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Exploring the Gene Pool of Silver Fir in Southern Germany on the Search for Climate-Smart Seed Sources

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ABSTRACT

Central European populations of silver fir (*Abies alba* Mill.) grow under a relatively wide amplitude of environmental conditions. Assuming that forest tree stands are locally adapted, the use of forest reproductive material from sites with limited water availability is expected to increase drought tolerance in future forests. At the same time, genetic diversity is of utmost importance as the basis of adaptation to a changing environment. Currently, a selection of potential sources for climate-smart reproductive material of silver fir is under way in Southern Germany. It is based on a multidisciplinary approach elucidating the genetic variation, site conditions, as well as tree resilience based on a dendroecological approach. The analysis encompasses a large number of stands representing the whole range of the species' ecological requirements. The population genetic analysis based on molecular markers (nuclear microsatellites) provided important information concerning the gene pool of the species in Southern Germany. On one hand, it revealed genetic differentiation and transition zones between western and eastern clusters. On the other hand, the results indicated gradients and regional variation of genetic diversity. These patterns can be explained by post glacial recolonization and the peripheral character of the species at the northern limit of its distribution. Together with the outcomes of the site condition and dendroecological approaches, the results of the genetic analysis will be used to inform future provenance recommendations.

Keywords: *Abies alba*; genetic diversity; resilience; microsatellites; forest reproductive material

INTRODUCTION

In Central Europe, silver fir (*Abies alba* Mill.) is expected to cope better with the challenges posed by climate change than the more widely cultivated Norway spruce (*Picea abies* (L.) H. Karst.). Compared to the latter species, silver fir can better tolerate drought events and can also better benefit from mild winters in terms of growth (Vitali et al. 2018). Moreover, its current distribution only covers a limited part of its potential range in this region (Tinner et al. 2013). After a period of decline during the 20th century, which was mainly attributed to sulphur dioxide emissions, it is now generally experiencing a recovery (Elling et al. 2009). Recent results on growth decline and mortality induced by the drought in the summer of 2018 show that Norway spruce might be more vulnerable to such extreme drought events (Schuldt et al. 2020). Therefore, silver fir can be a suitable option for several sites, especially in submontane areas (Ruosch et al. 2016, Vitasse et al. 2019).

Silver fir's growth responses to drought greatly depend on within-species genetic variation (Martínez-Sancho et al. 2021). Common-garden trials have shown that growth performance, drought tolerance and resilience underly a strong ecotypic variation (Commarmot 1995, Dobrowolska et al. 2017, Hansen and Larsen 2004, Martínez-Sancho et al. 2021). Thereby southern origins are not per se better suited to drought events. For example, individuals from the Pyrenees showed reduced growth performance likely pointing to their poor genetic pool due to isolation processes (Martínez-Sancho et al. 2021). On the other hand, provenances from the eastern part of the distribution (Romanian Carpathian Mountains) have demonstrated good growth and tolerance to extreme drought events in Central Europe. Additionally, one southern Italian provenance was

among those with the best performances (Martínez-Sancho et al. 2021). However, its relatively early start in the growing season might cause frost damages (Martínez-Sancho et al. 2021). Therefore, certain Central European provenances which have shown a combination of good growth, drought tolerance and recovery after extreme drought events should also be considered in climate change scenarios (Konnert and Schirmer 2011, Martínez-Sancho et al. 2021), including Gschwend, Pfalzgrafenweiler, Alpirsbach and Siegsdorf (Ruetz 2003). Knowledge about adaptive variation, its genetic background and the related environmental factors is important in order to select seed sources for establishing climate resilient forests (Martínez-Sancho et al. 2021). To this end, it is important to consider and unravel complex patterns and interactions, especially with respect to soil and climatic parameters, which may display medium to small-scale differences. For instance, soil water storage capacity influences growth at the small scale (Mellert et al. 2023) and may greatly compensate climatic aridity (Mellert et al. 2018).

The high adaptive genetic variation of silver fir is, at least partly, the result of the species' post-glacial recolonization history. Silver fir reached the full extent of its current distribution range relatively late in the Holocene, only about 3000 years ago (Liepelt et al. 2009). Competition with other species and its late successional character are important factors that might have contributed to this delayed postglacial expansion (Tinner and Lotter, 2006). The gene pools of European silver fir have been greatly shaped by Holocene migration. Genetic evidence based on mitochondrial DNA markers reveals two contact zones between migration routes of Apennine and Balkan origin; one of them in the Northern Carpathians, the other one in Slovenia and Northern Croatia (Liepelt et al. 2002). Thus, all Central European areas northwest of these geographic spots have their maternal origin in Apennine glacial refugia (Liepelt et al. 2002, Ziegenhagen et al. 2005). Yet, silver fir exhibits highly heterogenous gene pools within this region, also due to complex migration routes within and around the Alps. Range-wide and regional population genetic studies have demonstrated a pronounced population genetic structure, decreasing genetic diversity with increasing distance from refugial areas, elevated diversity in suture zones of different migration routes and relatively sharp allele frequency gradients (Konnert and Bergmann 1995, Lewandowski et al. 2001, Liepelt et al. 2009).

The most commonly cited population genetic studies with high coverage of the distribution range of silver fir were conducted based on isozyme markers (Konnert and Bergmann 1995, Liepelt et al. 2009). Although highly variable microsatellite markers (SSRs) were developed or successfully transferred into silver fir (Cremer et al. 2006, Hansen et al. 2005, Postolache et al. 2014), these were either tested in a few populations across the range (Postolache et al. 2014, Teodosiu et al. 2019) or were only rarely applied to explore population genetic structure within parts of central Europe (e.g. Cvrčková et al. 2015). Given the high variability and resolution in detecting population genetic structure (Postolache et al. 2014), more SSR-based population genetic studies would improve the status of knowledge about the gene pool of silver fir. As recent studies highlight the link between post-glacial demographic history and adaptive capacity to drought stress, such studies become highly relevant.

The current study was carried out in the framework of current German regional and national research projects aiming to identify populations of silver fir which can serve as seed sources for climate-smart forests (Schmied et al. 2023). In this context, seed stands from both productive and relatively arid sites are investigated. Given the recently shown link between adaptive responses and (neutral) genetic variation, and the relevance in the framework of assisted gene flow (Martínez-Sancho et al. 2021), a population genetic study based on highly variable SSR markers is an important part of these projects. Here, we present current results of this investigation. In particular, i) we describe the population genetic structure of silver fir in Southern Germany, ii) we explore the spatial variation of genetic diversity across our study populations, and iii) we discuss the results in the context of the species' post-glacial migration history and future assisted gene flow strategies.

MATERIALS AND METHODS

Study Area and Populations

In total, we genotyped 781 silver fir trees from 16 populations in Central Europe. Eight of the populations represented certified seed stands, the other ones represented non-certified stands, distributed over the state of Baden-Württemberg, south-western Germany, the state of Bavaria, south-eastern Germany and the southern state of Carinthia in Austria, situated along the eastern alpine postglacial recolonization pathway (Table 1). The stands were selected along a gradient of water storage capacity of the soil, ranging from 30 mm to over 200 mm, and soil acidity, ranging from acidic soils low in nutrients in the southern Black Forest to basic, nutrient rich soils on the Swabian Jura. Thus, we reached high diversity in stand conditions. Despite the fact that all stands are located in the optimal climatic niche zone (0.7<CMI<1; CMI=climatic marginality index) according to Mellert et al. (2023), they cover a broad climatic spectrum (Table 1). Mean annual temperatures (T_{yr}) range from 6.2°C (Spaichingen) to 9.9°C (Haslach), and annual precipitation from 780 mm (Cham) to 1339 mm (Schrög). The Ellenberg quotient (EQ) allows a differentiation of the beech-dominated forest types in Central Europe including silver fir (Ellenberg 1988). Low values around 10, as in Schrög, are typical for montane beech-fir forests, where fir is co-dominant due to the humid-cold climate. Higher EQ values characterize beech forests where fir dominates only under certain site conditions (e.g., dense soils, waterlogging), as in Cham where boulders cover large areas of the site. The climatic spectrum, which reaches EQ values above 20, shows that the site collective comprises the warm and dry edge of fir distribution in southern Germany.

From each tree, either needles were collected from the tree crown using a shotgun or cambium was sampled from the basis of the trunk using a hollow punch. Samples were put in paper bags, which were then stored in plastic bags filled with silica gel in order to dry the tissue and sent to the lab for genotyping.

Laboratory Procedures

For the 12 populations from Baden-Württemberg, Germany, as well as for the Austrian population from Carinthia, DNA was isolated from the dried tissue samples in the molecular genetics lab of the Institute of Silviculture at BOKU, Vienna, using a commercial extraction kit (DNEasy Plant Minikit, Qiagen, Hilden, Germany). For the three Bavarian populations, DNA was extracted, using an inhouse protocol. DNA quality was assessed by means of 1.0% agarose gels and DNA concentration was quantified using a spectrophotometer (NanoPhototometer®, Implen). Subsequently, the samples were diluted in water to achieve a DNA concentration of 30 ng/µl.

Subsequently, we used the diluted DNA to amplify ten nuclear microsatellite loci by means of polymerase chain reaction (PCR). The following loci, initially developed for *Abies alba* or other fir species, were genotyped: Aag01, Aat01, Aat04, Aat06, Aat11, Aat15 (Postolache et al. 2014); NFF3, NFF7, NFH15 (Hansen et al. 2005); and SF1 (Cremer et al. 2006). The loci were split into two multiplexes. PCR primers were fluorescently labelled in order to allow later scoring by means of capillary electrophoresis. Sequences, fluorescent dyes and other details about the used primers are summarized in Table 2.

PCRs were carried out in a reaction volume of 10µl including: 5µl of 2x Qiagen multiplex PCR master mix

Table 1. List of study populations sampled for the genetic analysis. BW = Baden-Württemberg, BY = Bavaria, A = Austria, D = Germany, T_{yr} = mean annual temperature, P_{yr} = mean annual precipitation, T_{07} = mean monthly temperature for July, EQ = Ellenberg's climate quotient (Ellenberg 1988), CMI = climate marginality index (Mellert et al. 2023), based on WorldClim data (Fick and Hijmans 2017, period 1970-2000), N = sampling size.

Population ID	Location name	Latitude	Longitude	T_{yr}	P_{yr}	T_{07}	EQ	CMI	N
1110	Blumberg (BW, D)	47.78	8.52	7.7	946	17.0	18.0	0.83	50
2112	Lahr (BW, D)	48.30	7.93	9.9	1043	18.7	17.9	0.77	50
2122	Nagold (BW, D)	48.57	8.70	8.3	968	17.3	17.9	0.77	50
2131	Schwäbisch Gmünd (BW, D)	48.83	9.76	8.9	961	17.8	18.5	0.79	50
2212	Hägelberg (BW, D)	47.66	7.74	9.1	1097	18.0	16.4	0.83	50
2221	Eutingen (BW, D)	48.48	8.70	8.0	950	17.0	17.9	0.76	50
2222	Haslach (BW, D)	48.27	8.10	9.9	1146	18.7	16.3	0.83	51
2232	Wutöschingen (BW, D)	47.66	8.39	8.7	1026	18.1	17.6	0.80	50
2312	Schömberg (BW, D)	48.78	8.62	7.5	1336	16.1	12.1	0.90	50
2322	Dunningen (BW, D)	48.20	8.49	7.3	1034	16.3	15.8	0.84	50
2331	Gönningen (BW, D)	48.43	9.11	8.1	798	17.2	21.6	0.80	51
2332	Spaichingen (BW, D)	48.05	8.75	6.2	1105	15.1	13.7	0.95	50
1201	Biberkor (BY, D)	47.94	11.37	8.1	1098	17.0	15.5	0.99	48
1202	Schrög (BY, D)	47.80	12.80	6.8	1339	15.4	11.5	1.00	38
1212	Cham (BY, D)	49.24	12.70	7.7	780	17.2	22.1	0.82	44
AT	Mauthen (K, A)	46.66	12.99	7.7	1170	18.0	15.4	0.95	49

Table 2. Names, primer sequences, fluorescent dyes (Atto550 = yellow, HEX = green, FAM = blue), allele size range (according to the primer notes) in base pairs and PCR multiplexes and primer note for the ten used microsatellite loci.

Locus name	Forward primer sequence	Reverse primer sequence	Allele size range	Dye	Multiplex
NFF3	CCAATGGGTTGTCAGAGTGTT	GGCATTCGAGATTGCTTGAT	105-187	Atto550	1
NFF7	CCCAAACTGGAAGATTGGAC	ATCGCCATCCATCATCAGA	116-174	HEX	1
NFH15	CGCCTCCCTCCATTACTTC	TCGTCTAGAGAGGCGAAATTCT	98-138	FAM	1
SF1	TTGACGTGATTAACAATCCA	AAGAACGACACCATTCTCAC	208-229	FAM	1
Aag01	GCTTATTCTCACTGCTCGCC	ATGACTTGAAGGTGGATGCC	193-250	Atto550	2
Aat01	CCATGTCTCCGATTTCCAGT	GGCCTAACGAAAGCAGAATC	103-127	HEX	2
Aat04	CCATGTATGGTGCTCCTCCT	CCTTCATTGCAGAAAAGCAA	158-191	FAM	2
Aat06	TTATGCGGAGCAGTTCTGTG	TGTTGCTGGCGTACTGGTAG	196-214	HEX	2
Aat11	AGCGTTGATTGGAAGCAGTC	GAAGCATGGTGTCGTTGTTG	255-270	HEX	2
Aat15	AGGAGGAGGTTCAGCATGTC	CTTGCTCTCTGACCCAGTTG	361-373	FAM	2

(Qiagen, Hilden, Germany), 3µl of RNAse-free water, 1µl of template DNA and 1 µl of 10x primer mix which was specific for each multiplex. In multiplex 1, the primer concentration in the 10x primer mix was 2 µM for loci NFH15 and SF1, 4 µM for locus NFF3 and 1 µM for locus NFF7, resulting in 0.2, 0.4 and 0.1 µM in the reaction volume, respectively. In multiplex 2, the primer concentration in the 10x primer mix was 4 µM for locus Aag01, 2 µM for loci Aat01, Aat06 and Aat15; and 1 µM for loci Aat04 and Aat11, resulting in 0.4, 0.2 and 0.1 µM in the reaction volume, respectively. The PCR program for multiplex 1 included an initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 30 s, annealing at 57°C for 90 s and elongation at 72°C for 30 s with a final elongation step at 60°C for 30 min. For multiplex 2, the annealing temperature was 62°C with all other program settings being the same. The success of PCR reactions was tested on 1.5% agarose gels, which were also used to select the dilution ratio of the PCR products for subsequent capillary electrophoresis.

The capillary electrophoresis was carried out in a genetic analyser (SeqStudio, Thermo Fischer, Waltham, Massachusetts, USA) in order to determine the length of PCR products (alleles) in base pairs. Allele scoring was performed by applying a fragment length analysis using the GeneMapper v.6.0 (Thermofischer) software. Allele binning was performed manually. The genotype lists were exported for use in population genetic analyses.

Population Genetic Analysis

In order to quantify the genetic diversity within populations, we first calculated the multilocus average and effective number of alleles (n_a and n_e, respectively), observed and expected heterozygosity (H_o and H_e, respectively) and inbreeding coefficient (F_{IS}) per population using the GenAlEx v.6.5 software (Peakall and Smouse 2012, 2006). To assess the genetic differentiation among populations, pairwise F_{ST} values were calculated and their significance was tested by applying 999 permutations of genotypes pairwise between populations, using the G-statistics function of GenAlEx. To visualize the pattern of genetic differentiation, we carried out a Principal Coordinate Analysis (PCoA; Orlóci 1978) with the same software. Mantel tests were carried out with the R-package VEGAN v. 2.5-7 (Oksanen et al. 2017) to address whether genetic differentiation (F_{ST}) and geographic distance are correlated across populations and within subsets (Southern Germany and Baden-Württemberg only). Finally, we applied a Bayesian cluster analysis by means of the STRUCTURE software (Falush et al. 2007, Pritchard et al. 2000), in order to address the population genetic structure. In particular, we used the STRAUTO v1.0 application (Chhatre and Emerson 2017) in conjunction with GNU parallel (Tange 2011) to achieve automation and parallelization of STRUCTURE runs on a 40-core standalone computer. We opted for 50,000 burn-in replications followed by 100,000 MCMC iterations and adopted the admixture model with correlated allele frequencies. We also used the LOCPRIOR model (Hubisz et al. 2009) by setting one location per population (*locispop* option). We set consecutive values of K assumed clusters from 1 to 10, performing 20 independent runs for each K-value. We further processed the data in the CLUMPAK online platform (Kopelman et al. 2015) which implements the CLUMPP method (Jakobsson and Rosenberg 2007) in order to identify different cluster modes (in case of multimodality) and average the membership proportions of individuals to clusters across runs, and with STRUCTURE HARVESTER (Earl & vonHoldt 2012) in order to identify the uppermost level hierarchy of population genetic structure by means of the statistic delta K (ΔK; Evanno et al. 2005).

RESULTS

All used loci were polymorphic, displaying an average number of 5.6 to 6.6 alleles with the numbers varying among populations (Table 3). The highest number of alleles over loci per population (n_a) were observed in populations from the Black Forest in the westernmost part of our study area. With one exception, that of the population 2122-Nagold, these stands exhibited n_a-values of 6.0-6.6 (Table 3). Populations from the Swabian Jura (including 1110-Blumberg, 2322-Dunningen, 2332-Spaichingen, 2331-Gönningen and 2131-Schwäbisch Gmünd) showed n_a-values between 5.5 and 5.8, similar to the three stands from Bavaria and the one stand from Carinthia (Southern Austria). In terms of the effective number of alleles (n_e), the highest values (n_e=3.1-3.31) were found in the three Bavarian populations (1201-Biberkor, 1202-Schrög and 1212-Cham), as well as in the easternmost population of the Swabian Jura (2131-Schwäbisch Gmünd). In the western part of the study area, but also in the southern Austrian stand, n_e-values were generally lower (n_e=2.32-3.0). The genetic diversity in terms of observed (H_o) and expected heterozygosity (H_e) displayed the same trends as the effective number of alleles. However, two populations, 2112-Lahr and 2122-Nagold, showed less heterozygous individuals than expected, which is mirrored in their high inbreeding coefficients (Table 3).

Pairwise F_{ST}-values ranged from 0.004 to 0.089. In general, the highest genetic differentiation was observed between the population AT-Mauthen (Carinthia, southern Austria) and all other populations, with all pairwise comparisons being significant. Also, the three Bavarian populations were significantly differentiated from all other stands (P=0.001 for all comparisons), but also from each other (Table 4). Within Baden-Württemberg, some comparisons were significant with the easternmost population 2131-Schwäbisch Gmünd being significantly differentiated from all other stands. These patterns are also reflected in the scatterplot produced by applying PCoA using the pairwise F_{ST}-distances (Figure 1). The Mantel tests showed a significant correlation between genetic differentiation (in terms of F_{ST}) and geographic distance across all populations (R=0.610, P=0.001), within the subset of all 15 southern German populations (i.e. excluding population AT-Mauthen; R=0.5425, P=0.001), but not within the state of Baden-Württemberg (i.e. also excluding the three Bavarian populations; R=0.1235, P=0.182).

Table 3. Diversity measures averaged over loci ± standard error over loci for each of the study populations. n_a = number of alleles; n_e = effective number of alleles; H_o, H_e = observed and expected heterozygosity, respectively; F = inbreeding coefficient. BW = Baden-Württemberg; BY = Bavaria; A = Austria; D = Germany.

Population ID	n_a	n_e	H_o	H_e	F
1110 - Blumberg (BW, D)	5.80 ± 1.24	2.76 ± 0.48	0.51 ± 0.07	0.54 ± 0.07	0.06 ± 0.03
2112 - Lahr (BW, D)	6.00 ± 1.29	2.81 ± 0.50	0.49 ± 0.07	0.55 ± 0.07	0.15 ± 0.05
2122 - Nagold (BW, D)	5.60 ± 1.13	2.50 ± 0.39	0.47 ± 0.07	0.52 ± 0.06	0.14 ± 0.06
2131 - Schwäbisch G. (BW, D)	5.50 ± 1.23	3.10 ± 0.71	0.53 ± 0.08	0.54 ± 0.08	0.01 ± 0.02
2212 - Hägelberg (BW, D)	6.60 ± 1.71	2.89 ± 0.59	0.52 ± 0.08	0.54 ± 0.07	0.05 ± 0.04
2221 - Eutingen (BW, D)	6.30 ± 1.49	3.00 ± 0.61	0.55 ± 0.07	0.56 ± 0.06	0.04 ± 0.04
2222 - Haslach (BW, D)	6.40 ± 1.32	2.40 ± 0.39	0.47 ± 0.07	0.50 ± 0.06	0.07 ± 0.04
2232 - Wutöschingen (BW, D)	5.60 ± 1.11	2.32 ± 0.39	0.47 ± 0.07	0.48 ± 0.06	0.04 ± 0.05
2312 - Schömberg (BW, D)	6.10 ± 1.26	2.72 ± 0.56	0.47 ± 0.08	0.50 ± 0.08	0.07 ± 0.05
2322 - Dunningen (BW, D)	6.60 ± 1.31	2.44 ± 0.42	0.50 ± 0.08	0.49 ± 0.07	0.03 ± 0.06
2331 - Gönningen (BW, D)	5.80 ± 1.25	2.76 ± 0.52	0.51 ± 0.08	0.52 ± 0.08	0.01 ± 0.02
2332 - Spaichingen (BW, D)	5.70 ± 1.21	2.59 ± 0.38	0.52 ± 0.05	0.55 ± 0.05	0.06 ± 0.03
1201 - Biberkor (BY, D)	5.80 ± 1.19	3.14 ± 0.57	0.59 ± 0.06	0.59 ± 0.07	-0.01 ± 0.02
1202 - Schrög (BY, D)	5.70 ± 1.40	3.31 ± 0.78	0.59 ± 0.06	0.58 ± 0.06	-0.02 ± 0.04
1212 - Cham (BY, D)	5.80 ± 1.35	3.13 ± 0.73	0.51 ± 0.08	0.54 ± 0.08	0.04 ± 0.04
AT - Mauthen (K, A)	5.80 ± 1.22	2.71 ± 0.52	0.51 ± 0.08	0.51 ± 0.08	0.01 ± 0.03

Table 4. Pairwise F_{ST} values for all population pairs (F_{ST} values below diagonal, probability values above diagonal).

	1110	2112	2122	2131	2212	2221	2222	2232	2312	2322	2331	2332	1201	1202	1212	AT
1110		0.141	0.064	0.001	0.753	0.128	0.059	0.001	0.100	0.043	0.484	0.160	0.001	0.001	0.001	0.001
2112	0.007		0.057	0.001	0.492	0.013	0.033	0.001	0.087	0.008	0.432	0.060	0.001	0.001	0.001	0.001
2122	0.009	0.009		0.005	0.341	0.057	0.271	0.002	0.070	0.066	0.171	0.171	0.001	0.001	0.001	0.001
2131	0.015	0.013	0.013		0.002	0.001	0.001	0.001	0.002	0.001	0.018	0.001	0.001	0.001	0.001	0.001
2212	0.004	0.005	0.006	0.013		0.247	0.065	0.002	0.160	0.075	0.637	0.583	0.001	0.001	0.001	0.001
2221	0.007	0.010	0.009	0.015	0.006		0.009	0.001	0.027	0.008	0.028	0.049	0.001	0.001	0.001	0.001
2222	0.008	0.010	0.006	0.018	0.008	0.011		0.001	0.048	0.160	0.048	0.014	0.001	0.001	0.001	0.001
2232	0.017	0.016	0.018	0.027	0.012	0.022	0.017		0.001	0.006	0.001	0.005	0.001	0.001	0.001	0.001
2312	0.008	0.008	0.008	0.014	0.007	0.009	0.009	0.018		0.115	0.462	0.001	0.001	0.001	0.001	0.001
2322	0.009	0.011	0.008	0.018	0.008	0.011	0.007	0.012	0.008		0.073	0.040	0.001	0.001	0.001	0.001
2331	0.005	0.006	0.007	0.009	0.005	0.009	0.009	0.017	0.005	0.008		0.079	0.001	0.001	0.001	0.001
2332	0.007	0.009	0.007	0.015	0.005	0.009	0.010	0.011	0.012	0.009	0.008		0.001	0.001	0.001	0.001
1201	0.030	0.026	0.038	0.023	0.030	0.033	0.042	0.045	0.040	0.041	0.029	0.025		0.007	0.001	0.001
1202	0.023	0.022	0.030	0.018	0.025	0.024	0.032	0.039	0.032	0.034	0.026	0.021	0.012		0.015	0.001
1212	0.043	0.041	0.054	0.031	0.045	0.043	0.054	0.062	0.050	0.055	0.043	0.043	0.024	0.013		0.001
AT	0.077	0.068	0.083	0.061	0.072	0.077	0.089	0.081	0.084	0.086	0.076	0.071	0.046	0.048	0.057	

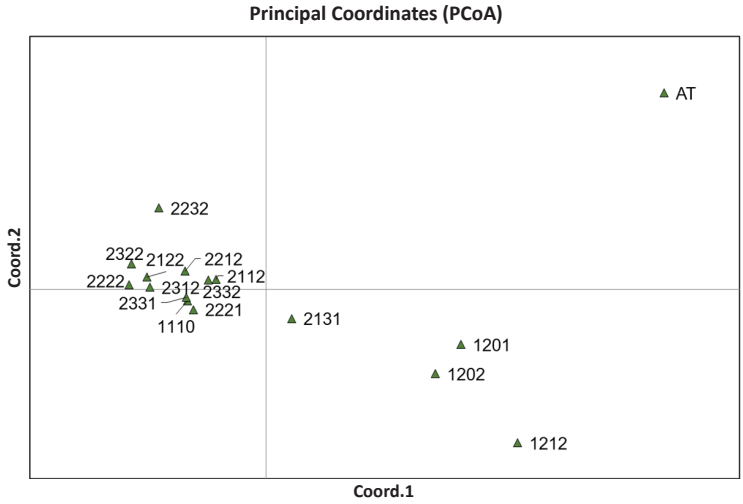

Figure 1. Scatterplot of the Principal Coordinate Analysis (PCoA) based on the pairwise F_{ST} values. The first and second coordinates account for 50.07 and 16.33% of the total variance, respectively.

The STRUCTURE analysis produced meaningful results for an assumed number of K=2 up to K=4 clusters. ΔK was maximum for K=2 and displayed a secondary peak for K=4 (Supplementary Material 1), while all runs within a K-value were unimodal (resulted in the same clustering pattern). To illustrate the spatial distribution of the genetic structure, we plotted the results for K=2 and K=4 on maps (Figure 2, Figure 3). For K=2, a west-east differentiation could be observed. The southern Austrian stand was assigned to an eastern cluster both at the population and individual level. The three Bavarian populations showed mixed affinity to an eastern and a western cluster. The easternmost population of the Swabian Jura (2131-Schwäbisch Gmünd) also showed partial membership (membership proportion q=0.197) to the eastern cluster both at the population and the individual level. All other stands from the western part of our study area showed a high membership proportion (q≥0.926) to the western cluster with the exception of populations 2112-Lahr and 2212-Hägelberg. These populations received somewhat lower membership proportions to the western cluster (q=0.861 and 0.886, respectively) due to heterogeneity at the individual level. In particular, most individuals of these populations were identified as members of the western clusters. However, a small number of trees from these populations received relatively high scores of membership proportion (q>0.6) to the eastern cluster, which was uncommon for other individuals in the same or neighbouring stands (Figure 2).

At K=4, the southern Austrian population significantly differed from all other silver fir stands (not the case at K=3; results for K=3 not shown). The southern German populations showed again a west-east differentiation. A cluster, illustrated with sky blue in Figure 3, was mostly represented in the west. A further cluster, for which we used dark green colour in Figure 3, was more prevalent in the east. The three Bavarian populations presented high membership proportions to this cluster. Along the Swabian Jura we could observe a transition zone with increasing membership coefficients to the eastern cluster from southwest (1110-Blumberg, 2332-Spaichingen) towards northeast (2331-Gönningen, 2131-Schwäbisch Gmünd). Some affinity to this cluster was also observed for stands 2112-Lahr and 2212-Hägelberg at the westernmost part of the Black Forest. However, this was against due to some single individuals in these populations which were assigned to the eastern cluster with high membership proportions, which was unusual for other individuals in the same or neighbouring stands. Finally, a fourth cluster, for which we used dark blue colour, did not show an obvious geographic pattern.

DISCUSSION

The ten used microsatellite markers proved to be useful in order to identify diversity gradients and distinguish among different genetic clusters of silver fir in our study area in Central Europe. Although a sufficient number of nuclear microsatellites for this species have been available for more than a decade (Cremer et al. 2006, Hansen et al. 2005, Postolache et al. 2014), relatively few studies in central Europe have made use of them so far (e.g. Cvrčková et al. 2015). In contrast, several SSR-based studies dealing with population genetics in peripheral populations of silver fir and its related species appeared in this time period (Belletti et al. 2017, Dalmaris et al. 2022, Sancho-Knapik et al. 2014, Teodosiu et al. 2019). Thus, conclusions about diversity gradients and the population genetic structure of silver fir in Central Europe are mostly based on older studies applying isozymes (Breitenbach-Dorfer et al. 1997, Konnert

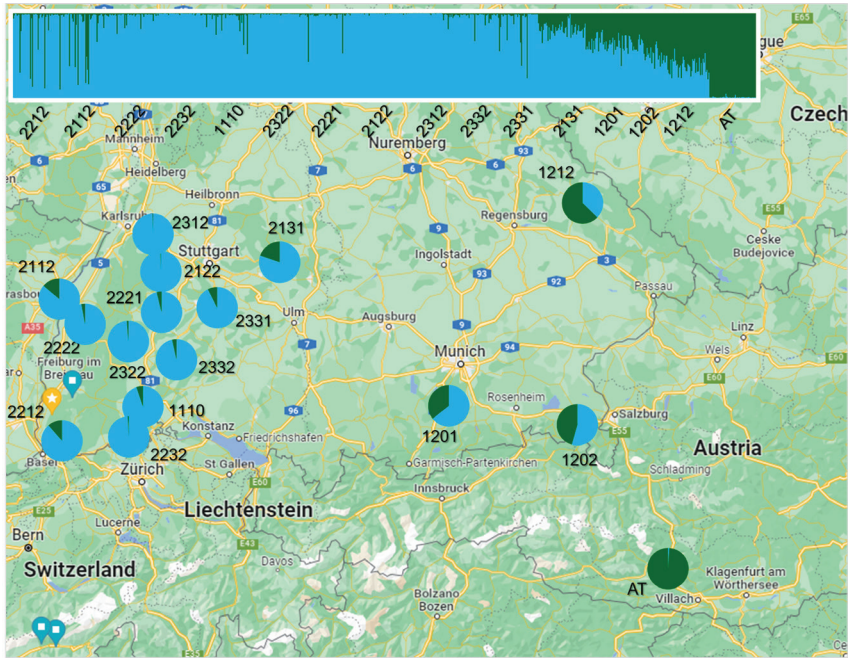

Figure 2. STRUCTURE results for K=2 assumed clusters. The clusters are illustrated with different colours. Individuals in the bar plot are illustrated with one bar each. At the population level, the pie charts depict the membership proportion to the clusters.

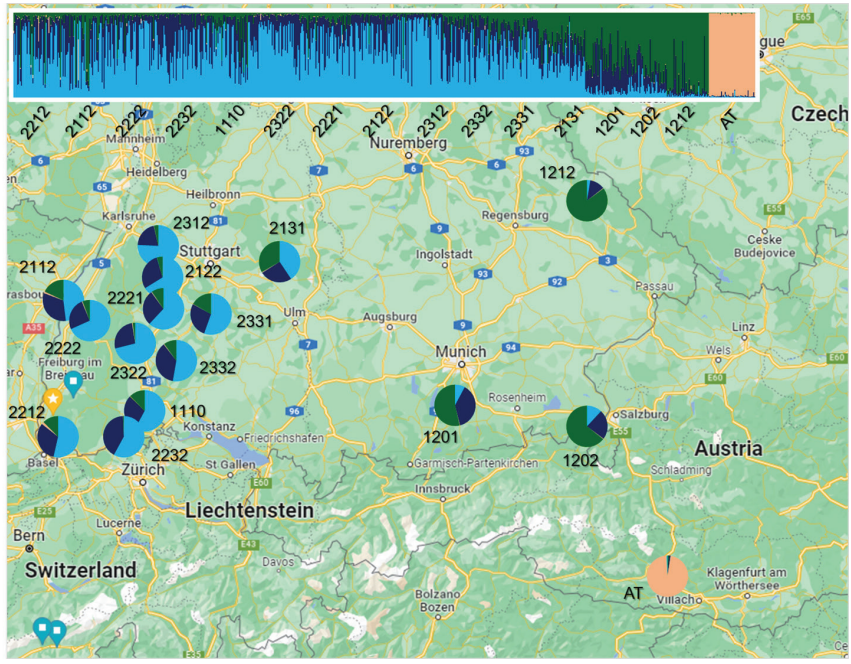

Figure 3. STRUCTURE results for K=4 assumed clusters. The clusters are illustrated with different colours. Individuals in the bar plot are illustrated with one bar each. At the population level, the pie charts depict the membership proportion to the clusters.

and Bergmann 1995, Lewandowski et al. 2001, Liepelt et al. 2009). Given that the microsatellite markers we used are more variable than isozymes, our study complements these previous investigations and refines our knowledge about the genetic diversity and structure of the species in Central Europe.

With respect to genetic variation, one of our main findings is the decreasing trend of the average number of alleles per locus towards the east. This is in contrast with other measures of diversity like the effective number of alleles and heterozygosity, which take the allele frequencies into account. These tend to be higher in the east and especially in Bavaria. In general, the number of alleles is expected to decrease with increasing distance from the glacial refugia, while high genetic diversity may occur in suture zones of different postglacial recolonization pathways (Konnert and Bergmann 1995, Liepelt et al. 2009, Petit et al. 2003). Therefore, we attribute the higher values of this diversity measure in the west to the lower distance of the Black Forest (westernmost part of our study area) from the refugial populations along the relatively short Jura (western alpine) migration route (Liepelt et al. 2009). The lower allele numbers in the three Bavarian and the one Carinthian (southern Austrian) population of our study might have resulted from genetic drift during recolonization. Indeed, the west-east orientation of the Alps at their eastern flank and the occurrence of several parallel mountain chains posed a significant barrier which forced silver fir to expand through narrow valleys and cover a much longer distance during Holocene expansion (Liepelt et al. 2009). This result was not affected by variation in sample size among populations as calculation of rarefied allelic richness (Petit et al. 1998) did not lead to significant changes of the aforementioned pattern (results not shown).

As opposed to the average number of alleles per locus, we found higher values of effective allele number and heterozygosity in the east, namely in Bavaria, but not in Carinthia. We attribute this result to the contact of the western and eastern Holocene migration routes and admixture of the respective gene pools in the region of Bavaria (while Carinthia is situated at a more southern region, completely within the eastern route). A similar observation was made by Konnert and Bergmann (1995), Liepelt et al. (2009) and Breitenbach-Dorfer et al. (1997) based on isozymes. However, in those studies, the maximum values of diversity measures were observed in the Black Forest area, while Bavarian stands were less diverse with a further decrease of genetic diversity eastwards in southeastern Austria and northwards in the Bavarian Forest along the border between Germany and Czechia and further reduction towards the Ore and Beskid Mountains. The genetic diversity values in the population 1212-Cham are comparable with the other two Bavarian populations which is not in line with the hypothesis of a drastic genetic erosion along the eastern migration route towards north. Further recent SSR-based results from Czechia also do not conform with this hypothesis (Cvrčková et al. 2015). Thus, the zone of genetic admixture between the different circum-alpine migration routes might be situated further to the east than as hypothesized by Konnert and Bergmann (1995), while more studies are needed to address whether population at the northernmost margin of silver fir's range has been affected by past population bottlenecks.

The transition from one gene pool to another is also supported by the F_{ST}-based analysis of genetic differentiation, but also by the Bayesian clustering results. The genetic and geographic distances are significantly correlated when observing the entire study area with or without the rather isolated Carinthian AT-Mauthen population. This population appears to be genetically differentiated from the three Bavarian stands, which can be explained by the significant landscape barriers that exist between them. However, it is genetically more similar to the three Bavarian populations than to any other stand of our study, which agrees with the hypothesis that the eastern alpine recolonization pathway has influenced the gene pool of silver fir in Bavaria. On the other hand, populations in the west appear to be relatively homogenous with low pairwise F_{ST}s, lack of isolation by distance (within Baden-Württemberg) and to the same clusters in similar proportions by STRUCTURE analysis. Nevertheless, a gradual differentiation of the eastern populations along the Swabian Jura, and especially the easternmost stand 2131-Schwäbisch Gmünd, can be observed in some of the results (especially STRUCTURE for K=4). These are genetically somewhat closer to the Bavarian populations, which is in line with their geographic location. Furthermore, the range of silver fir in the Swabian Jura is discontinuous so that stands in the centre and southwest of this mountain range (e.g. 2331-Gönningen, 2332-Spaichingen) are separated by more than 30 km (where silver fir is lacking) from occurrences in the northeast (including 2131-Schwäbisch Gmünd). The latter is rather connected to silver fir populations in the northeast, which is in line with its partial affinity to the Bavarian stands. On the contrary, the genetic differentiation of this stand from all other populations in Baden-Württemberg may have resulted through its isolation from further stands towards southwest. These results indicate that genetic structure might be also present at a smaller scale (i.e. within Baden-Württemberg). Increasing the number of loci and populations could better resolve such fine-scale genetic structure.

Finally, in two stands in the Black Forest (2112-Lahr and 2212-Hägelberg), a small number of individuals displayed high membership proportion to an eastern genetic cluster. This suggests transfer of reproductive material from an eastern origin into these two stands. Unfortunately, we could not trace back the origin of these stands. However, trade of reproductive material is known to have taken place for centuries in Europe, which is particularly true for conifers (Hamberger 2011). Also, we assume that the presence of a seed production enterprise (Staatsklenge Nagold) with a history of more than 150 years in short distance from several of our stands in Baden-Württemberg may also have influenced the genetic variation of silver fir in this region. This might be the case, for instance, in stand 2122-Nagold which displayed decreased genetic diversity and elevated inbreeding coefficient. Establishment with reproductive material from a reduced number of trees could be a possible

reason behind this. Even if natural regeneration has been more common in silver fir than e.g. in Norway spruce (*Picea abies*), plantation and seed transfer should be considered as another important factor shaping the gene pool of our study species.

CONCLUSIONS

Our study confirms that the gene pool of silver fir in central Europe is structured and characterized by gradients of genetic diversity which have resulted from postglacial migration history of the species in this area, which supports that our populations are mostly autochthonous. Not only molecular markers, but also common garden trials demonstrate the high genetic variation of silver fir in Central Europe (Dobrowolska et al. 2017). Results based on both molecular and quantitative genetic approaches suggest that a rather high resolution of seed zones (i.e. a rather high number of geographically limited seed zones) is meaningful as it accounts for the aforementioned regional genetic differences. At the same time, certain Central European provenances from relatively dry sites may display an increasing significance as seed sources for future climate smart forests (Konnert and Schirmer 2011). While previous common-garden based studies had a rather interregional and range-wide scope, our study focuses on medium (regional) scale adaptation by including both dry and productive sites situated in geographic proximity to each other. A dendroecological approach implemented in the framework of the same project suggests that silver fir on dry sites shows an increased resilience to drought events (Lamprecht 2022) and copes better with persistent climate changes compared to Norway spruce (Schmied et al. 2023). Combination of dendroecological, molecular and quantitative genetic studies will provide a robust basis to define seed sources for climate resilient forests of silver fir.

Author Contributions

BF, HGM, MS und KHM conceived and designed the related project, NL carried out the stand selection and supervised the project activities in Baden-Württemberg, while KHM, MS and BF undertook these tasks in Bavaria. BF and CN designed and supervised the genetic analyses, CN performed the statistical analysis and wrote the manuscript with contributions from all coauthors.

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Conflicts of Interest

The authors declare no conflict of interest.

Supplementary Materials

Supplementary File 1 - Results of the Evanno method. Table and Figures of ln-probability of data, Delta K and further statistics calculated by applying the Evanno method with use of the software STRUCTURE HARVESTER.

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