Genetic variation of silver fir (*Abies alba* Mill.) in the Palatinate Forest

Isabelle Katharina Mittenbühler[1], Oliver Gailing*,[1,2,3], Markus Müller*,[1,2]

[1]Forest Genetics and Forest Tree Breeding, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany.
[2]Center for Integrated Breeding Research (CiBreed), University of Göttingen, Göttingen, Germany.
[3]Center of Biodiversity and Sustainable Land Use (CBL), University of Göttingen, Büsgenweg 1, 37077 Göttingen, Germany

*Corresponding authors: (MM) Tel.: +49 551 39 28238, mmuellef@gwdg.de; (OG) Tel.: +49 551 39 23536, ogailin@gwdg.de

Abstract

Silver fir in the southern Palatinate Forest grows naturally on dry sandy sites in one of the areas of Germany with the lowest precipitation. This region represents the northwestern edge of its natural range. In the present study, six EST-SSRs were used to genetically characterize a representative old-growth stand and its regeneration. Due to the pilot nature of the study, the results refer only to the silver fir stand examined and therefore cannot provide generally valid statements about the entire population. Compared to studies using similar methods, the stand shows an average genetic variation for Central European silver firs. The average number of alleles (Na) is 6.5 alleles locus^{-1}, while the average number of effective alleles (Ne) is 3.79 alleles locus^{-1}. Relatively high observed and expected heterozygosity values were measured. At 61.8 %, the current heterozygosity is very similar to the expected heterozygosity of 61.2 %. No significant differences in genetic variation were observed between generations. The genetic information was passed on to the next generation without the loss of allelic variants. The fact that more private alleles were found in the natural regeneration indicates a high external pollen input. There was no evidence of isolation, genetic drift, or inbreeding. No family structures were recognizable on the basis of the investigation of the fine-scale spatial genetic structure. Based on the results, the population is not currently classified as endangered, which is why *in situ* conservation is considered appropriate.

Key words: *Abies alba*, EST-SSRs, Genetic Variation, Microsatellites, Palatinate Forest, Spatial Genetic Structure

Introduction

From historical scientific sources, paleontological studies, stand descriptions, and archived forest management documents, it can be shown that silver fir (*Abies alba* Mill.) was a fairly common tree species in mixed Central European forests at higher elevations (Haupt 1988). However, there is probably no other native tree species with a greater discrepancy between its potential natural and its actual distribution (Braun and Gómez 1994). In Germany, its natural occurrence is mainly restricted to the southern part of the country (Schroeder 1988), whereby it is classified as regionally endangered (Wolf 2002). In the federal state of Rhineland-Palatinate, the current natural occurrence of *A. alba* is limited to the Palatinate Forest near the German-French border (cf. Eder 1988). This occurrence represents the northwestern edge of its natural range.

Given the climate that already prevails in the Southern Palatinate today, combined with the steady increase in dry years and the relatively high precipitation requirements of silver fir highlighted in the relevant literature, this tree species is currently outside its climatic suitability range in this area. It is known that the conditions in the glacial refugia and their postglacial remigration history have affected the genetic structure of *A. alba* (see Konnert 1994, Gómez and Braun 1995, Konnert 1995, Gómez 1998, Vendramin et al., 1999, Konnert and Hussendörfer 2004, Liepelt et al., 2009, Konnert and Schirmer 2011, Neophytou 2015). In the past, the peculiarities of the genetic structure of silver fir have led to the view that this tree species has insufficient adaptability (e.g., Larsen 1986, Larsen 1988, Konnert 1992). With regard to the geographical distance to its core distribution area and thus also to its ecological center, reduced genetic variation is expected in the genetic makeup of the Palatinate silver fir population. At the same time, there is the possibility of adaptation to drought due to its successful establishment on dry, sandy sites.

To date, however, there is a lack of knowledge about the genetic constitution of the population to categorize the necessary conditions that will enable it to adapt sufficiently to future climatic changes. Earlier studies on *A. alba* used isoenzyme analyses for this purpose (e.g., Bergmann and Gregorius 1993). In the meantime, highly variable nSSRs (e.g. Cremer et al. 2006, Oreshkova et al. 2023) as well as EST-SSRs (EST = Expressed Sequence Tags) have been designed for *A. alba* (Postolache et al. 2014). Mosca et al. (2012) and Roschanski et al. (2013, 2015, 2016) have dealt with the development of SNPs in candidate genes in relation to *A. alba*. Recently, next generation sequencing (NGS) methods have been used to investigate the the genetic basis of adaptation to environmental changes (Behringer et al. 2025, García-García et al. 2025). Further, new genomic resources were generated such as a reference genome

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Mosca et al. (2019) as well as chloroplast and mitochondrial genomes (Li et al. 2019, Kersten et al. 2022).

Microsatellite markers are suitable for the study of small-scale diversity, especially in populations with low variation (cf. Cremer et al. 2006), due to their highly variable characteristics and the possibility of comparison with a large number of studies using similar methods. In the present study, selected EST-SSRs were used to carry out an initial genetic characterization of a fir stand representative of the Palatinate Forest. With regard to the genetic potential, meaningful conservation measures are to be derived

Material and Methods

Selection of sample areas

The selected study area (Fig. 1) is located in the southwest of Rhineland-Palatinate on the southern edge of the Palatinate Forest. Functionally, it is assigned to the German part of the Pfälzerwald-Voges du Nord cross-border biosphere reserve. C-dated pollen profiles near Wilgartswiesen, approx. 14 km northwest of the study area, confirm the natural remigration of silver fir in this part of the Palatinate Forest during the Subboreal, Late Warm Period (ca. 2500 BC) at the same time as beech and hornbeam (Schloß 2017). A second pollen profile near Trippstadt, about 30 km to the northwest of the study area, on the other hand, showed that silver fir only appeared here with the beginning of modern forestry (Wolters 2007). According to this, silver fir did not succeed in colonizing the entire Palatinate Forest in the course of its remigration. At around 180,000 hectares (Hohmann et al., 2018), the Palatinate Forest is considered the largest contiguous forest area in Germany (Fenkner-Gies and Gauer 2005, Hohmann et al., 2018). The soil-forming parent substrate in the Palatinate Forest is mostly Triassic sedimentary rock known as red sandstone (Hohmann et al., 2018). Some of the fir stands in the southern Palatinate Forest grow on shallow brown soils. The topsoil horizon of these sites is characterized by moderately silty sand, whereas the mineral subsoil horizon consists of pure sand (cf. Mittenbühler 2020). These very dry sites are characterized by low usable water storage capacities (nWSK 3) (cf. Mittenbühler 2020). The climate in the area is characterized by subcontinental, warm-temperate, and low-precipitation zones (cf. Fenkner-Gies and Gauer 2005). The average annual precipitation calculated for the district over a period of 20 years is 738 mm year^{-1} (Table 1). The months of the growing season account for 412 mm year^{-1} (Table 1). An earlier study comparing the growth of silver firs in a dry location with that in a more humid location showed that the tree species physiologically adapts to the dry climate of the region (Mittenbühler 2020): The silver firs studied hardly differed in their stress tolerance. The growth of silver fir on the dry site was even less severely reduced on average in dry years, meaning that the persistent drought stress of recent years has hardly affected its growth.

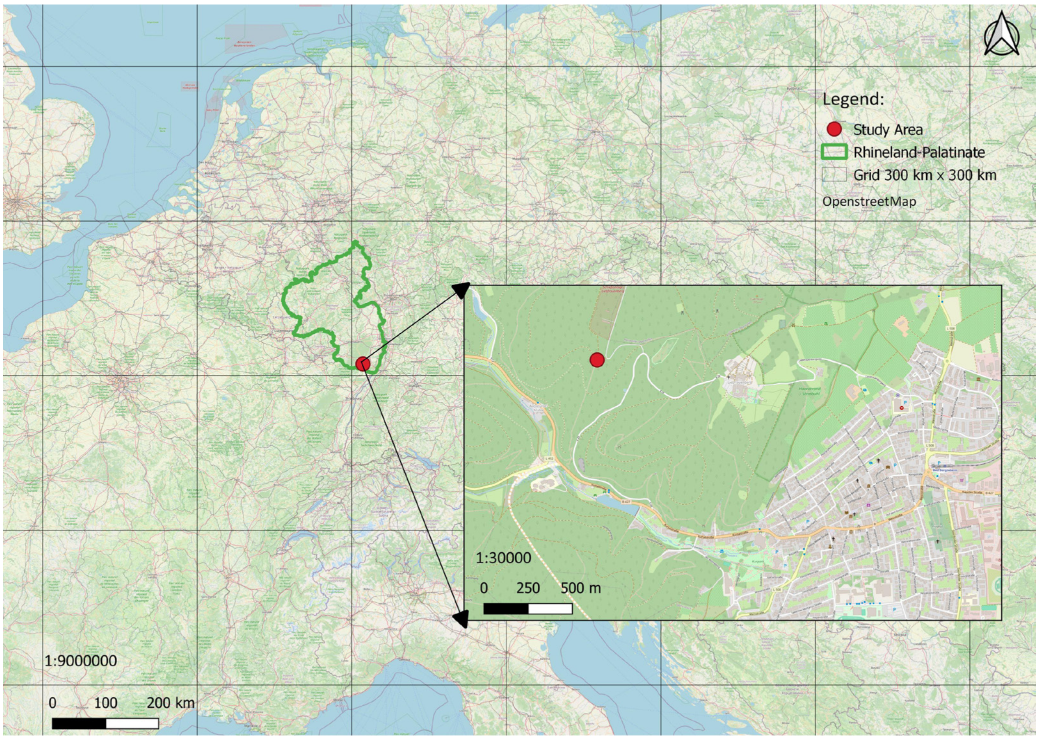

Figure 1
Overview map of the topographical location of the study area. Map of Germany with the study area marked (red dot). Background map shows WGS 84 grid lines with a 300 km spacing; scale bar indicates 0-100-200 km. Map in the foreground has a scale of 0-250-500 m. Source: Own illustration; background map: OpenstreetMap (2024), © OpenstreetMap, CC BY-SA 2.0, (https://creativecommons.org/licenses/by-sa/2.0/), URL: http://tile.openstreetmap.org/{z}/{x}/{y}.png (cited 18/12/2024)

Table 1: Climate data for the district of "Südliche Weinstrasse"

Climate data:	district of „Südliche Weinstraße“
Annual mean temperature:	11.5 °C
Temperature in July:	20.8 °C
Temperature in January:	2.6 °C
Temperature FVP:	16.3 °C
(Temperature > 10°C)	
Precipitation:	738 mm year^{-1}
Precipitation FVP:	412 mm year^{-1}
Length FVP:	approx. 150 – 165 days
Altitude range:	collin (150 – 300 m)
Continentality:	subcontinental

FVP: forest growing season

In addition to fir (*A. alba*), tree species typical of the Palatinate Forest are Scots pine (*Pinus sylvestris* L.), beech (*Fagus sylvatica* L.), spruce (*Picea abies* (L.) Karst.), sessile oak (*Quercus petraea* (Matt.) Liebl.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), European larch (*Larix decidua* Mill.) and sweet chestnut (*Castanea sativa* Mill.).

Field and laboratory work

The stands were selected by means of local inspections and taking into account current and historical forest management plans. Stands excluded if insufficient information was available on the completeness of natural development or if they were not regenerated across the entire area. Using the QGIS program (developed by the QGIS development team, version 3.28.4 Firenze), two randomly sampled rectangular observation strips were set up in the sample areas, and all old trees of classes 1 + 2 at Kraft (1884) and the nearest seedlings (minimum diameter of 2 cm) were recorded. In total, 88 old trees and 88 corresponding seedlings were selected. The sample collection was carried out from January 10 to 11, 2024 by employees of the Research Institute of Forest Ecology and Forestry of Rhineland-Palatinate (FAWF, Trippstadt). The samples were processed and analyzed between January 29, 2024 and February 9, 2024 in the laboratories of the Department of Forest Genetics and Forest Tree Breeding at the University of Göttingen. A DNeasy 96 Plant Kit (QIAGEN) was used for DNA extraction of the samples. Eight EST-SSRs primers (AB 6, AB 7, AB 10, AB 13, AB 14, AB 17, AB 19 and AB 40) were selected for genetic characterization (Table 2). These were recently developed as part a genetic study by the Department of Forest Genetics and Forest Tree Breeding at the University of Göttingen using the transcriptome data published by Roschanski et al. (2013). Two different fluorescent dyes were used for colour labelling (carboxyfluorescein (6-FAM): AB 6, AB 7, AB 10, AB 14) and hexachlorofluorescein (HEX): AB 13, AB 17, AB 19, AB 40). Instead of labelling individual primers, an M13-specific sequence (5‘-CACGACGTTGTAAACGAC-3’) was used, as suggested by Schuelke (2000), which colour-codes the 5' end of the forward primers. The 5‘ end of the reverse primers was labelled with a PIG-tail (5’-GTTTCTT-3'). The PIG-tail method used here was developed by Brownstein et al. (1996) with the aim of increasing the accuracy of genotyping. The primers were first tested individually and then using various multiplex analyses.

Table 2: PCR primer sequences

Primer No.		Accession no.	PCR primer (5′ – 3′)	sequences	Size range (bp)	total length	Fluorescent dye
			forward	reverse	reapeat motif		
AB 6	contig 28516	JV143388.1	TCACTGGGTCATGTTTAGGATGA	AAACGACCAGAAAGCCCTCT	200 –235	TA $_{(23)}$	6-FAM
AB 7	contig 30769	JV144084.1	TCGCTGAAGGTCTGATAGATGT	ACAGACTGGATGGCACTTGA	254 –285	TA $_{(22)}$	6-FAM
AB 10	contig 08889	JV151323.1	TGAGTTCCTGGTTTCGTCTGA	AGAATGCCAGAATGTCAAA-CAGT	155 –195	AT $_{(17)}$	6-FAM
AB 13	contig 10403	JV151817.1	AGGAGTTGGAGGAATTGGCA	GTCAGAAGAGGAGTCGAG-GAG	239 –255	GGA $_{(33)}$	HEX
AB 14	contig 11256	JV152090.1	TCCGTAGCCCCTCTCTCATA	TCCGTGGTTCCATCTCACAA	231 –258	AT $_{(16)}$	6-FAM
AB 17	contig 19808	JV155481.1	GCCAGAGCCAAAGTTTCAGG	CGACCAGTGATTGCCAAACA	124 –150	TA $_{(21)}$	HEX
AB 19	contig 20177	JV155613.1	GCTGAACTTTTGGAGGCCTC	GCAAGGCAGCAAGAAGACAA	135 - 189	CT $_{(26)}$	HEX
AB 40	contig 17312*	JV140842.1	TAACGTGCTCTTATCGGTGC	ACAAGTTCTAGCAGCAGTTGA	240 –266	GGAAAG $_{(25)}$	HEX

Fluorescent dyes used: carboxyfluorescein (6-FAM): AB 6, AB 7, AB 10, AB 14 und hexachlorofluorescein (HEX): AB 13, AB 17, AB 19, AB 40 *Contigs refer to Roschanski et al. (2013)

To prepare the PCR, the extracted DNA template was mixed with H_2O (4 µl DNA + 36 µl H_2O) in a ratio of 1:10. For the preparation of the PCR solution, 7.1 µl, or for a multiplex analysis, 6.4 µl H_2O, 1.5 µl PCR buffer, 1.5 µl $MgCl_2$ (25 mM), 1.0 µl dNTP (2.5 mM of each dNTP), 1.0 µl M13 adapter (5 µM), 0.2 µl primer forward (5 µM) and 0.5 µl primer reverse (5 µM) as well as 0.2 µl Taq polymerase (5 U/µl) were mixed. Biometra thermocyclers (TProfessional Thermocycler) were used to carry out the PCRs. The touchdown program consisted of an initial denaturation at 95 °C for 15 minutes. This was followed by 10 touchdown cycles of one minute denaturation at 95 °C, primer hybridization (annealing) at 60 °C (- 1 °C after each cycle) and an elongation step at 72 °C. This sequence was followed by 25 cycles, each with one minute of denaturation at 95 °C, one minute of primer hybridization at 50 °C and an elongation step at 72 °C, before a constant temperature of 72 °C was maintained for 20 minutes for a final elongation. The samples were then cooled down to 16 °C. The amplification products were diluted 1:50 with H_2O. A mixture of 1,200 µl HiDi Formamide (Applied Biosystems) and 1.6 µl GeneScan 500 Rox (Applied Biosystems) internal size standard was prepared. The samples were then denatured in the PCR cycler at 95 °C for 3 minutes. A fluorescence-based capillary sequencer (ABI PRISM® 3130xl Genetic Analyser, Applied Biosystems) was used to separate the amplification products. The microsatellite fragments separated by the sequencer were automatically identified and genotyped in the GeneMapper® program version 4.0 (Applied Biosystems) and then visually inspected and assigned to allele sizes. After genotyping, primers AB 7 and AB 17 were excluded from further analysis due to the frequent occurrence of stutter bands.

Data analysis

Probability of identity was assessed with GenAlEx version 6.51b2 (Peakall and Souse, 2006). MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004) was used to check for the presence of null alleles. The indices of genetic variation (number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He)), the fixation index (Fst), the parameters of genetic distance (G'st(Nei), G'st(Hed), Dest), the AMOVA, the codominant genotypic distance and the PCoA based on it were calculated using the software GenAlEx version 6.51b2. GenAlEx version 6.51b2 was also used to test for Hardy-Weinberg equilibrium and to calculate the p-values of the differentiation measures. Based on 1000 permutations the inbreeding coefficient of the F statistic (Fis) and its p-values were calculated separately with SPAGeDi version SPAGeDi1-5d, build02-06-2017 (Hardy and Vekemans 2002). For locus-wise tests (HWE and F-statistics), we controlled for multiple testing using Bonferroni correction. All calculations were performed using the default settings. A Kruskal-Wallis test was carried out in RStudio (Version RStudio-2024.12.1-563) to compare the genetic variation parameters (Na, Ne, Ho, He) between old trees and natural regeneration. The SGS was analyzed using the pairwise kinship coefficient according to Loiselle et al. (1995) using SPAGeDi version SPAGeDi1-5d, build02-06-2017). With regard to the average dispersal distance of the seeds, which according to Kutter (2007) is 22 m, 10 distance classes were assigned. With regard to the kinship coefficient, at least 50 pairwise comparisons were carried out for each distance class. The number of permutations was set to 1,000. Sp value, which indicates the strength of the SGS, was estimated using the formula $Sp = -b/(1-F1)$ (Vekemans and Hardy 2004), where b denotes the regression slope of the Loiselle coefficient and F1 denotes the average kinship coefficient in the first distance class (Vekemans and Hardy 2004).

Results

Genetic variation

Probability of identity for the six EST-SSRs was 0.00001. The results from MICROCHECKER show that none of the loci exhibit a high probability of null alleles. Deviation from Hardy-Weinberg equilibrium was significant for AB 13 and AB 14.

The genetic variation of the old growth (adult), natural regeneration (young), and the entire stand (total) was analyzed (see Table 3). For individual markers, the number of alleles (Na) varies between 4 and 12 with a mean value of 6.5 alleles. The number of effective alleles (Ne) is between 1.3 and 8.5 with a mean value of 3.79 alleles. Ho varies between 0.25 and 0.89 with a mean value of 0.62. He is between 0.23 and 0.88 with a mean value of 0.61. The Kruskal-Wallis test indicated that old growth and natural regeneration do not differ significantly from each other with regard to the parameters of genetic variation (Na, Ne, Ho, He). The fixation index (Fis) ranges from 0.21 and - 0.21 with a mean value of - 0.02, and does not differ significantly from zero.

Table 3: Indices of genetic variation at the 6 loci; mean values (Mean) of the parameters of genetic variation and standard error (SE). Old growth (adult, n = 88) and regeneration (young, n = 88) as well as for the entire stand (total, n = 160)

Pop	Locus	Na	Ne	Ho	He	Fis
Adult	AB 10	4	1.63	0.47	0.39	-0.20*
	AB 40	5	4.08	0.77	0.75	-0.02
	AB 6	8	5.35	0.78	0.81	0.04
	AB 13	5	1.52	0.27	0.34	0.21
	AB 14	4	2.29	0.63	0.56	-0.10
	AB 19	12	7.51	0.80	0.87	0.08
	Mean	6.33	3.73	0.62	0.62	0.01
	SE	1.28	0.97	0.09	0.09	0.03
young	AB 10	5	1.30	0.25	0.23	-0.08
	AB 40	5	4.02	0.74	0.75	0.02
	AB 6	9	5.46	0.82	0.82	0.01
	AB 13	5	1.63	0.36	0.39	0.07
	AB 14	4	2.18	0.63	0.54	-0.16
	AB 19	12	8.49	0.89	0.88	0.00
	Mean	6.67	3.85	0.61	0.60	-0.02
	SE	1.28	1.13	0.10	0.11	0.03
total	Mean	6.5	3.79	0.62	0.61	-0.00
	SE	0.87	0.71	0.06	0.07	0.02

Pop = population, Na = mean number of alleles, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity, Fis = inbreeding coefficient, *significant after Bonferroni correction ($p < 0.005$)

Genetic differentiation

The genetic differentiation between old growth and regeneration was analyzed. Based on both the F-statistics and G-statistics, no significant genetic differentiation between old growth and regeneration was found (Tab. 4).

Table 4
F-statistics and G-statistics for the genetic differentiation between old growth and natural regeneration at the 6 loci, mean values (Mean) and standard deviation (SE)

Locus	Fst	Fis	G'stN	G'stH	Dest
AB 10	0.017*	-0.137*	0.029*	0.028*	0.013*
AB 40	0.007	-0.007	0.008	0.029	0.025
AB 6	0.001	0.022	0.000	0.000	0.010
AB 13	0.002	0.132	0.000	0.000	0.000
AB 14	0.000	-0.133	0.000	0.000	0.000
AB 19	0.003	0.039	0.001	0.004	0.004
Mean	0.005	-0.003	0.003	0.005	0.004
SE	0.003	0,017	0.003	0.007	0.005
p-values	0.08	0.856	0.098	0.098	0.098

Parameters of the F-statistic: Fst and the G-statistic: G'st(Nei), G'st(Hed), Dest, *significant after Bonferroni correction ($p < 0.005$)

Based on pairwise G-statistics for Fst, 999 permutations, only slight differences between old growth and regeneration were determined. The Fst value measured here is 0.005 ($p = 0.08$).
The variability of genetic distances between individuals was also visualized using a PCoA (Fig. 2). The PCoA shows a relatively centered coordinate system within a homogeneous point cloud with a mix of individuals from old growth and regeneration.

Fine-scale spatial genetic structure (SGS)

With regard to the fine-scale spatial genetic structure, no family structures are recognizable in either the old growth or regeneration (Fig. 3). It is striking that a negative relationship coefficient exceeds the confidence interval for the old growth at a distance of 50 meters. When analyzing the entire stand, family structures were detected at distances of 20 and 40 m, respectively.

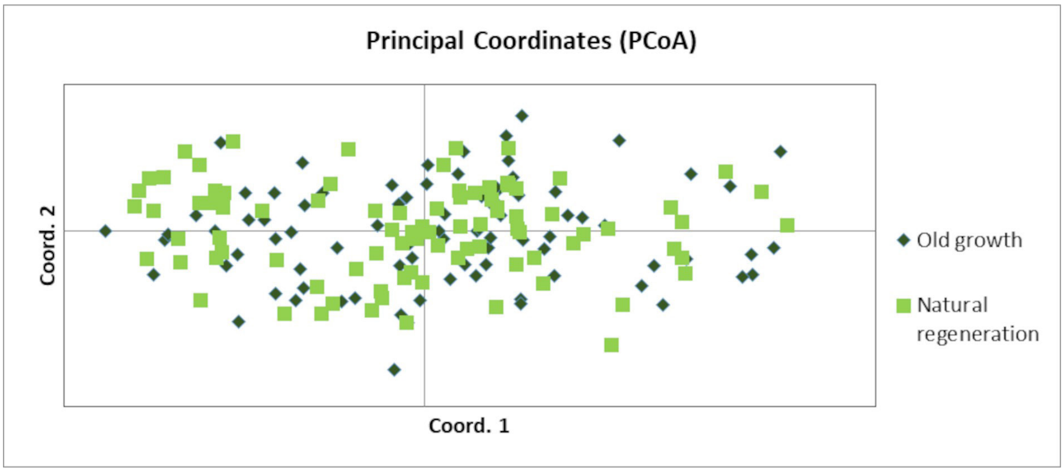

Figure 2
Principal Coordinate Analysis (PCoA) of the stand. Old growth: dark green diamonds, regeneration: light green squares, plotted on the coordinates x (1) and y (2). Basis of the calculation: Codominant Genotypic Distance

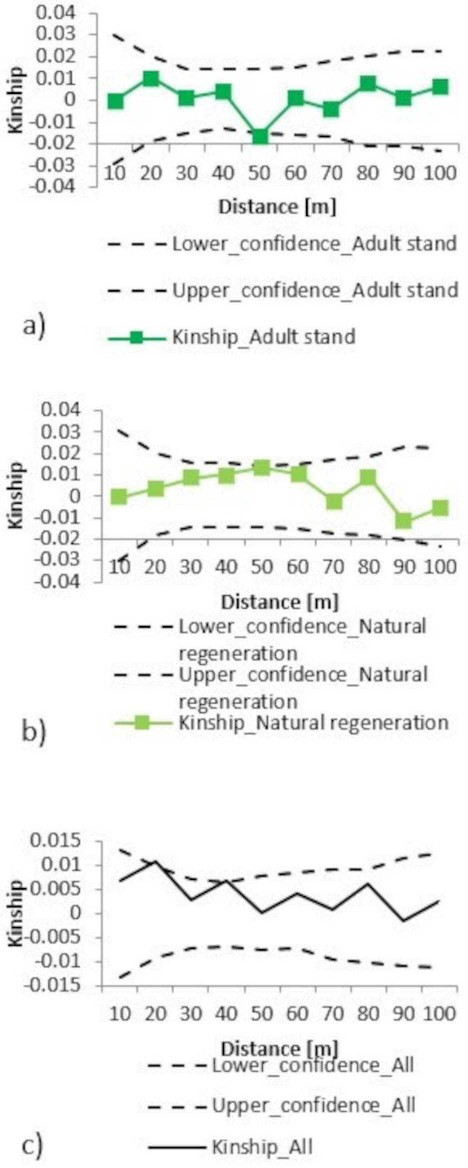

Figure 3 Fine-scale spatial genetic structure of the old growth, the natural regeneration, and the entire stand. At least 50 pairwise comparisons were carried out for each distance class. Dotted line: confidence intervals, green line: kinship coefficient. Plotted over the spatial distance (m). If the kinship coefficient is consistently within the confidence intervals, no family structures are recognizable. a) A negative kinship coefficient is recorded for the old growth at a distance of 50 m (Sp-Statistic: 0.0002). b) There are no indications of family structures in the regeneration (Sp-Statistic: 0.0047). c) With regard to the entire stand, it is noticeable that a positive kinship coefficient exceeds the confidence intervals at a distance of 20 and 40 m (Sp-Statistic: 0.0032).

Discussion

Comparatively high level of genetic variation

The findings refer to the investigated stand of silver fir trees in the Palatinate Forest. It cannot be ruled out that other regional stands may differ from this. The results were categorized by comparing them with the results of other studies using similar methods. This made it possible to determine that the Palatinate silver fir stand has an average genetic makeup for Central European fir populations (see Table 5). However, a direct comparison was not possible due to the differences between the markers. While an average of 6.7 alleles per locus (3.1-10.1 for different populations) was determined in other studies, this value amounts to 6.5 alleles per locus in the Palatinate silver fir stand. Likewise, the effective number of alleles is 4.0 (2.8- 5.4) alleles per locus in other studies, which is comparable to the value of the Palatinate silver fir stand, which is 3.8 alleles per locus. Due to the fact that EST-SSRs are located in genes and are therefore more conserved, they are considered less polymorphic compared to nSSRs (Ellis and Burke 2007), so that a relatively high genetic variation was determined in this study.

Table 5
Comparison of results from different *A. alba* studies

Reference	Na	Ne	Ho (%)	He (%)	Population
Postolache et al., 2014 (16 EST-SSRSs)	7.0*	n. a.	37.8*	38.2*	Italy (Apennines), Bulgaria, Romania
Cremer 2009 (6 nSSRs)	8.1*	3.1*	47.1*	56.7*	Northern Black Forest
Cremer et al., 2006 (11 nSSRs)	5.2	n. a.	31.1*	53.2*	Germany, France, Bulgaria
Cvrčková et al., 2015 (8 nSSRs)	10.1*	5.4*	69.1*	75.3*	Czech republic
Šeho et al., 2022 (15 nSSRs)	8.8	4.5	60.4	60.2	Romania
Popović et al., 2017 (9 nSSRs)	3.1	n. a.	n. a.	n. a.	Serbia
Oreshkova et al., 2023 (14 nSSRs)	4.5	2.8	44	56	unknown
Mean value[1]	6.7	4.0	48.3	56.6	
In this study	6.5	3.8	61.8	61.2	

* based on the authors' calculations, [1]the mean values were calculated from the studies listed in the table (unweighted arithmetic mean).

In the comparison of old growth and regeneration, the Palatinate silver firs hardly differ in terms of their genetic make-up. The breadth of genetic variation in the old growth is therefore passed on to the next generation without any demonstrable losses. The natural regeneration even had more private alleles (6) than the old growth (4). Both parameters of genetic variation (Na and Ne) are even higher in the regeneration than in the old growth. The genetic variation of the Palatinate silver fir stand is relatively high, which was determined by the degree of heterozygosity (see Table 5). While an observed heterozygosity (Ho) of 48.3 % (31.1 %-69.1 %) was determined on average in the comparative studies, this value amounts to 61.8 % in the Palatinate silver fir stand. The expected heterozygosity (He) in the other studies averages 56.6 % (38.2 %-75.3 %), while in the Palatinate silver fir stand, this value is 61.2 %. The silver fir stand from the Palatinate Forest therefore not only has more heterozygous individuals than the stand in the neighboring Black Forest but also reveals high diversity in an international comparison. The analyzed old growth from the Palatinate Forest shows a degree of heterozygosity that hardly differs from that of their seedlings. Nevertheless, a slight decrease in the percentage of heterozygosity can be observed, albeit not significant. A significant drift-related loss of alleles was not observed. The genetic structure of the Palatinate silver fir stand does not show the characteristic features of an isolated population.

Low genetic differentiation between adult and seedling generation

Based on the F-statistics, the genetic distance parameters, the pairwise G-statistics, the AMOVA, and the PCoA, no significant genetic differentiation between old growth and regeneration was detected. The consistency of the genetic structure shows that old growth and regeneration genetically behave like a single population, in which random mating prevails (cf. Cremer 2009, Peakall and Smouse 2012). Influences such as isolation, which disrupt the balance between drift and gene flow and lead to genetic differentiation due to reduced gene flow (cf. Templeton et al., 2001), can be ruled out. Similar observations were made by Cremer (2009) on the basis of analyses of three silver fir stands from the northern Black Forest.

Absence of SGS

Analyzing the entire stand (i.e., adult trees and seedlings together) revealed family structures as expected. These outcomes can be seen as evidence of the relationship between the mature trees and the seedlings. Kutter (2007) describes the average dispersal distance for fir seeds as 22 m, which aligns with our results. However, such structures were not found in the two subpopulations of old growth and regeneration. The population density and the number of effective neighbors are deemed sufficient, which means there is currently no risk of genetic impoverishment from drift and inbreeding caused by single dominant seed parents or varying selection conditions.

Economic utilization could have led to the breakup of family structures in the old growth through the removal of related individuals. According to Hattemer and Ziehe (2018), the absence of family structures may also indicate that the stand was artificially established by planting, but this hypothesis can be ruled out in the present case based on research into the stand history.

Cremer (2009) investigated SGS in three silver fir stands from the northern Black Forest. No significant family structures in adult firs were found. She concludes that high gene flow processes have equalized the local, selection-related genetic differentiation and led to genetic homogeneity (cf. Slatkin 1987). She cites the low genetic differentiation of the stands as further evidence of homogenizing gene flow. In the Palatinate Forest, too, local bottlenecks in the seedling pool were presumably compensated for by extensive pollen inputs - an effect that goes hand in hand with the increase in genetic variation (cf. Cremer 2009).

Conclusions

To characterize a Palatinate silver fir stand and investigate its population genetic structure, a representative stand was selected, and DNA analysis using six gene-based microsatellite markers was conducted. Observations of the genetic variation in the stand and comparison of the results with similar studies indicate high genetic variation in the gene pool, suggesting current resilience to prevailing conditions. Nevertheless, the mid- or long-term local survival of the populations also depends on future conditions. In the context of rapid climate change, monitoring of genetic resources is proposed to assess long-term impacts and to be able to respond in a timely manner to any changes in genetic variation (cf. Finkeldey and Hattemer 2010). The present study determined a similarly high level of genetic diversity for the investigated fir stand in Rhineland-Palatinate as that observed in Central European silver fir populations. An unusually high level of genetic variation was observed in this fir stand, considering its location within a marginal population and its geographical distance from the center of its distribution. No characteristics indicating isolation were found. The mixing of genetic material from different migration routes can be ruled out on the basis of the findings on postglacial remigration (cf. SGD SÜD and FAWF 2005). It is possible that seedlings (cf. Keiper 1930, Eder 1988) have provided a genetic refreshment. No family structures were recognizable on the basis of the analysis of the fine-scale spatial genetic structure. Especially in the context of the lack of differentiation of the stand, the finding suggests homogenizing gene flow processes, favored by high pollen inputs, as shown by the presence of private alleles in the natural regeneration. Given the adaptation to wind pollination, it is also conceivable that a genetic exchange could occur with the stands in the Vosges. A comparison of old growth and regeneration shows no evidence of drift-induced allele loss or inbreeding. It is to be expected that local genetic bottlenecks in the seedling pool will be compensated for by external pollen inputs, as the natural regeneration already has more private alleles. Based on fine-scale genetic structure analysis, there were no indications of an insufficient number of effective neighbors, individual dominant seed parents, or varying selection conditions. The old growth and regeneration behave like a single population under random mating. The stand is characterized by different age classes, a strikingly lush natural regeneration, and a reproductive system similar to panmixis, so that the complete transmission of genetic breadth/variation of the mother trees to their regeneration is to be expected (cf. Hattemer and Ziehe 2018), and there is also the possibility of sustainable management in the foreseeable future. Based on the available findings, *in situ* conservation is considered sufficient. Due to the fact that a current threat to the stand can be ruled out, further measures are not necessary. The results of the study refer to a reference population located in the center of a proven autochthonous population. Due to the wide pollen dispersal and natural regeneration processes, homogenization is assumed in the core area. Differentiations between the local fir stands in the Palatinate Forest are assumed to be due to artificial introduction, especially outside the core area. To recognize possible differentiations, comparisons with the genetic structure of fir trees from the northern and southern Black Forest and the Vosges are essential. With regard to the conditions in the growing region, adaptation to drought stress seems possible. To gain insights into the characteristics of their drought stress tolerance, further investigations such as physiological studies, but also analyses of adaptive variation on the basis of SNPs or transcriptomics are required.

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