fingerprinting of selected samples, reporting to the relevant control authorities and ideally the implementation of enforcement measures, including penalties.

4.1. Source of plant material

During tissue isolation from offspring samples, particular care was taken to thoroughly separate maternal tissue, such as seed coats, endosperm, or megagametophyte, which can confound parentage analysis. Therefore, a solid understanding of seed anatomy is essential before attempting to isolate suitable material. While DNA extraction from fresh green plant tissue such as leaves or cambium is standard practice, such materials were unavailable for mother tree fingerprinting in this study. Therefore, various material types were first tested to overcome the problem of inferior DNA quality and quantity. The condition and age of the material were identified as critical factors. Degraded DNA was observed in larch cones older than ten years, which negatively affected the success of the genetic analyses. Seeds of *Larix*, *Picea*, and *Pinus* have a long shelf life and nursery plants may enter the market a decade or more after harvest, when the extraction of high-quality DNA from aged mother material may have become infeasible. This could be prevented by freezing material for long-term storage.

4.2. Verification of origin

The parentage analysis was the primary tool in this study to match each offspring genotype to its putative mother tree. Using this approach, we successfully assigned between 72% and 94% of the offspring to their respective mother trees. Additionally, we were able to identify cases in which offspring could not be assigned, due to missing maternal genotypes in the reference dataset. This method also provided detailed insight into the number of contributing mother trees, as it operates at the level of individual trees. When applying STRUCTURE and discriminant analyses to our data, which are commonly used in the ZüF certification system (Cremer et al. 2022; Konnert & Cremer 2024), samples could be reliably assigned to their respective populations.

However, approaches such as STRUCTURE and discriminant analysis primarily use multilocus genotype data to assign individuals to broader genetic clusters or populations rather than to specific mother trees (Pritchard et al. 2000; Jombart et al. 2010). In contrast, parentage analysis uses multilocus genotypes to identify the parents of particular offspring among candidate parents thereby enabling direct inference of parent-offspring relationships at the individual level rather than the population level (Jones & Ardren 2003; Smouse & Sork 2004; Christie et al. 2017). Parentage analyses are highly sensitive to incomplete sampling of candidate parents, whereas assignment-based methods are more sensitive to low population differentiation (Jones et al. 2010; Christie et al. 2017). Nevertheless, parentage analysis can also be substantially affected by genotyping error, which may lead to false exclusion of true parents unless the analysis is configured to account potential imperfections in the data, such as the error rate (Marshall et al. 1998; Kalinowski et al. 2007; Jones et al. 2010). Therefore, careful sampling and accurate genotyping are essential to obtain reliable results when identifying parent-offspring relationships.

4.3. Duplicate reference samples

Using parentage analysis, we could also detect duplicates amongst the reference samples. From a technical standpoint, it is thus a necessary step to perform a duplication check before conducting parentage analysis. Such duplicates arise when harvesters collect multiple samples from the same mother tree, typically in situations where too few seed-bearing trees are available to meet the legal requirement of harvesting from 10 or 20 distinct mother trees. This practice is unlawful and can be identified through genetic fingerprinting, as identical genotypes reveal that multiple samples originate from the same individual. Collecting seeds from fewer than the required number of mother trees is a serious concern as it reduces the genetic diversity of the offspring, a particularly undesirable outcome under climate change. To avoid such practice mother trees could be labelled in the field to avoid any miss-identification (ZüF 2023). Seed harvesters should be engaged through targeted campaigns and workshops that clearly communicate the importance of maintaining high genetic diversity.

4.4. Single-tree sampling

If reference samples were collected from each mother tree, then parentage analysis would allow for a 100% match. However, in our field testing, the percentage of offspring that could be assigned to the mother trees was mostly between 72% and 94%. There are multiple potential explanations for this discrepancy such as incomplete genotyping profiles or in a worst-case scenario that seeds or plants from another population have been mixed in the batch.

Another contributing factor may be the incomplete collection of mother tree samples, due to oversight, logistical challenges, time pressure, or inconsistent practices. When reference fingerprints are missing, they cannot be accounted for in the assessment and offspring may be falsely identified as not matching the declared origin. This potentially casts doubt on the compliance of legally harvested materials. In our opinion, submission of incomplete mother tree samples can generally be attributed to two primary factors. In species like *Quercus* or *Acer*, where seeds are collected off the ground or are wind-dispersed, it is often unclear which individual tree

the seeds originated from. In such cases, it is advisable to collect samples from more rather than fewer mother trees to ensure accurate representation. In other cases, the number of mother trees declared on the master certificate corresponds only to the mandatory minimum number of mother trees, even though seeds were collected from a larger number of trees. This is likely due to the considerable logistical effort involved in recording all contributing trees during collection.

Material coming from clonal seed orchards may also introduce an additional potential error. In seed orchards it is common practice to submit only one reference sample per harvested clone to reduce time and effort. However, this streamlined procedure relies on accurate field labelling of clones. In a separate (in prep.) genetic study, instances of mislabelling of clones and outgrowth of rootstock were identified within the tested *Prunus* seed orchards. Misidentification in clonal seed orchards has been reported as a common issue (Przybylski et al. 2019).

Beyond sample quantity, sample quality is equally critical. In this study missing mother tree samples for *Quercus* and *Abies* were identified potentially due to samples arriving in a deteriorated or mouldy condition. To avoid such losses, regular feedback to harvesters on their sample submissions are essential, to continuously improve collection and handling practices. For optimal results, the samples should be shipped as soon as possible after collection. If immediate shipment is impossible, samples must be stored in a cool, ventilated area and turned regularly to prevent fungal growth.

4.5. Practical implications

Identity controls can be organized in different ways e.g. through voluntary certification schemes such as those presently practiced in Germany while another feasible option would be to integrate them into existing federal control systems. By incorporating sampling into routine (legal) inspections at nurseries and seed facilities conducted by federal authorities, all stakeholders along the supply chain can be effectively covered. This pragmatic approach builds on established structures, avoids additional administrative burdens, and therefore represents an efficient solution. Importantly, it may benefit small-scale nurseries, which often lack the resources to meet extensive additional financial and administrative requirements of certification schemes. Given their key role in supplying locally adapted provenances, it is essential to avoid measures that could accelerate their decline.

A fundamental limitation of the system is its reliance on reference samples, which restricts its applicability to domestically produced material. Only FRM from Germany meets comparable standards, whereas FRM from other countries generally lacks equivalent traceability mechanisms. This creates asymmetries within the European market and may disadvantage domestic producers subject to stricter verification requirements.

Generally, to avoid excessive burdens, regulatory requirements should be calibrated carefully, as overly stringent rules could drive the acquisition of FRM from other countries, thereby reducing domestic value creation and potentially undermining the use of locally adapted material. For Austria this would be particularly relevant given existing shortages of deciduous species such as *Acer platanoides* L., *Prunus avium*, *Quercus* spp., and *Tilia cordata* Mill. that are needed for forest conversion.

Closer collaborations between the relevant control bodies across countries is therefore needed to ensure consistent origin tracking and harmonised standards of control. Until such coordination is achieved, careful communication is essential to avoid unintended signaling effects. Limiting identity controls to domestic material may inadvertently lead consumers to perceive local products as less reliable, thereby counteracting the intended benefits of the system.

Stakeholder acceptance is crucial for successful implementation. While proof of compliance with quality standards can provide a competitive advantage, particularly in export markets, it may also expose producers to increased scrutiny. Clear communication, awareness-raising among forest owners and producers, and transparent reporting (e.g. anonymized annual results) are necessary to build trust and ensure broad support.

5. Conclusions

The fingerprinting methods described here are suitable for routine, large-scale verification of FRM. They serve several purposes: 1) Verification of the accurate declaration of forest reproductive material and 2) Compliance with the minimum number of harvested mother trees assuring high genetic diversity. As such they represent a significant milestone in strengthening consumer protection. However, verification testing relies heavily on the availability and quality of reference samples. Harvesters must therefore submit the single-tree seed samples in the correct quantities and of sufficient quality. It is neither practical nor intended to analyse all FRM on the market, instead analysis should focus on a representative subset (ca. 5% to 10% is feasible).

Genetic fingerprinting based on adaptive-neutral markers such as microsatellites is well suited for differentiating individual genotypes (trees) and should be viewed as a supplement to existing FRM regulations. Our results indicate that 8–9 primers are sufficient for reliable parentage analysis, making this approach cost-effective in operation. In practice, however, it is often insufficient enforcement of existing regulations that undermines traceability. Strengthening compliance and regulatory

oversight is therefore just as important as introducing new technical tools such as genetic fingerprinting.

Given the cross-border nature of FRM trade, it would be advantageous to work towards a harmonised, Europe-wide framework to ensure consistency, transparency, and effective implementation.

Supplementary material is available on-line via the following QR code:

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Appendix

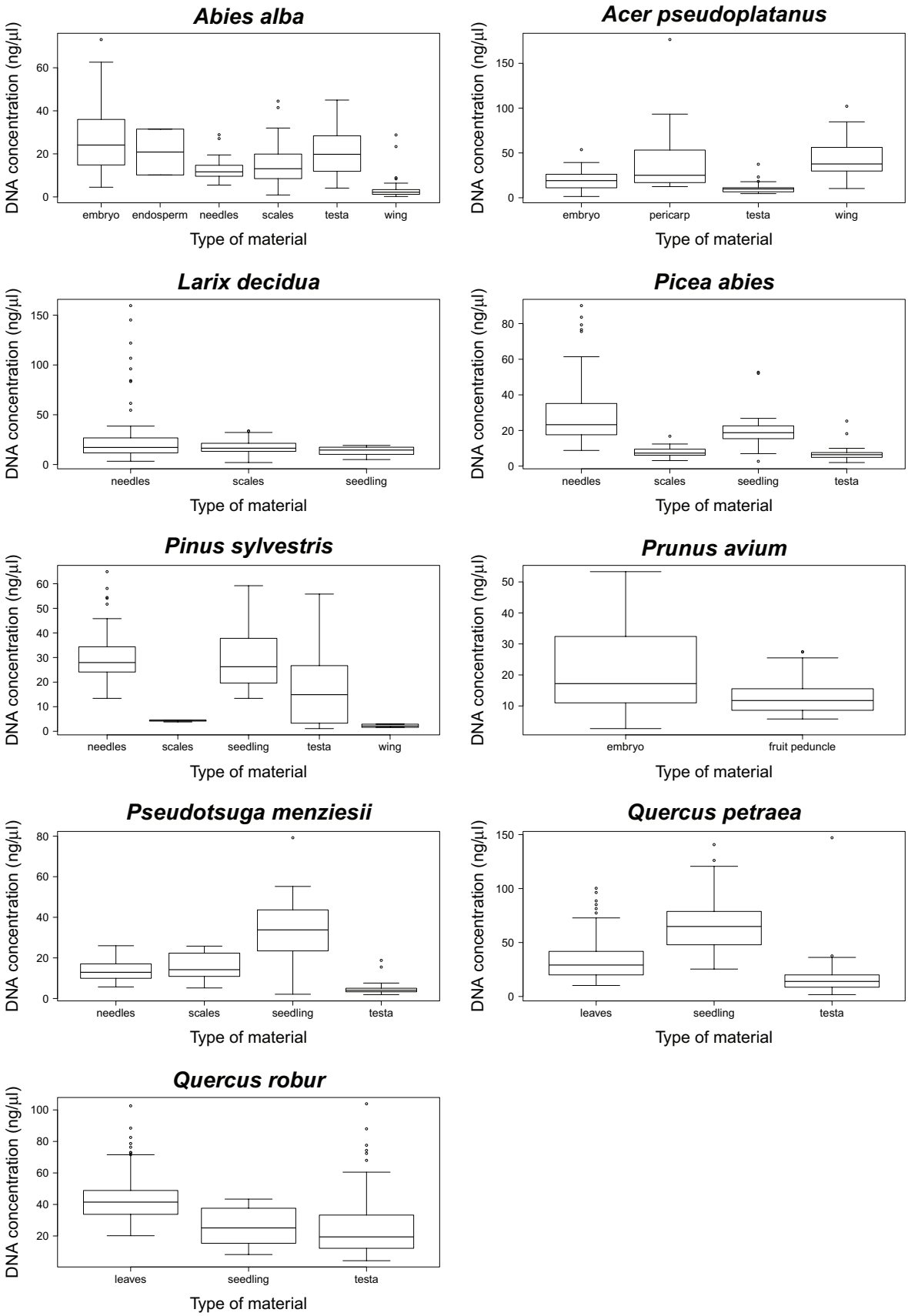


Fig. A1. DNA concentration of different types of material.

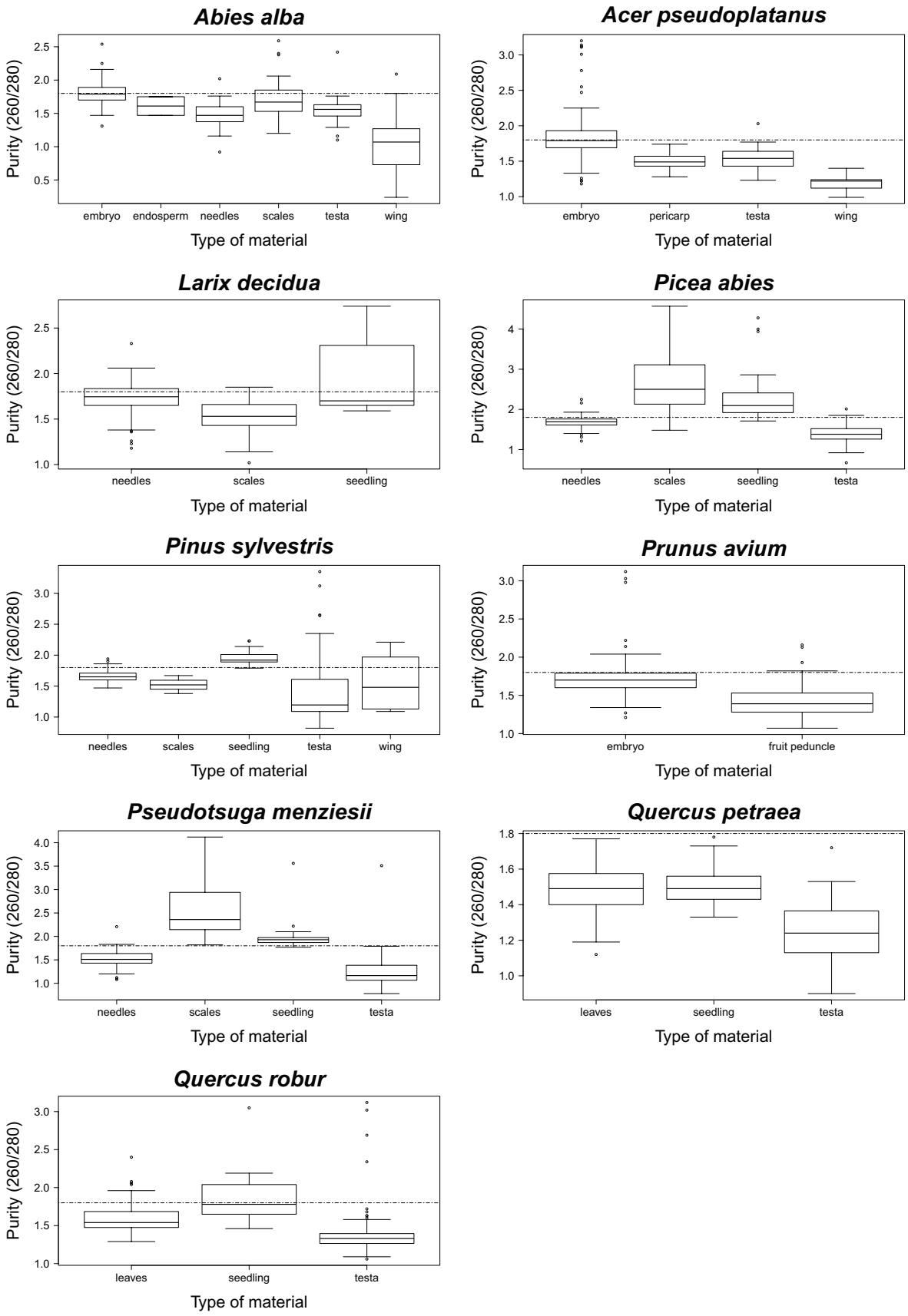


Fig. A2. DNA purities of different types of material. The optimal DNA purity of 1.8 is presented by the dashed line.

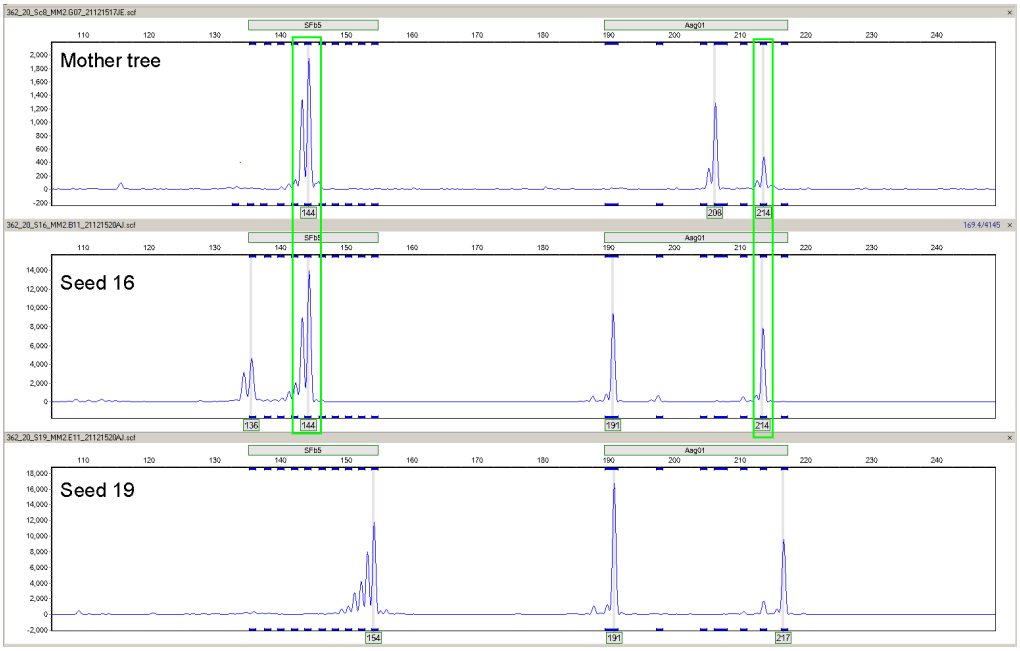


Fig. A3. Example of a microsatellite profile of *Abies alba*. The mother tree 8 and seed 16 share the allele 144 for the marker SFb5 and the allele 214 for the marker Aag01. Seed 19 does not have any shared alleles with mother tree 8 and hence is not its offspring.

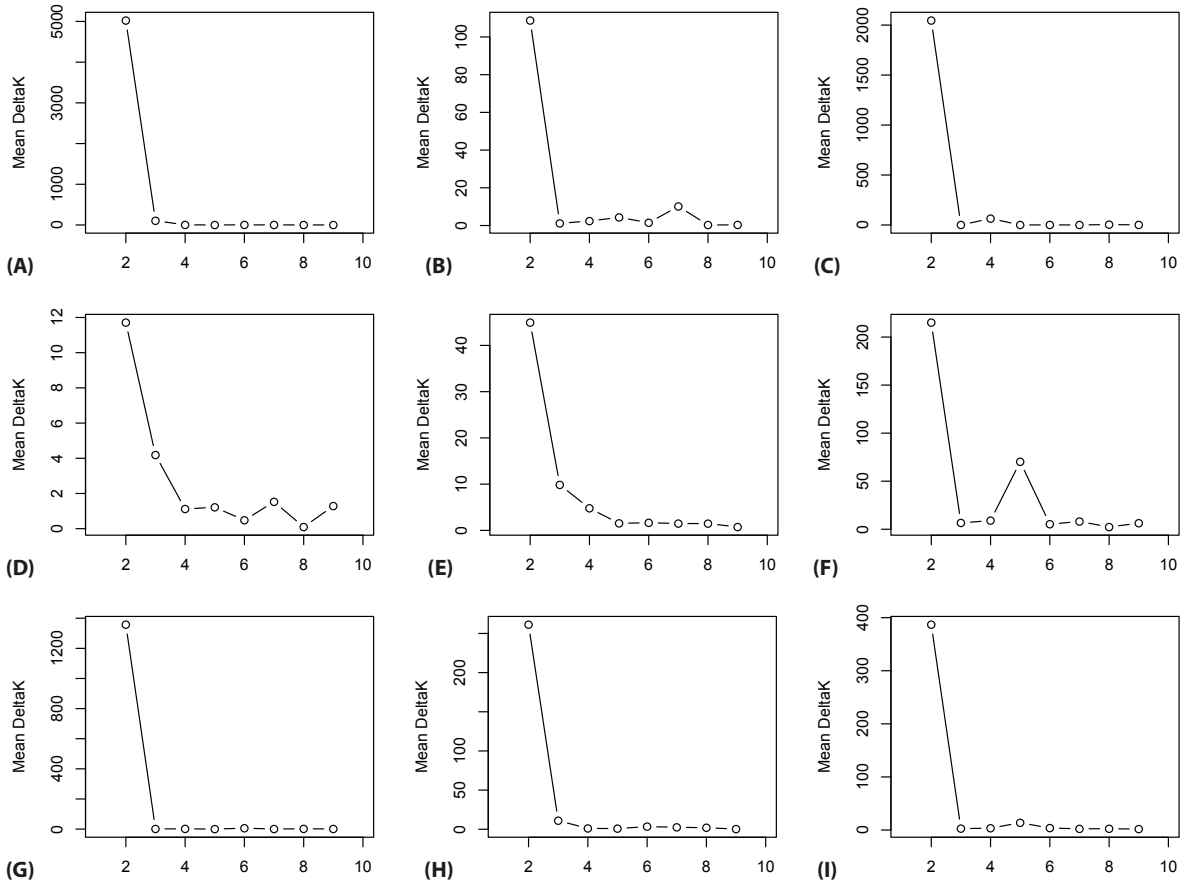


Fig. A4. Scatter plot to determine best K value using log probability (ΔK) method for: (A) *Abies alba*, (B) *Acer pseudoplatanus*, (C) *Larix decidua*, (D) *Picea abies*, (E) *Pinus sylvestris*, (F) *Prunus avium*, (G) *Pseudotsuga menziesii*, (H) *Quercus petraea*, (I) *Quercus robur*; the highest value of ΔK represents the optimum K value (K = 2) (Evanno et al. 2005).