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Genetic Variation of European Beech Populations and Their Progeny from Northeast Germany to Southwest Switzerland

Markus Müller [1,*], Laura Cuervo-Alarcon [1], Oliver Gailing [1], Rajendra K.C. [1], Meena Suyal Chhetri [1], Sarah Seifert [1], Matthias Arend [2], Konstantin V. Krutovsky [1,3,4,5] and Reiner Finkeldey [6]

1 Forest Genetics and Forest Tree Breeding, Faculty of Forest Sciences and Forest Ecology, University of Goettingen, Büsgenweg 2, 37077 Göttingen, Germany; lcuervo@forst.uni-goettingen.de (L.C.-A.); ogailin@gwdg.de (O.G.); rkc.gen@gmail.com (R.K.C.); meena234suyal@gmail.com (M.S.C.); sseifer@gwdg.de (S.S.); kkrutov@gwdg.de (K.V.K.)
2 Physiological Plant Ecology, Department of Environmental Sciences, Faculty of Science, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland; matthias.arend@unibas.ch
3 Vavilov Institute of General Genetics, Russian Academy of Sciences, 3 Gubkina Str., 119333 Moscow, Russia
4 Laboratory of Foresty Genomics, Genome Research and Education Center, Siberian Federal University, 50a/2 Akademgorodok, 660036 Krasnoyarsk, Russia
5 Department of Ecosystem Science and Management, Texas A&M University, 2138 TAMU, College Station, TX 77843-2138, USA
6 University of Kassel, Mönchebergstrasse 19, 34109 Kassel, Germany; praesident@uni-kassel.de
* Correspondence: mmuellef@gwdg.de; Tel.: +49-551-39-14281

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Abstract: Climate change can adversely affect the growth of European beech (*Fagus sylvatica* L.) across its entire distribution range. Therefore, knowledge of the adaptive potential of this species to changing climatic conditions is of foremost importance. Genetic diversity is the basis for adaptation to environmental stress, and the regeneration phase of forests is a key stage affecting genetic diversity. Nevertheless, little is known about the effect of climate change on the genetic diversity of adult trees compared to their progeny. Here, we present genetic diversity data for 24 beech populations ranging from northeast Germany to southwest Switzerland. Potentially adaptive genetic variation was studied using single nucleotide polymorphism (SNP) markers in candidate genes that are possibly involved in adaptive trait variation. In addition, more than 2000 adult trees and 3000 of their seedlings were genotyped with simple sequence repeat (SSR) markers to determine selectively neutral genetic diversity and differentiation among populations. All populations showed high SSR and SNP variation, and no differences in genetic diversity were found between adult trees and their offspring. The genetic differentiation between adults and seedlings within the same stands was also insignificant or very low. Therefore, we can conclude tentatively that the transfer of genetic variation among tree generations, currently, is not much affected by climate change, at least in the studied beech populations.

Keywords: European beech; genetic variation; climate change; adaptive potential

1. Introduction

Climate change has different effects on forests, such as a prolonged vegetation season [1], increased tree mortality, or altered productivity [2–5]. A reduced growth of European beech (*Fagus sylvatica* L.), one of the economically and ecologically most important forest tree species in Europe, was observed

not only at its range limits [6,7], but also in the center of its distribution area [8–11]. Thus, the adaptive potential of beech to changing environmental conditions is of considerable importance. The adaptive potential is directly related to the level of genetic diversity in relevant genes, and it provides the basis for adaptation and resilience to environmental stress and change [12]. Neutral genetic diversity may also indirectly reflect population fitness being a good indicator of effective population size and total diversity [13]. In general, European beech has a high genetic diversity, revealed in different studies based on various genetic markers, such as isozymes, microsatellites or simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) and amplified fragment-length polymorphisms (AFLPs) [14–17]. Nevertheless, little is known about the current impact of climate change on the transfer of genetic information from adult populations to their seedlings. Climate change might influence the reproductive phenology of trees, and therefore negatively affect the quantity and quality of seeds [18,19]. Regeneration is a key stage affecting genetic diversity in natural forests [20], and different studies revealed a negative impact of climate change on the regeneration of different tree species including beech in some areas [21–24]. The more trees involved in reproduction, the higher the genetic diversity of a seed crop might be [20]. Hence, reproductive failure of parental trees can reduce the genetic diversity of their progeny in forest stands. There are a few recent studies that investigated the genetic structure of different beech generations. For instance, Westergren et al. [25] analyzed the influence of an irregular shelterwood management system on the genetic structure of natural regeneration in two beech stands in Slovenia. Sandurska et al. [26] investigated the genetic diversity of adult beech trees and their seedlings in forest stands, showing indications for ongoing ecological succession from oak to beech-dominated forests. Bilela et al. [14] studied the genetic composition of adult trees and seedlings of two populations differing in microclimatic conditions using isozymes and SSRs. In these studies, no significant differences in genetic diversity were detected between beech generations. Nevertheless, these studies were designed to investigate the impact of different factors such as microclimate, succession or forest management on the genetic structure of beech and were conducted in a limited number of populations. To our knowledge, only one large-scale study compared the genetic structure of adult beech populations and beechnuts [27]. Based on a set of isozymes, the authors found similar genetic diversity values for both adult trees and beechnuts. Nevertheless, samples from adult trees and beechnuts were mostly taken from different populations, so that possible changes in genetic structure among generations within populations could not be monitored.

Here, we investigated the genetic diversity within and population structure among 24 beech populations distributed from northeast Germany to southwest Switzerland based on 2102 adult trees and 3186 seedlings. We combined data from different studies and complemented them with new genotyping data [15,17,28–31]. The previous studies focused on aspects such as SNP-trait/-environment associations, management impact on genetic structures or the genetic variation of beech on the local scale. A combination and further analysis of the data made it possible to compare the genetic structure of different tree generations based on a large sample size and a large geographic range. Neutral genetic diversity was estimated at SSRs both in all adult trees and 2357 seedlings. Since neutral genetic diversity should not be used as a surrogate of adaptive genetic variation [32], potentially adaptive genetic variation was inferred for 3157 seedlings based on SNP markers.

2. Materials and Methods

2.1. Study Sites and Plant Material

Mature beech populations located at 24 different sites ranging from northeast Germany to southwest Switzerland (Rhone valley and Rhine valley) and growing under different geographic and environmental conditions were studied (Figure 1, Table 1). For an easier assignment of populations to geographic locations in this study, the German populations were labeled with the prefix “DE” and the Swiss populations with the prefix “CH”. The Swiss populations were further labeled with “W” or “E”

corresponding to their location in western ("W") or eastern ("E") Switzerland. The altitude ranged from 64 m a.s.l. (population DE-SEW5) to 800 m a.s.l. (populations CH-W-Ardon and CH-W-Chamoson). The mean annual temperatures (period 1961–1990) varied from 6.2 °C (population DE-HA) to 9.3 °C (populations CH-E-Malans, CH-E-Mastrils, CH-E-Sargans, and CH-E-Mels), and the mean annual precipitation (period 1961–1990) from 532.1 mm (populations DE-SEW5 and DE-SEW9) to 1326 mm (population DE-HA). The populations mainly represented pure beech stands, sometimes mixed with a few other tree species, such as oak. Three of the 24 populations (DE-AEW8, DE-SEW9, and DE-HEW10) are not under forest management anymore, because they are located in nature conservation areas. Climate data for the period 1961–1990 were obtained from climate stations located near these populations, and downloaded from the websites of the German Meteorological Service (Deutscher Wetterdienst) and the Federal Office of Meteorology and Climatology (MeteoSwiss). Based on the downloaded variables, three additional variables were calculated: Mean vegetation season temperature and precipitation during May to September, and the Ellenberg's climatic quotient (EQ), which is used to describe the climatic limit for beech in Europe. Regions with an EQ value below 20 represent pure beech climates, while beech competitiveness slowly decreases in regions with EQ values between 20 and 30; beech disappears in regions with EQ > 30 [33]. The Ellenberg's climatic quotient was calculated as follows [32,33]:

$$\mathrm{EQ} = \frac{\text{Temperature of July } (^{\circ}\mathrm{C})}{\text{Annual precipitation } (\mathrm{mm})} \times 1000 \quad (1)$$

In each population, leaves from adult trees were collected as described in References [15,28,30]. The research plots were used for sampling of the adult populations in Germany (DE-SEW5, DE-SEW9, DE-HEW6, DE-HEW10, DE-AEW5, and DE-AEW8), in which the most exhaustive sampling was conducted (ca. 200 adult trees per plot) in 2008 [15]. In the other German populations (DE-GL, DE-GS, DE-US, DE-CL, DE-CS, and DE-HA) ca. 100 adult trees per population were randomly selected and sampled in 2009 (the population DE-HA was sampled in 2011) [28]. In each of the Swiss populations, 25 adult trees ca. 50 m apart from each other were sampled in 2014 [30]. Further, leaves of seedlings from all populations were collected. In each of the populations located in Switzerland, 16–31 adult trees ca. 50 m apart from each other were selected, and 2–4 ca. 20 cm tall seedlings growing underneath them were sampled in 2011 [31]. For the German populations, leaf samples were taken from seedlings raised from beechnuts in a greenhouse at Göttingen University as described in [17,34]. Briefly, beechnuts of the populations DE-SEW5, DE-SEW9, DE-HEW6, DE-HEW10, DE-AEW5, and DE-AEW8 were collected in September/October 2011. In each of the populations, each 200 beechnuts were collected from under 100 randomly selected adult trees. After drying, the beechnuts were stored at −10 °C until January 2012. The beechnuts were germinated and the established seedlings (from which leaf samples were taken) were planted out in the forest in September/October 2012 [17]. Beechnuts of the other German populations DE-GL, DE-GS, DE-US, DE-CL, DE-CS, and DE-HA were collected in fall 2009. In total, 100 beechnuts from each previously sampled adult tree (see above) were collected. After drying, the beechnuts were stored at −10 °C until January 2010. After germination and establishment of the seedlings in the greenhouse, leaf samples were taken and the plants were panted out in the forest in the fall of 2010 [34]. An overview of the sample number and sample year can be found in Supplementary Material Table S1.

2.2. DNA Isolation

Total DNA was extracted from leaves using the DNeasy 96 Plant Kit (Qiagen, Hilden, NRW, Germany). Amount and quality of the DNA were analyzed using 1% agarose gel electrophoresis with 1X TAE (Tris-Acetate-EDTA) as running buffer. DNA was stained with ethidium bromide or Roti-Safe GelStain (Roth, Karlsruhe, Baden-Wuerttemberg, Germany), visualized by UV illumination (Herolab,'Wiesloch, Baden-Wuerttemberg, Germany) and compared to the Lambda DNA size standard (Roche, Mannheim, Baden-Wuerttemberg, Germany).

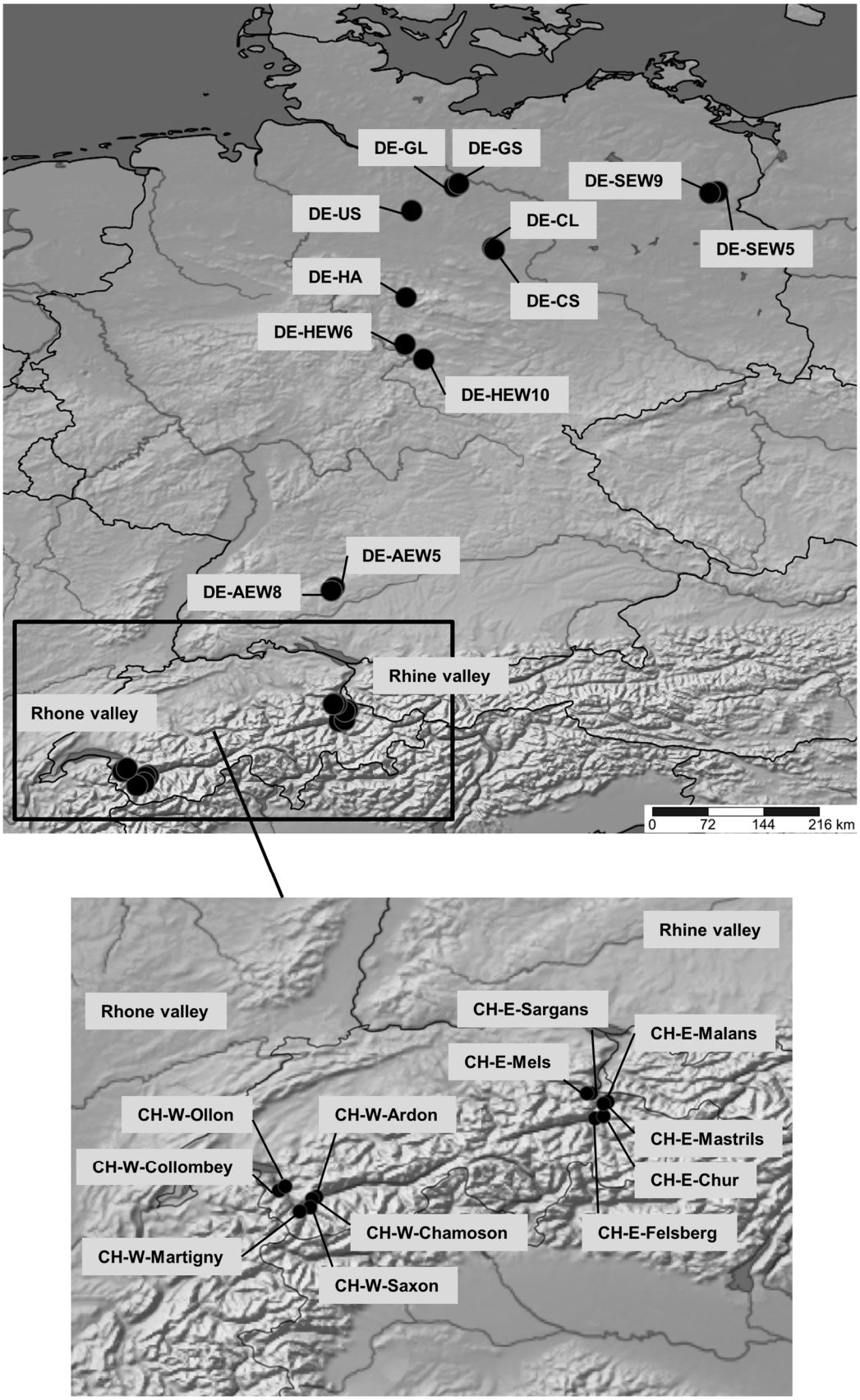

Figure 1. Distribution map of the studied beech populations.

Table 1. Population name and location, number of sampled individuals and environmental variables for the studied beech populations.

Population	Location	Number		Altitude, m a.s.l.	Annual Temperature, °C	Mean Vegetation Season Temperature, °C	Mean Number of Summer Days [a]	Mean Number of Heat Days [b]	Mean annual Precipitation, mm	Mean Vegetation Season Precipitation, mm	EQ [c]
		Adult Trees	Seedlings								
DE-SEW5	53°3′25.3″ N 13°53′7.3″ E	200	200	64	8.3	15.38	33.4	5.6	532.1	273	32.9
DE-SEW9	53°2′40.5″ N 13°48′36.4″ E	198	200	79	8.3	15.38	33.4	5.6	532.1	273	32.9
DE-GL	53°07′ N 10°49′ E	100	228	85	8.6	15.24	27.5	4.6	545.2	272	31.5
DE-GS	53°09′ N 10°52′ E	103	144	85	8.6	15.24	27.5	4.6	545.2	272	31.5
DE-US	52°50′ N 10°19′ E	100	211	117	8.1	14.58	26.8	4.8	800.5	352	20.6
DE-CL	52°24′ N 11°16′ E	101	266	72	8.5	15.2	33.1	6.5	562.8	278	30.6
DE-CS	52°23′ N 11°17′ E	104	230	75	8.5	15.2	33.1	6.5	562.8	278	30.6
DE-HA	51°49′ N 10°15′ E	99	152	458	6.2	12.6	8	0.1	1326	548	10.9
DE-HEW10	51°5′24″ N 10°27′44.8″ E	200	200	378	7.5	14.06	18.7	1.7	662.6	306	24
DE-HEW6	51°15′50″ N 10°14′27.4″ E	200	200	435	7.5	14.06	18.7	1.7	662.6	306	24
DE-AEW5	48°25′10.6″ N 9°24′52.9″ E	198	200	788	6.5	13.14	20.5	1.6	963	477	16.1
DE-AEW8	48°22′57.3″ N 9°22′56.6″ E	199	200	766	6.5	13.14	20.5	1.6	963	477	16.1
CH-E-Felsberg	46°51′ N 9°28′ E	25	62	725	9.2	15.8	40	5.4	798	430	22.6
CH-E-Chur	46°52′ N 9°32′ E	25	63	750	9.2	15.8	40	5.4	798	430	22.6
CH-E-Malans	46°59′ N 9°34′ E	25	64	650	9.3	16	36.6	3.5	1095	583	16.6
CH-E-Mastrils	46°58′ N 9°32′ E	25	62	600	9.3	16	36.6	3.5	1095	583	16.6
CH-E-Sargans	47°3′ N 9°26′ E	25	63	700	9.3	16	36.6	3.5	1318	647	13.8
CH-E-Mels	47°3′ N 9°24′ E	25	60	700	9.3	16	36.6	3.5	1318	647	13.8
CH-W-Ardon	46°13′ N 7°14′ E	25	63	800	9.2	16.5	55.3	10.8	598	233	31.9

Table 1. *Cont.*

Population	Location	Number		Altitude, m a.s.l.	Annual Temperature, °C	Mean Vegetation Season Temperature, °C	Mean Number of Summer Days [a]	Mean Number of Heat Days [b]	Mean annual Precipitation, mm	Mean Vegetation Season Precipitation, mm	EQ [c]
		Adult Trees	Seedlings								
CH-W-Chamoson	46°12′ N 7°12′ E	25	64	800	9.2	16.5	55.3	10.8	598	233	31.9
CH-W-Saxon	46°8′ N 7°11′ E	25	64	750	9.2	16.5	55.3	10.8	598	233	31.9
CH-W-Martigny	46°6′ N 7°6′ E	25	64	600	9.2	16.5	55.3	10.8	843	319	22.7
CH-W-Collombey	46°16′ N 6°56′ E	25	63	600	8.9	15.5	35.1	2	1032	492	17.4
CH-W-Ollon	46°18′ N 6°59′ E	25	63	650	8.9	15.5	35.1	2	1032	492	17.4

[a] with maximum temperature ≥25 °C; [b] with maximum temperature ≥30 °C; [c] Ellenberg's climatic quotient.

2.3. SSR Genotyping

SSR data were obtained from several published studies [15,17,28–31,34]. Data of the adult populations DE-SEW5, DE-SEW9, DE-HEW6, DE-HEW10, DE-AEW5, and DE-AEW8 were obtained from Rajendra et al. [15] (data set ID "11560" available from the Biodiversity Exploratories Information System, https://www.bexis.uni-jena.de/PublicData/About.aspx). Seedling data of the same populations were obtained from Müller et al. [17] (available at the TreeGenes Database, https://treegenesdb.org, under the accession number TGDR062). Data for the adult populations DE-GL, DE-GS, DE-US, DE-CL, DE-CS, and DE-HA were obtained from Seifert [28]. Seedling data of the same populations were obtained from Müller et al. [34], and Müller and Finkeldey [29]. SSR data of the adult and seedling populations from Switzerland were obtained from Cuervo-Alarcon et al. [30] (available at the TreeGenes Database under the accession number TGDR073). The data were complemented with additional genotyping data (Supplementary Material Data file S1) of seedlings of the populations DE-AEW5 and DE-HEW10 (in total, 150 individuals). In all studies, the same SSR genotyping protocol was used. Briefly, nine highly polymorphic SSR markers were selected from published data: six random genomic SSRs (FS3-04 [35], mfs11 [36], sfc0018, sfc0161, sfc1063, and sfc1143 [37]), and three EST markers (GOT066, FIR065, and FIR004 [38]), were pooled into three different sets for PCR multiplexing (set1: all sfc loci, set 2: FS3-04 and mfs 11, set 3: GOT066, FIR065, and FIR004). After PCR, the microsatellite fragments were analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Fragments were scored using GeneMapper 4.0 (Applied Biosystems, Foster City, CA, USA).

2.4. SNP Genotyping

SNP data were partly obtained from previous studies [17,30,31,34] and combined with new data generated in this study. SNP data of the populations DE-SEW5, DE-SEW9, DE-HEW6, DE-HEW10, DE-AEW5, and DE-AEW8 were obtained from Müller et al. [17] (available from the TreeGenes Database (https://treegenesdb.org) under the accession number TGDR062), and complemented with SNP data of additional 600 individuals of these populations (Supplementary Material Data file S1). Data of the populations DE-GL, DE-GS, DE-US, DE-CL, DE-CS, and DE-HA were obtained from Müller et al. [34] (available from the TreeGenes Database under the accession number TGDR040). SNP data of the populations from Switzerland were obtained from Cuervo-Alarcon et al. [30,31] (available from the TreeGenes Database under the accession number TGDR073). SNPs that were used in all these studies were selected to allow population comparisons. The SNPs were located in candidate genes related to bud burst timing and drought stress tolerance [39]. In total, a set of 27 SNPs was used for genotyping (five non-synonymous SNPs, 11 synonymous, seven non-coding, and four SNPs from untranslated regions (UTR)) (Table 2). The flanking sequences of the SNPs were sent to LGC Genomics Ltd. (Teddington, Middlesex, UK) for primer design and genotyping using the PCR-based KASP (Kompetitive Allele Specific PCR) genotyping assay (Hoddesdon, UK).

2.5. Data Analysis

The GenAlEx 6.5 software [40,41] was used to calculate expected heterozygosity (H_e) and observed heterozygosity (H_o) in adult populations (based on the SSR data) and seedlings (based on both SSR and SNP data), as well as genetic differentiation (pairwise G_{ST}, [42,43]) among adult populations, among seedlings, and between adult populations and seedlings based on the SSR data and 999 permutations. Furthermore, a Mantel test for the correlation of geographic distance with genetic distance measured as pairwise G_{ST} and based on SSR data of the adult trees was conducted with this software and visualized with the R package ggplot2 [44]. FSTAT 2.9.4 [45] was applied to estimate allelic richness (A_R) using rarefaction, F_{IS} [46] with FDR (false discovery rate) correction for multiple testing [47] implemented in the p.adjust R function [48] and to test for significant differences of A_R, H_o, H_e, and F_{IS} between adult populations and seedlings based on SSR data and 999 permutations. FSTAT was also

used to calculate F_{IS} based on SNPs for the seedlings using the same FDR correction as described above. Pairwise comparisons of the genetic diversity indices between adult and seedling cohorts from the same populations were conducted using a Kruskal-Wallis test with multiple comparisons implemented in the R package pgirmess 1.6.7 [49]. Linkage disequilibrium (LD) was estimated using the Genepop 4.2.1 software [50] for both SSR (seedlings and adult populations) and SNP data (seedlings). The frequency of null alleles for the SSR loci was also estimated with this software. The effective population size (N_e) of the adult populations and seedlings was estimated based on SSRs using the bias-corrected version of the method based on LD [51–53] with a critical frequency of rare-alleles of 0.01 as implemented in the NeEstimator V2 software [54]. The Bayesian model-based clustering method implemented in the STRUCTURE 2.3.4 software [55] was applied to infer population structure among adult populations and seedlings based on SSRs, and additionally for the seedlings based on SNPs, considering the admixture model and correlated allele frequencies. The Markov chain Monte Carlo (MCMC) iterations included a burn-in period of 50,000 iterations followed by 100,000 iterations. Potential clusters (K) from 1 to 30 were tested using 10 replicates for each cluster run. The ΔK method by Evanno et al. [56] was applied to determine the most likely number of K using the STRUCTURE HARVESTER 0.6.94 program [57]. The CLUMPAK software [58] was used for summation and graphical representation of the STRUCTURE results. Outlier analyses for SSR and SNP loci were conducted with LOSITAN 1.0 [59] with 70,000 simulations, a FDR of 0.1, the stepwise mutation model for SSRs, and the infinite allele model for SNPs. The "populations" 1.2.32 software [60] was used to build a neighbor-joining (NJ) dendrogram for the adult populations based on SSRs and for the seedlings based on SSRs and SNPs. In both cases, Nei's distance [61] was used. Bootstrap values were based on 1000 permutations across loci. The dendrogram was visualized with the Tree Explorer implemented in the MEGA 7.0.26 software [62].

Table 2. SNPs used for genotyping.

Name	Type	Gene
CP10_65	Synonymous	*Chloroplast chaperonin like*
CP10_67	Non-synonymous	
CP10_377	Non-coding	
CP10_442	Non-coding	
CP10_503	Synonymous	
CP10_749	Synonymous	
CP10_1317	Non-coding	
CP10_1428	Non-synonymous	
CysPro_118	Synonymous	*Cystein proteinase*
CysPro_202	Synonymous	
CysPro_728	UTR	
CysPro_783	UTR	
DAG_81	UTR	*DOF zinc finger protein*
DAG_289	Non-coding	
DAG_1059	Synonymous	
His3C1_292	Non-coding	*Histone 3*
His3C2_104	Synonymous	
His3C2_186	Non-coding	
His3C2_260	Synonymous	
NAC_854	Non-synonymous	*NAC transcription factor*
NAC_962	Synonymous	
NAC_1300	UTR	
PP2C_315	Non-synonymous	*Protein phosphatase 2C*
PP2C_391	Synonymous	
PP2C_791	Non-synonymous	
PP2C_941	Non-coding	
PP2C_1200	Synonymous	

3. Results

3.1. SSR Analysis

The observed heterozygosity (H_o) ranged from 0.547 (population CH-E-Chur) to 0.643 (DE-GL) in the adult populations and from 0.563 (CH-W-Collombey) to 0.635 (CH-E-Mels) in the seedlings (Table 3). The expected heterozygosity (H_e) ranged from 0.542 (CH-W-Ollon) to 0.646 (DE-SEW5) in the adults and from 0.576 (CH-E-Mastrils) to 0.645 (DE-SEW5) in the seedlings. The lowest allelic richness (A_R) for the seedlings was found in CH-W-Chamoson (A_R = 5.4) and the highest in DE-GS (8.4). The lowest A_R for the adult populations was found in CH-E-Chur and CH-W-Martigny (5.2) and the highest in DE-GS and DE-SEW5 (6.6). However, the differences in genetic diversity were minor and non-significant. The genetic diversity indices for the different SSR markers are presented in Supplementary Material Tables S2 and S3. The genetic diversity indices were not statistically different between adult populations and their seedlings.

The mean fixation index (F_{IS}) was relatively low: 0.016 and 0.020 for the adult and seedling populations, respectively, but in one of the adult (DE-GS) and six seedling populations it was significantly different from zero (Table 3).

Table 3. Genetic diversity indices based on SSRs.

Population	Seedlings					Adults				
	N	A_R	H_o	H_e	F_{IS}	***N***	A_R	H_o	H_e	F_{IS}
DE-SEW5	100	7.3	0.599	0.645	0.076 *	200	6.6	0.636	0.646	0.018
DE-SEW9	100	7.3	0.580	0.611	0.057 *	198	6.1	0.620	0.616	−0.005
DE-GL	148	7.6	0.610	0.633	0.041 *	100	6.2	0.643	0.635	−0.008
DE-GS	129	8.4	0.622	0.628	0.014	103	6.6	0.602	0.638	0.062 *
DE-US	149	7.8	0.565	0.600	0.062 *	100	6.2	0.594	0.595	0.005
DE-CL	134	7.7	0.596	0.603	0.016	101	6.5	0.581	0.599	0.036
DE-CS	142	8	0.631	0.635	0.009	104	6.2	0.599	0.619	0.036
DE-HA	152	8.2	0.627	0.638	0.020	99	6.4	0.612	0.630	0.033
DE-HEW10	179	7.6	0.609	0.641	0.053 *	200	6.1	0.607	0.626	0.033
DE-HEW6	100	7.3	0.618	0.623	0.013	200	6.4	0.608	0.614	0.012
DE-AEW5	169	7.9	0.585	0.625	0.066 *	198	6.4	0.621	0.610	−0.015
DE-AEW8	100	7.2	0.589	0.600	0.023	199	6.1	0.627	0.621	−0.006
CH-E-Felsberg	62	6.9	0.579	0.588	0.024	25	6.2	0.600	0.605	0.029
CH-E-Chur	63	6.9	0.591	0.592	0.010	25	5.2	0.547	0.547	0.022
CH-E-Malans	64	6.5	0.606	0.597	−0.008	25	6.0	0.600	0.573	−0.027
CH-E-Mastrils	62	6.3	0.573	0.576	0.012	25	5.4	0.573	0.560	−0.004
CH-E-Sargans	63	6.9	0.626	0.602	−0.032	25	6.1	0.596	0.604	0.035
CH-E-Mels	60	7.4	0.635	0.616	−0.023	25	6.4	0.582	0.582	0.020
CH-W-Ardon	63	6.9	0.587	0.588	0.009	25	5.7	0.582	0.587	0.028
CH-W-Chamoson	64	5.4	0.580	0.579	0.007	25	5.4	0.591	0.578	−0.002
CH-W-Saxon	64	6.7	0.583	0.592	0.023	25	5.4	0.573	0.595	0.057
CH-W-Martigny	64	6.6	0.604	0.589	−0.017	25	5.2	0.573	0.581	0.033
CH-W-Collombey	63	7.2	0.563	0.590	0.054	25	6.0	0.604	0.603	0.018
CH-W-Ollon	63	6.8	0.607	0.591	−0.019	25	5.3	0.573	0.542	−0.037
Mean	98	7.2	0.599	0.608	0.020	88	6.0	0.598	0.600	0.016

N—number of individuals; A_R—allelic richness; H_o—observed heterozygosity; H_e—expected heterozygosity; F_{IS}—fixation index; * $p < 0.05$; no significant differences were observed in genetic diversity between adult and seedling generation.

LD for the analyzed loci ranged from 0% (populations DE-CS, DE-AEW5, CH-E-Chur, CH-E-Mastrils, and CH-W-Martigny) to 38.9% (DE-AEW8) in the adult populations (mean 9.7%), and from 0% (CH-E-Mastrils and CH-W-Ollon) to 19.4% (DE-SEW9) in the seedlings (mean 8.8%) (Supplementary Material Table S4). The mean frequency of null alleles was 2.8% in the adult populations and 1.8% in the seedlings (Supplementary Material Table S5).

The effective population size (N_e) ranged from 82.3 in the adult population DE-SEW9 to 711.6 in the seedling population CH-W-Saxon (albeit, for some populations N_e was "infinite", which indicates that there was no evidence for genetic drift caused by a limited number of parents [53]; Supplementary Material Table S6).

The Mantel test for correlation of geographic distance with genetic distance (measured as pairwise G_{ST}, [42,43]) in the adult populations was significant ($R^2 = 0.484$, $p < 0.001$) (Supplementary Material Figure S1).

No deviations from neutral expectations for the SSR loci were detected in the outlier analysis. The STRUCTURE analysis for the adult populations revealed the most likely number of $K = 4$ based on the delta K method [56]. The clustering of individuals showed a tendency of decreasing genetic similarity of populations from northeast to southwest, but the population DE-AEW8 deviated from this pattern and seems to be an outlier (Figure 2a). The NJ dendrogram showed similar results. The German and Swiss populations grouped into two different clusters with high bootstrapping support for the German cluster (76%) with population DE-AEW5 in a basal position (Figure 3). However, most clusters in the dendrogram were not well-supported by bootstrapping values. A similar dendrogram was also obtained for the seedlings (Supplementary Material Figure S2). The STRUCTURE analysis for the seedlings revealed the most likely number of $K = 2$ (Supplementary Material Figure S3). The same trend was also observed for the seedlings: Similarity between populations is decreasing from northeast to southwest with most differences observed between German and Swiss populations (Figure 2b). The mean pairwise G_{ST} values were 0.017 among adult populations, 0.015 among seedlings, and 0.015 between adults and seedlings (Supplementary Material Table S7). Adult and seedling cohorts from the same population were not significantly differentiated or revealed very low differentiation values (mean pairwise $G_{ST} = 0.002$; Table 4).

Table 4. Pairwise G_{ST} values between adult and seedling cohorts based on SSRs.

Population	G_{ST}
DE-SEW5	0.0022 *
DE-SEW9	0.0013 *
DE-GL	0.0020 *
DE-GS	0.0005
DE-US	0.0019 *
DE-CL	0.0005
DE-CS	0.0004
DE-HA	0.0025 *
DE-HEW10	0.0021 *
DE-HEW6	0.0011 *
DE-AEW5	0.0016 *
DE-AEW8	0.0059 *
CH-E-Felsberg	0.0001
CH-E-Chur	0.0033
CH-E-Malans	0.0007
CH-E-Mastrils	0.0015
CH-E-Sargans	0.0011
CH-E-Mels	0.0011
CH-W-Ardon	0.0010
CH-W-Chamoson	0.0050 *
CH-W-Saxon	0.0000
CH-W-Martigny	0.0025
CH-W-Collombey	0.0017
CH-W-Ollon	0.0008
Mean	0.0017

Note: * $p < 0.05$.

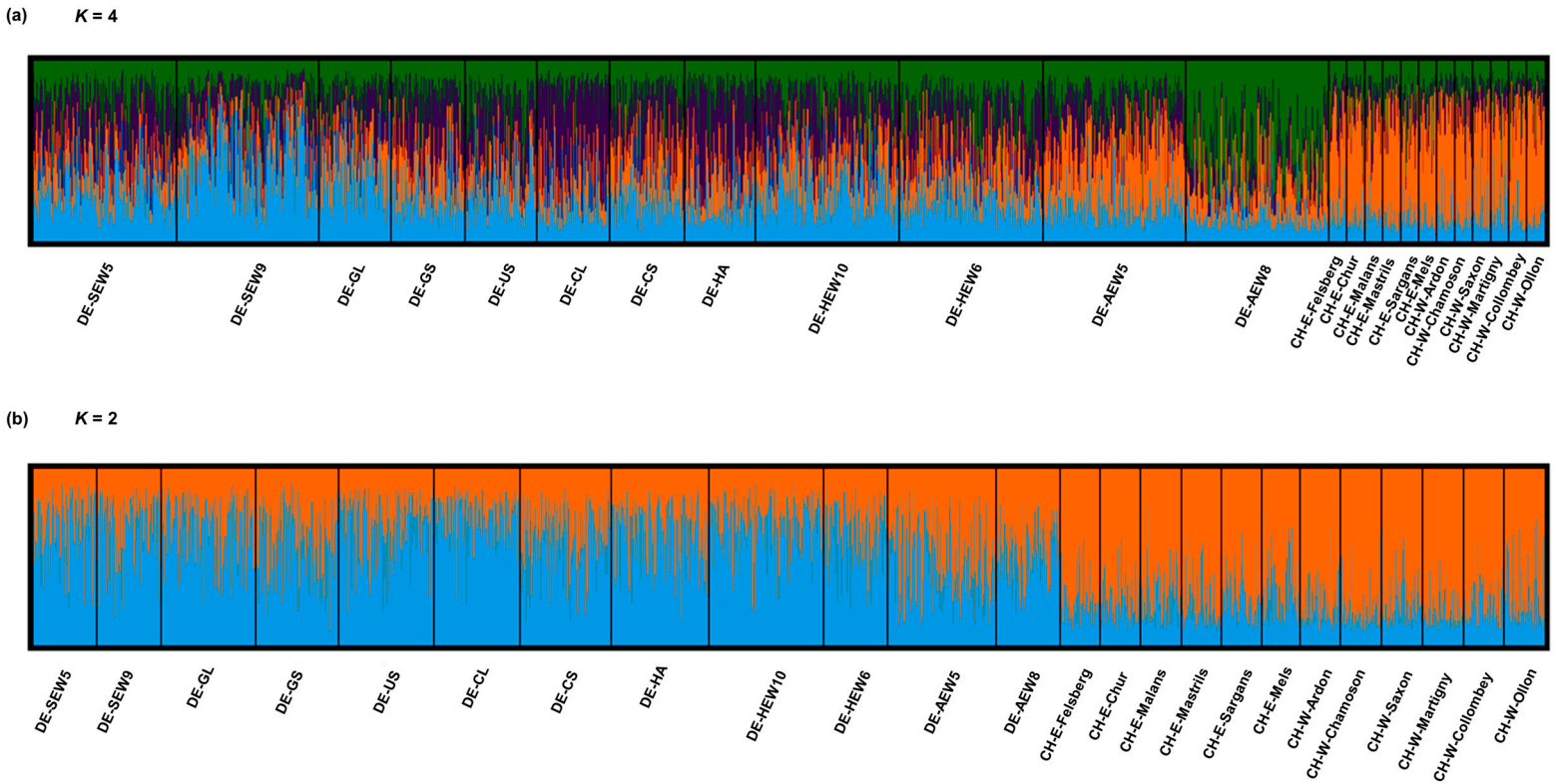

Figure 2. Clustering of individuals based on SSRs for the adult (**a**) and seedling (**b**) populations sorted from northeast to southwest.

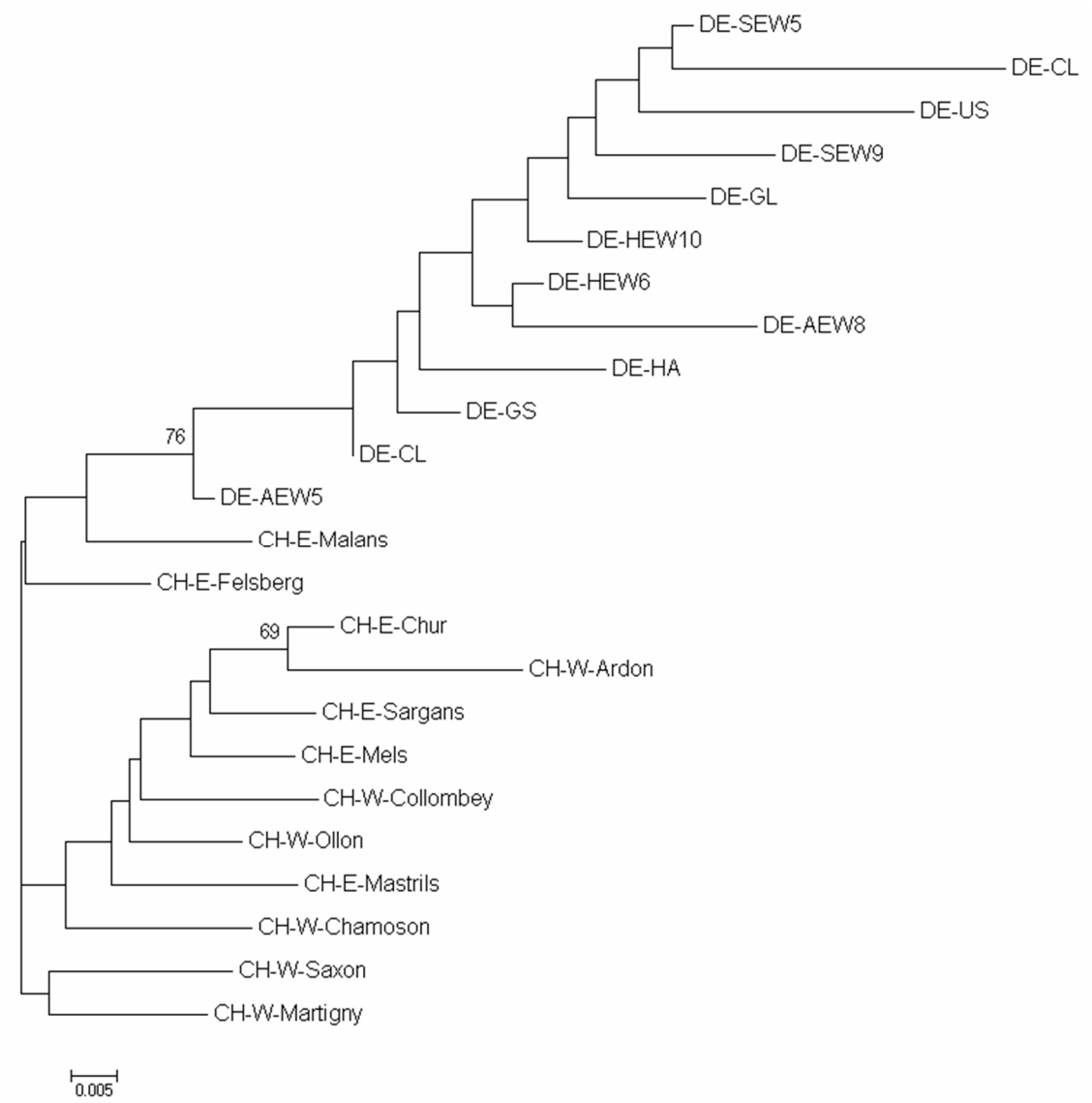

Figure 3. Neighbor-joining dendrogram of the adult populations based on SSRs. Bootstrapping values ≥50 are shown.

3.2. SNP Analysis

The observed heterozygosity in the seedlings ranged from 0.192 (CH-W-Saxon) to 0.290 (CH-E-Felsberg) and the expected heterozygosity from 0.226 (CH-W-Saxon) to 0.288 (CH-W-Chamoson). The mean F_{IS} value was 0.011 and was significantly different from zero only in two populations: DE-AEW8 and CH-W-Saxon (Table 5).

LD for the SNP loci ranged from 8.3% in CH-W-Ollon to 21.9% in DE-GL (mean LD = 13.3%). Similar to the seedling dendrogram based on SSRs, the dendrogram based on SNPs showed differentiation of the German and Swiss populations, albeit not well-supported by bootstrapping values (Supplementary Material Figure S4). The STRUCTURE analysis revealed the most likely number of $K = 5$, but did not reveal strong population structure. The Swiss populations together with the two German populations, DE-AEW5 and DE-AEW8, showed a slight differentiation from the other German populations (Supplementary Material Figure S5). The mean pairwise G_{ST} value was 0.016 among the populations (Supplementary Material Table S5). No F_{ST} outlier SNPs were detected.

Table 5. Genetic diversity indices based on SNPs.

Population	N	H_o	H_e	F_{IS}
DE-SEW5	200	0.266	0.258	−0.027
DE-SEW9	200	0.259	0.253	−0.023
DE-GL	228	0.273	0.279	0.025
DE-GS	144	0.269	0.270	0.008
DE-US	211	0.280	0.284	0.015
DE-CL	266	0.246	0.248	0.011
DE-CS	230	0.273	0.278	0.020
DE-HA	123	0.270	0.278	0.033
DE-HEW10	200	0.248	0.249	0.006
DE-HEW6	200	0.271	0.277	0.023
DE-AEW5	200	0.281	0.277	−0.012
DE-AEW8	200	0.254	0.275	0.078 *
CH-E-Felsberg	62	0.290	0.278	−0.036
CH-E-Chur	63	0.288	0.278	−0.029
CH-E-Malans	64	0.242	0.246	0.024
CH-E-Mastrils	62	0.269	0.271	0.016
CH-E-Sargans	63	0.282	0.273	−0.028
CH-E-Mels	60	0.244	0.252	0.042
CH-W-Ardon	63	0.276	0.250	−0.095
CH-W-Chamoson	64	0.279	0.288	0.037
CH-W-Saxon	64	0.192	0.226	0.156 *
CH-W-Martigny	64	0.231	0.233	0.015
CH-W-Collombey	63	0.249	0.252	0.019
CH-W-Ollon	63	0.249	0.241	−0.025
Mean	131.5	0.262	0.263	0.011

* $p < 0.05$.

4. Discussion

4.1. Neutral Genetic Variation

All SSR genotyping was conducted in the same laboratory and scoring of alleles followed the same scheme making sure that all alleles were identically genotyped and allowing accurate comparisons across multiple populations, which can be problematic when SSR data generated by different laboratories have to be combined and compared [63]. High levels of genetic diversity were found in all studied populations. The mean observed (H_o) and expected (H_e) heterozygosity values were 0.598 and 0.600 for the adult populations, and 0.599 and 0.608 for their seedlings, respectively. These values are similar to those found in other studies of beech (e.g., [14,36,64]). The fixation index (F_{IS}) was close to zero in most populations (mean F_{IS} was 0.016 and 0.020 in the adult and seedling populations, respectively). Only one adult population (DE-GS) showed a positive F_{IS} value significantly different from zero, while this was the case in six seedling populations. Nevertheless, positive F_{IS} values were only revealed for single SSR markers and not constantly across all loci, suggesting that they were not due to inbreeding, but rather due to null alleles that indeed were detected for a few SSR markers. However, null alleles were at very low frequency and were not expected to largely bias the population genetic parameters [65]. In general, no statistically significant differences of the genetic diversity indices were detected between adult stands and seedlings. This finding is in agreement with other beech studies that compared the genetic diversity across generations [14,25,26]. Large effective population sizes can be expected in forest tree species, which was also indicated by our results that further demonstrated no reduction of effective population sizes in the seedlings compared to the adult populations.

The sample size in this study varied among populations. Different sample sizes can especially affect the observed number of alleles. Therefore, we used rarefaction to calculate the allelic richness (Ar) (Table 3). We further calculated the observed and expected heterozygosity (H_o and H_e, respectively) not only based on the complete sample set, but also based on the same sample size for each population (25 randomly selected individuals of each population), and received similar results for

both sample sets (Supplementary Material Table S8). The same approach (25 randomly selected individuals per population) also generated a NJ dendrogram similar to the one based on the complete sample set (see below), albeit with lower bootstrapping support (Supplementary Material Figure S6). Therefore, there is no indication that the results are biased by different sample sizes. The genetic diversity estimated in the present study, however, might be partly biased upward, because seedlings from the German populations have been grown from beechnuts in a greenhouse. Hence, natural selection acting on seedlings as it occurs in forests was excluded. Therefore, the genetic diversity estimates for the seedlings of the German populations might rather be seen as the potential of genetic variation that is transferred from the adult trees to the seedlings, and not the genetic diversity of established seedlings in the forest. Nevertheless, reproductive failure of adult stands should also have been reflected in the genetic diversity of greenhouse seedlings, if existent.

The genetic differentiation was rather low among adult populations (G_{ST} = 0.017) and seedlings (G_{ST} = 0.015). The lowest differentiation was observed between adult and seedling cohorts from the same populations (G_{ST} = 0.002), indicating an effective vertical gene transfer. The STRUCTURE analysis revealed a tendency of decreasing genetic similarity among adult populations from northeast to southwest. This is most likely an effect of isolation by distance, which was confirmed by a Mantel test. Also, re-colonization after the last glacial period could have contributed to this pattern. Thus, most of the (northern) German populations may have originated from a southeastern refuge, whereas the Swiss and southwestern German populations may also have been influenced from a refuge in the western Alps [15,66,67]. The NJ dendrogram also showed a separation of German and Swiss populations with high bootstrapping support for the German cluster. This is likely not an effect of different silvicultural management methods in Switzerland and Germany, since forest management is similar in both countries. In the bar plot derived from the STRUCTURE analysis the population DE-AEW8 was the most separated from the other populations and deviated from the pattern of decreasing similarity from northeast to southwest. The population DE-AEW8 has been unmanaged for almost 80 years [15], which could have led to a different genetic structure of the population. The populations DE-SEW9 and DE-HEW10 have also been unmanaged, albeit the exact time point of management cessation is unknown for them [15]. While DE-HEW10 showed no different pattern than the other populations, DE-SEW9 seemed to be slightly more differentiated from the other northern populations (Figure 2a). The seedlings showed lower population structure than the adult populations in the STRUCTURE bar plot, but a decrease of genetic similarity from north to south was also visible. For the seedlings, no "outlier populations" were revealed by the STRUCTURE analysis. Lower population structure in seedlings as compared to adult stands can be expected, since adult stands should have experienced ongoing natural and artificial selection during their life time. In general, there was low population structure among the beech populations. This is in agreement with other studies on European beech (e.g., [68,69]). Previous studies on the same beech populations also revealed low population structure, albeit partially more differentiation among populations was detected than here [29–31]. These studies were conducted on a regional scale, while in the present study, many populations along a large geographic gradient from northern Germany to Switzerland were analyzed. Therefore, small-scale population structure might remain undetected due to the larger differentiation of populations along the geographic gradient.

4.2. Potentially Adaptive Genetic Variation

Although neutral genetic diversity (heterozygosity) was found to be correlated with population fitness [13], it should not be used as a surrogate of adaptive genetic variation [32]. Therefore, we applied genic SNP markers to infer potentially adaptive diversity of the beech populations. We selected the common SNPs also studied in the previously published studies to be able to compare potentially adaptive genetic variation among the 24 analyzed beech seedling populations based on more than 3100 individuals. In total, 24 of the 27 SNPs have shown signs of selection in the beech populations in the previous studies [17,30,31,34], and hence, may be involved in adaptation or, in case of SNPs in

non-coding regions, may be linked to adaptive functional SNPs. The mean observed and expected heterozygosity of the populations was 0.262 and 0.263, respectively. These values are similar to the values reported in other studies that analyzed the same or other beech populations in Germany or Switzerland [17,30,31,34,70] and indicate a high adaptive genetic diversity of beech that is comparable with other tree species (e.g., H_e = 0.25 [71] and 0.188 [72] in *Pinus taeda* L.; 0.208 in *Quercus robur* L., 0.252 in *Q. petraea* (Matt.) Liebl., 0.274 in *Q. pubescens* Willd., 0.283 in *Q. frainetto* Ten. [73]; 0.270 in *Picea glauca* (Moench) Voss [74]). Genotyping by sequencing (GBS) methods such as RAD-seq, however, are becoming feasible for non-model forest tree species [75,76], and may provide more precise estimates of adaptive genetic variation in beech in the future.

F_{IS} values were significantly different from zero only in two populations. Hence, similar to the SSR data, there seems to be no pronounced inbreeding in the studied populations. The dendrogram based in SNP data was also similar to the one based on SSRs. Genetic differentiation between German and Swiss populations was observed, albeit not well-supported by bootstrapping values. The two German populations, DE-AEW5 and DE-AEW8, grouped rather with the Swiss populations than with the German ones. The population structure for the SNPs revealed by the STRUCTURE analysis was lower as compared to the structure revealed by the SSRs. Only a slight differentiation between the southern and northern populations was detected. The mean G_{ST} value based on the SNPs (0.016) was also similar to the one based on the SSRs (0.015). Hence, the potentially adaptive nature of the SNP markers did not result in a higher genetic differentiation among populations, but their within population variation was lower than the one based on SSRs. This could be a sign of purifying selection, which, however, was not revealed by the outlier analysis.

5. Conclusions

Beech populations from northeast Germany to southwest Switzerland across a wide range of climatic and geographic conditions for this species were studied. Relatively high neutral and potentially adaptive genetic diversities were found in all populations, which may be a good basis for the adaptation to climate change. No differences in the genetic diversity parameters were found between the adult populations and their seedlings. Thus, at least in the studied beech populations, the transfer of genetic variation across generations currently seems not to be limited by potentially negative factors, such as climate change. Nevertheless, a reduced growth of beech has recently been observed even in the center of its distribution range [9–11]. Therefore, a reduced fitness of beech populations in some areas with ongoing climate change cannot be ruled out for the future and still needs to be thoroughly studied. The diversity estimates in this study only reflect a fraction of the adaptive and neutral genetic variation in the beech genome. Hence, more genome-wide data, including methylation sensitive markers that could contribute to an understanding of the role of epigenetics in adaptation, should be used in further studies to confirm the observations presented here.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/9/8/469/s1, Data file S1: previously unpublished SNP and SSR data; Figure S1: Mantel test for correlation of geographic distance with genetic distance measured as pairwise G_{ST} between adult tree populations; Figure S2: Neighbor joining dendrogram of the seedling populations based on SSRs and Nei's distance (Nei 1972). Bootstrapping values $\geq$50 are shown; Figure S3: Plots of delta *K* and log likelihood for each *K* based on SSRs for the adult (**a**) and seedling (**b**) populations; Figure S4: Neighbor joining dendrogram for the seedling populations based on the SNP markers and Nei's distance (Nei 1972). Bootstrapping values $\geq$50 are shown; Figure S5: Clustering of individuals for different *K* (**a**), plots of delta *K* (**b**) and log likelihood for each *K* (**c**) based on SNPs for the German and Swiss seedling populations; Figure S6: Neighbor-joining dendrogram of the adult populations based on SSRs and 25 randomly selected individuals per population. Bootstrapping values $\geq$50 are shown. Table S1: Sampling details of the study; Table S2: Genetic diversity indices for the different SSRs over all populations; Table S3: F_{IS} values of the different populations displayed for the different SSR markers; Table S4: Percentage of SSR pairs with significant linkage disequilibrium; Table S5: Frequency of null alleles at the SSR loci; Table S6: Estimated effective population sizes (N_e); Table S7: Pairwise G_{ST} values among (**a**) adult populations, (**b**) seedlings, and (**c**) between adult and seedling cohorts based on SSRs, as well as for the seedlings based on SNPs (**d**). *p* Values above the diagonal; Table S8: Observed heterozygosity (H_o) and expected heterozygosity (H_e) based on SSRs for 25 randomly selected individuals per population.

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