

Genetic and adaptive trait variation in seedlings of European beech provenances from Northern Germany

Markus Müller^{1*}, Reiner Finkeldey^{1,2}

¹ University of Goettingen, Faculty for Forest Sciences and Forest Ecology, Forest Genetics and Forest Tree Breeding, Büsgenweg 2, 37077 Göttingen, Germany;

² University of Kassel, Mönchebergstraße 19, 34109 Kassel*

Corresponding author: Markus Müller, E-mail: mmuellef@gwdg.de

Abstract

European beech (*Fagus sylvatica* L.) will most likely be threatened by climate change. Therefore, more knowledge of its adaptation potential to changing environmental conditions is required. Several studies showed differences in adaptive traits for beech populations from across Europe, but there is also growing evidence for microevolutionary processes occurring within short geographic distances in this species, or even a lack of local adaptation. Hence, a more regional perspective for adaptation research in beech might be appropriate. Here, we investigated the performance (height, height increment and mortality) and genetic structure based on microsatellite markers of 2400 beech seedlings from provenances growing along a temperature and precipitation gradient in Northern Germany in a common garden experiment. Differences for all traits were found among provenances. Provenances from lower precipitation areas did not perform better than provenances from higher precipitation areas at the common garden site, which was located near the lower precipitation limit of beech. Further, neighboring provenances growing on either sandy or loamy soils showed more different adaptive trait variation than distant provenances. We detected minor genetic structure among provenances and a high genetic diversity within them. Thus, small-scale adaptive trait variation in beech can occur, despite low but significant genetic population structure among provenances.

Keywords: *Mortality, adaptive traits, climate change, genetic diversity*

Introduction

European beech (*Fagus sylvatica* L.) is a foundation tree species of various forest ecosystems and covers a large continuous geographic range in Europe (Knapp et al. 2008). Its importance will even increase in the future, due to current forest conversion programs in which pure conifer stands are converted into pure beech stands or mixed deciduous stands including beech (Tarp et al. 2000, Scharnweber et al. 2011). Nevertheless, climate change-related alterations of productivity or species distribution have already been observed for this species (Peñuelas and Boada 2003, Charru et al. 2010), and further range shifts of beech have been predicted (Meier et al. 2011, Hanewinkel et al. 2013). Even in the center of its distribution range, beech seems already be negatively affected by climate change (Knutzen et al. 2017). Several studies detected differences among beech provenances from across Europe in adaptive traits such as drought tolerance, frost resistance, or bud burst timing (von Wühlisch et al. 1995, Višnjić and Dohrenbusch 2004, Robson et al. 2012). For instance, it was shown that provenances from Central and Southeast Europe are more frost resistant than provenances from Southern Europe (Višnjić and Dohrenbusch 2004), and provenances from dryer environments are better adapted to drought than provenances from moister environments (Rose et al. 2009, Dounavi et al. 2016). Nevertheless, recent studies showed that microevolutionary processes in beech can occur within short geographic distances (Pluess and Weber 2012, Pluess et al. 2016). Indications for water availability-related adaptation in neighboring beech populations, for instance, were found by Pluess and Weber (2012), despite high gene flow. Also, there can be a lack of local adaptation in beech (Hofmann et al. 2015). Therefore, more knowledge about the regional adaptive trait and genetic differentiation of beech is required.

Here, we investigated the genetic diversity and performance of transferred seedlings of six beech provenances in Northern Germany. Beech provenances from a temperature and

precipitation gradient were selected and a common garden experiment comprising 2400 seedlings was placed on the dry end of the gradient with a mean annual precipitation close to the lower limit of beech. Hence, the performance (measured as height, height increment, and mortality) of genetic material transferred from high to low precipitation areas could be evaluated. Furthermore, neighboring provenances growing on either sandy or loamy soils were selected along the gradient to cover variation in water storage capacity and to investigate small-scale differentiation in beech provenances.

We tested the hypotheses that (I) there is adaptive trait variation among the investigated beech provenances, with differences on the local level, (II) provenances from lower precipitation areas perform better than provenances from higher precipitation areas under low precipitation conditions, (III) all investigated beech provenances show a high genetic diversity, and (IV) low but significant genetic structure is detectable among provenances.

Material and Methods

Study sites

Beech provenances from four different study sites in Northern Germany were selected (Fig. 1). They were jointly investigated by the collaborative project "KLIFF" (climate impact and adaptation research in Lower Saxony; <http://www.kliff-niedersachsen.de>), and have previously been described by Müller et al. (2015). At three of these study sites (Calvörde, Göhrde, and Unterlüß) two provenances with different soil properties were selected to cover variation in water storage capacity: one provenance on a sandy soil, hereafter termed "sand", and one provenance on a loam-richer soil, hereafter termed "loam". The different provenances were the following: Calvörde sand (CS), Calvörde loam (GL), Göhrde sand (GS), Göhrde loam (GL), Unterlüß sand (US), Unterlüß loam (UL). The provenance UL was not included in the common garden experiment (see below) due to an insufficient number of seedlings for this provenance. Additionally, a provenance in the Harz Mountains (Harz, (Ha)) near the village Bad Grund was investigated to include a provenance growing under high mean annual precipitation and low mean annual temperatures. All provenances were 2009 between 97 (CS) and 142 years (GL) old and either pure beech stands or beech stands with mixture of a few oak trees. The provenances were located at elevations of 72 m a.s.l. (CL) to 458 m a.s.l. (Ha). Mean annual temperatures ranged from 7.2 °C (Ha) to 9.2 °C (CS) and mean annual precipitation ranged from 543 mm (CL) to 1,170 mm (Ha) (Table. 1). Geographic distance among provenances ranged from 2.2 km between CL and CS to 154.0 km between GS and Ha (Supplementary Material 1).

Common garden experiment

In total, 707 adult trees of the beech provenances described above have been investigated in a previous study (Seifert 2012). The trees were randomly distributed throughout the provenances. To establish a common garden experiment with

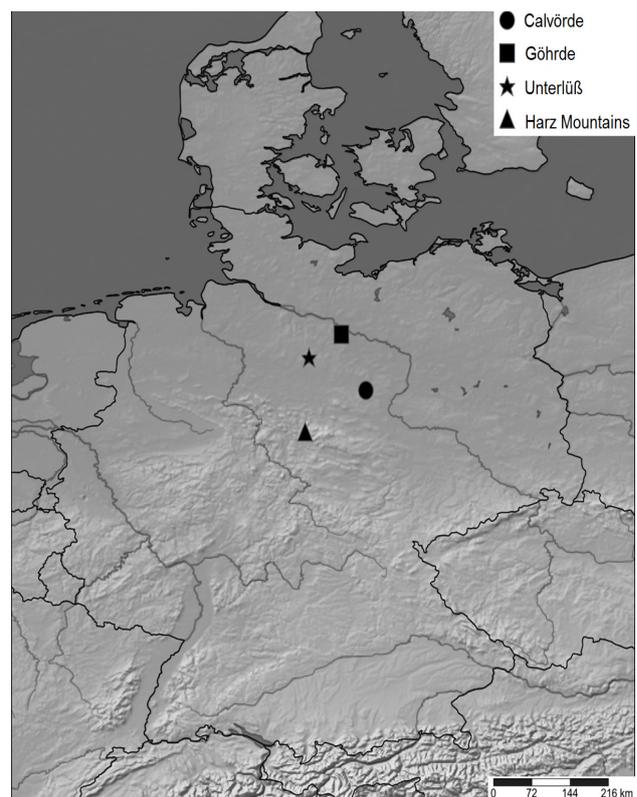


Fig. 1 Location of the four study sites Calvörde, Göhrde, Unterlüß and Harz Mountains. The map was created using Simple-Mappr (Shorthouse 2010)

offspring of these provenances, 100 beechnuts from beneath each previously sampled tree were collected in fall 2009. Beechnuts from the sampled trees were not kept separate within provenances, hence they cannot be assigned to single mother trees. Seedlings out of these beechnuts were raised in a greenhouse as described by Seifert (2012). The seedlings of the different provenances were randomly placed in the greenhouse. The seedlings were planted out in a common garden experiment in fall 2010 using a randomized complete block design with four replications as described in Müller et al. (2015). Briefly, from each provenance 400 vital individuals of the established seedlings in the greenhouse were randomly selected and planted out in the beech stand Calvörde sand. Each block contained 600 seedlings. In each block, each provenance was planted in 20 rows (with 14 cm of distance between them) each containing 5 seedlings (with 11 cm of distance between them). The seedlings were protected against damage by deer.

Measurements and observations of seedling adaptive traits

Height of all living seedlings was measured in fall 2010 (planting), 2011 and 2012. The height was measured to 0.5 cm accuracy from the ground to the beginning of the terminal bud along the stem axis of each seedling. Seedlings with damaged terminal shoots were excluded from the analysis (in total, 252 individuals (zero in 2010; 177 in 2011; 252 in 2012)). Seedling

Table 1
Characteristics of the seed source beech provenances

Provenance	Calvörde sand (CS)	Calvörde loam (CL)	Göhrde sand (GS)	Göhrde loam (GL)	Unterlüß sand (US)	Harz Mountains (Ha)
Position	52°23' N 11°17' E	52°24' N 11°16' E	53°09' N 10°52' E	53°07' N 10°49' E	52°50' N 10°19' E	51°49' N 10°15' E
Mean temperature ¹ [°C]	9.2 / 14.5	9.1 / 14.5	8.7 / 13.9	8.7 / 13.8	8.5 / 13.6	7.2 / 12.4
Mean precipitation ¹ [mm]	544 / 294	543 / 294	665 / 347	675 / 349	766 / 374	1170 / 660
Water storage capacity mineral soil [mm 120 cm ⁻¹]	81 ^a	140 ^a	80 ^a	78 ^a	79 ^a	na
Stand age [years]	97 ^a	131 ^a	133 ^a	142 ^a	115 ^a	136 ^b
Elevation [m a.s.l.]	75 ^a	72 ^a	85 ^a	85 ^a	117 ^a	458 ^b

¹period 1971-2000 (regionalized by C. Döring from the national weather stations network data base provided by DWD, Deutscher Wetterdienst), the left value refers to the mean annual precipitation sum or mean annual temperature, respectively, while the right value refers to mean precipitation or mean temperature in the vegetation period April – September; ^aHertel et al. (2013); ^bSeifert (2012), na: data not available

height increment was calculated by subtracting the seedling height at planting (2010) from the seedling height in fall 2012 for each individual in the common garden experiment. The mortality of the seedlings (death of the whole seedling) was visually assessed and recorded in early summer 2011, 2012 and 2013.

Selection of seedlings for genotyping

SSR-genotyping of seedlings was conducted to determine their neutral genetic variation. For that, 100 individuals per provenance (i.e., 25 within each block) were randomly selected for genotyping (in total, 600 seedlings). Microsatellite data for the adult provenances were provided by Seifert (Seifert 2012).

DNA isolation

In July 2010, one leaf per seedling from the common garden experiment was sampled and stored at -20 °C until DNA extraction. Total DNA was extracted from these leaves using the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany). Amount and quality of the DNA were analyzed by 1 % agarose gel electrophoresis with 1 X TAE as running buffer. DNA was stained with ethidium bromide or Roti-Safe GelStain (Roth, Karlsruhe, Germany), visualized by UV illumination and compared to a Lambda DNA size marker (Roche, Mannheim, Germany).

Microsatellite analysis

The microsatellite analysis was conducted as described in Müller et al. (2015). Briefly, nine highly polymorphic microsatellite markers were used (FS3-04, Pastorelli et al. (2003); mfs11, Vornam et al. (2004); sfc0018, sfc0161, sfc1063, sfc1143, Asuka et al. (2004); EST markers GOT066, FIR065, FIR004, Durand et al. (2010)), and the primers were pooled into three different sets

for multiplexing (set1: all sfc loci, set 2: FS 3-04 and mfs 11, set 3: GOT066, FIR065, FIR004). After PCR, the microsatellite fragments were separated on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA). Scoring of fragments was conducted with the Genotyper 3.7 software (Applied Biosystems, Foster City, USA).

Data analysis

Adaptive data

Provenances were tested for differences in seedling height and seedling height increment using linear mixed-effects models (“lmer” function in the lme4 R package (Bates et al. 2015)), with seedling height or seedling height increment as response variable, provenances as fixed effect, and blocks and provenance-block interactions as random effects. Non-significant interactions were excluded from the model. The “glht” function within the “multcomp” R package (Hothorn et al. 2008) with Tukey’s post hoc test was applied to detect differences among provenances. A false discovery rate (FDR) threshold of 0.05 was applied to correct for multiple testing. Differences between the mortality rate of the provenances were tested using generalized mixed-effects models (“glmer” function in the lme4 R package (Bates et al. 2015)) with a binomial distribution. Mortality was the response variable, provenances were fixed effects, and blocks and provenance-block interactions were random effects in the model. Non-significant interactions were excluded from the model. Differences among provenances were determined using the “glht” function as described above with the same FDR threshold. Model assumptions were checked visually using diagnostic plots of residuals. Spearman’s rank correlation coefficient was used to determine correlations between the traits “mortality”, “height” and “height increment” with the environmental conditions at the provenance origins

“mean annual precipitation”, “mean precipitation in the vegetation period”, “mean annual temperature”, “mean temperature in the vegetation period”, and “water storage capacity in the mineral soil” (Table 1). All statistical analyses were conducted in R 3.3.1 (R Core Team 2016). Boxplots for seedling height and seedling height increment were created with STATISTICA version 13 (Dell Inc., Round Rock, USA).

Microsatellites

Linkage disequilibrium, frequency of null alleles, and Hardy-Weinberg proportions were estimated using the Genepop 4.2.1 software (Rousset 2008) based on SSR data of the adult provenances and seedlings. Markov chain parameters for the test of linkage disequilibrium and deviations from Hardy-Weinberg equilibrium were the following: 10,000 demorization steps, 100 batches and 5,000 iterations per batch. For the seedlings the genetic diversity indices number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e) and fixation index (F) were estimated using the GenAlEx 6.501 software (Peakall and Smouse 2006, Peakall and Smouse 2012). A Kruskal-Wallis test with multiple comparisons implemented in the STATISTICA 13 software (Dell Inc., Round Rock, USA) was used to compare these parameters with previously obtained data from the adult provenances (origin of the seedlings; (Seifert 2012)). Additionally, to test the representativity of the seedlings for the adult provenances, an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram, based on Nei’s distance (Nei 1972) was calculated using the “populations” 1.2.32 software (Langella 1999). Bootstrap values based on 1,000 permutations were also calculated with this program. The dendrogram was visualized with the TreeView 1.6.6 software (Page 1996) using the phylogram tree style. Pairwise G_{ST} among (Nei 1987, Meirmans and Hedrick 2011) adult provenances was calculated with GenAlEx 6.501 (Peakall and Smouse 2006, Peakall and Smouse 2012) using 999 permutations for the significance test. The Bayesian model-based clustering method implemented in the STRUCTURE 2.3.3 software (Pritchard et al. 2000) was applied to infer population structure among adult provenances. The no admixture model and correlated allele frequencies were selected. A burn-in period of 10,000 and Markov chain Monte Carlo (MCMC) replicates of 100,000 were used. Potential clusters (K) from one to ten were tested using ten iterations. The ΔK method by (Evanno et al. 2005) was applied to determine the optimal value of K using the STRUCTURE HARVESTER 0.6.93 program (Earl and vonHoldt 2012).

Results

Seedling adaptive traits

At planting, several provenances showed significantly different heights, whereby the median ranged from 11.0 cm for the provenances Ha, CL and GL to 14 cm for the provenance GS (Fig. 2a). In fall 2011, the median height ranged from 22.0 cm for the provenance CL to 25.5 cm for the provenance GS and US (Fig. 2b). In 2012 the provenances CL and GL showed the smallest

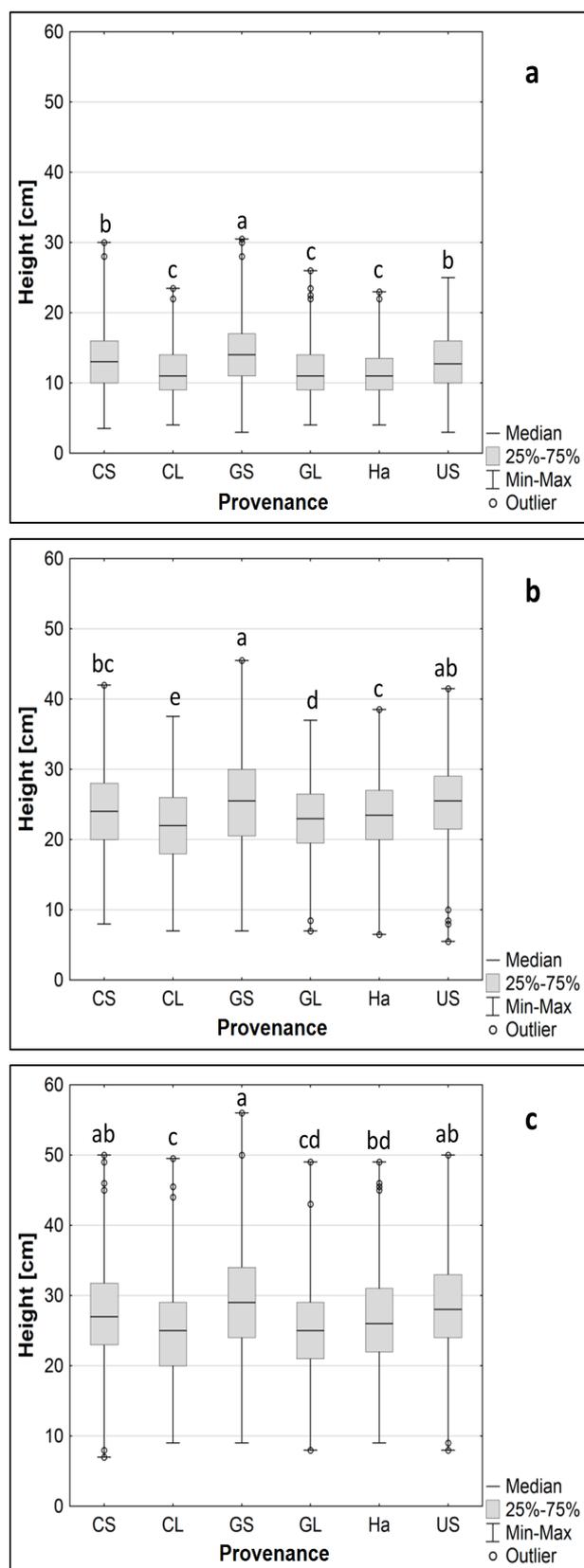


Fig. 2 Seedling height of the provenances in the common garden experiment at planting (2010) (a), the year 2011 (b), and the year 2012 (c). Different letters indicate significant differences among provenances ($p < 0.05$)

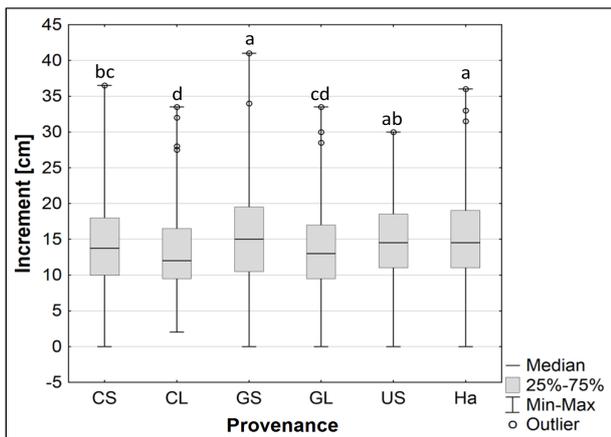


Fig. 3
Mean increment of the beech provenances in the common garden experiment from establishment (2010) to fall 2012. Different letters indicate significant differences among provenances ($p < 0.05$)

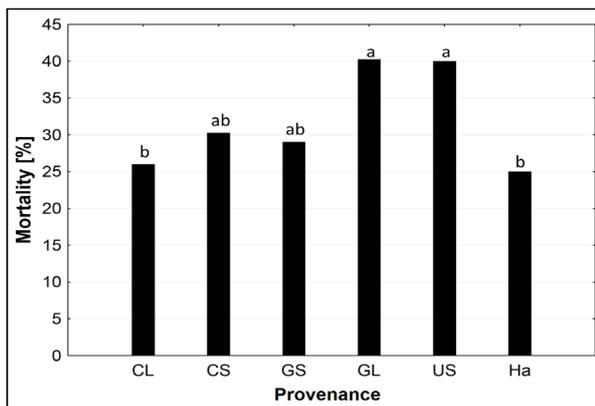


Fig. 4
Mortality rate [%] of the beech provenances in the common garden experiment in the year 2013. Different letters indicate significant differences among provenances ($p < 0.05$)

seedlings (median: 25 cm), whereas the provenance GS showed the largest seedlings (median 29.0 cm) (Fig. 2c).

Also the seedling increment differed between the provenances (Fig. 3), whereby the provenances GS and Ha showed the highest increment (median: 15.3 cm) during the observation period from the establishment of the experiment (2010) to fall 2012. The lowest increment was observed for the provenance CL (median: 13.1 cm). In early summer 2011, only a few seedlings (1-4) per provenance did not survive. In 2012, the mean mortality rate was low (8.2 %) but significantly different between the provenances. The highest mortality was observed for the provenance US (12.3 %) and the lowest one for the provenance GS (4.3 %). In the following year (2013) (Fig. 4), the mean mortality rate was 31.8 % and, hence, clearly higher than in 2012 and still significantly different between provenances. The highest mortality was recorded for the provenances US (40 %)

and GL (40.3 %). The lowest mortality was found for the provenance Ha (25 %). No statistically significant correlations were found between adaptive traits and the environmental conditions at the provenance origins.

Linkage disequilibrium, Hardy-Weinberg equilibrium, and null alleles

Significant linkage disequilibrium ($p < 0.05$) was observed for the markers in the different adult provenances and seedlings (Supplementary Material 2). No LD was found for the adult provenance CS and the seedlings of provenance GS, whereas the highest amount of LD was estimated for the adult provenance Ha (19.4 %). Based on the complete set of SSR loci, deviations from Hardy-Weinberg proportions were revealed only for the adult provenances Ha and GS, and the seedlings of the provenance Ha. Single SSR loci that showed deviations from Hardy-Weinberg proportions were sfc0161 (for the adult provenance US), sfc1063 (for the juvenile provenance Ha), FIR065 (for the juvenile provenances CL and CS), FIR004 (for the juvenile provenances GS and Ha, and the adult provenances GS, UL, and Ha), FS3-04 (for the juvenile and adult provenances US), and GOT066 (for the adult provenance GS).

The mean estimated null allele frequency was 3.6 % for the juvenile and 3.7 % for the adult provenances (Supplementary Material 3).

Genetic diversity indices

Genetic diversity indices differed between the different SSR loci, whereas the two EST-markers "GOT066" and "FIR004" as well as the locus "FS 3-04" showed lower diversity values than the remaining markers (Table 2). Only slight differences for the genetic diversity indices were observed between the different provenances (Table 3). The mean number of alleles was 8.2. The observed heterozygosity ranged from 0.571 (US) to 0.624 (Ha), and the expected heterozygosity ranged from 0.586 (US) to 0.635 (Ha). The mean fixation index was 0.015. No statistically significant differences between the adult and juvenile provenances for the genetic diversity indices were detected.

Genetic differentiation and population structure

An UPGMA analysis was conducted to test the representativity of the seedlings for the adult provenances. All seedlings grouped together with the respective adult provenance of origin in the dendrogram (Fig. 5). The bootstrap values ranged from 56 % for the group GS_adult and GS_juvenile and 100 % for the group CL_adult and CL_juvenile.

G_{ST} values among the adult provenances ranged from 0.002 between CS and GS, and 0.018 between CL and US, and CL and GL, respectively (Table 4). The mean distance between the provenances was 0.010. The STRUCTURE analysis revealed weak population structure among the different adult provenances, whereby the provenances US and UL were most differentiated from the other provenances (Fig. 6). The ΔK method (Evanno et al. 2005) revealed an optimal value of $K = 2$ (Supplementary Material 4).

Table 2

Genetic diversity indices for the investigated SSR loci over all analyzed seedlings of the different provenances, N_a : number of alleles, H_o : observed heterozygosity, H_e : expected heterozygosity, F: fixation index

Locus	N_a	H_o	H_e	F
sfc0018	10.3	0.736	0.731	-0.006
sfc0161	15.7	0.809	0.811	0.002
sfc1063	10.0	0.787	0.802	0.017
sfc1143	10.8	0.762	0.764	0.003
GOT066	3.2	0.145	0.135	-0.066
FIR065	4.0	0.674	0.692	0.024
FIR004	7.8	0.522	0.600	0.130
FS 3-04	3.8	0.299	0.308	0.040
mfs11	8.2	0.724	0.716	-0.013

Table 3

Genetic diversity indices for the seedlings of the different provenances, N: number of individuals, N_a : number of alleles, H_o : observed heterozygosity, H_e : expected heterozygosity, F: fixation index

Provenance	N	N_a	H_o	H_e	F
CL	100	8.2	0.594	0.603	0.009
CS	99	8.4	0.621	0.633	0.012
GL	100	7.9	0.615	0.626	0.012
GS	100	8.6	0.613	0.623	0.010
US	100	7.9	0.571	0.586	0.043
Ha	99	8.2	0.624	0.635	0.002
Mean	99.7	8.2	0.606	0.618	0.015

Table 4

G_{ST} values (Meirmans and Hedrick 2011, Nei 1987) among the analyzed adult provenances (in bold, below the diagonal). P-values are shown above the diagonal

	CL	CS	GL	GS	Ha	US	UL	
	0.000	0.001	0.001	0.001	0.001	0.001	0.001	CL
	0.012	0.000	0.001	0.010	0.001	0.001	0.001	CS
	0.018	0.007	0.000	0.001	0.001	0.001	0.001	GL
	0.011	0.002	0.007	0.000	0.001	0.001	0.001	GS
	0.012	0.005	0.011	0.005	0.000	0.001	0.001	Ha
	0.018	0.011	0.014	0.014	0.012	0.000	0.001	US
	0.012	0.007	0.014	0.009	0.007	0.003	0.000	UL

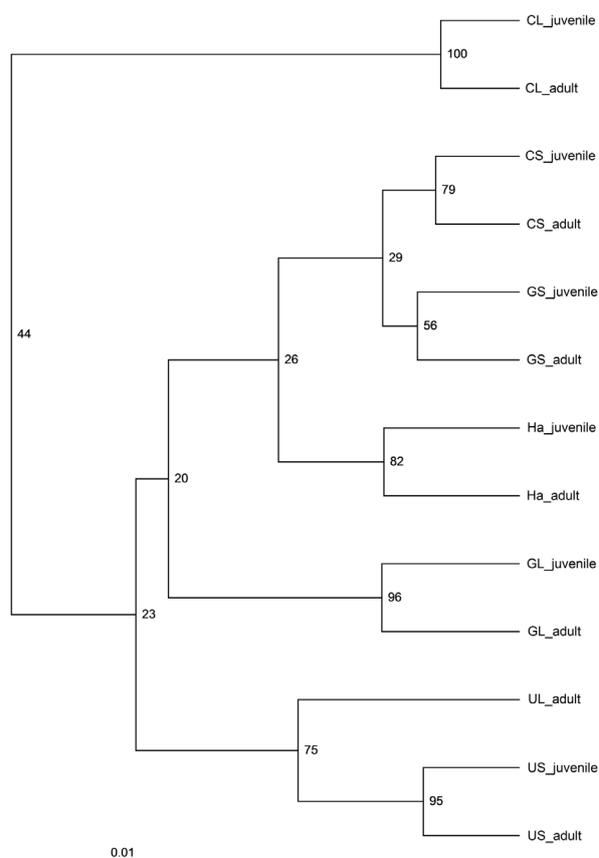


Fig. 5

UPGMA dendrogram based on Nei's genetic distance (Nei 1972) and bootstrap values [%] for the investigated adult and juvenile provenances

Discussion

Adaptive trait differentiation

Significant differences for all analyzed adaptive traits were found for the provenances in the common garden experiment. This is in agreement with data from other studies which also detected variation in plant height, height increment, and mortality among beech provenances (von Wühlisch et al. 2008, Liesebach 2012). Interestingly, the performance of seedlings from lower precipitation environments was not better than that of seedlings from higher precipitation environments. This is unexpected, since the common garden experiment was located in an area near to the lower precipitation limit of beech and there occurred even a dry period from March to May 2011 at the common garden site (Müller-Haubold et al. 2013). Knutzen et al. (2017) and Nguyen et al. (2017) also did not find an influence of precipitation at provenance origins on the growth rate of saplings in a drought stress experiment using saplings from partly the same provenances as here, despite differences in drought response among provenances. Nevertheless, we did not measure drought stress parameters or below ground biomass, and therefore the level of drought stress possibly

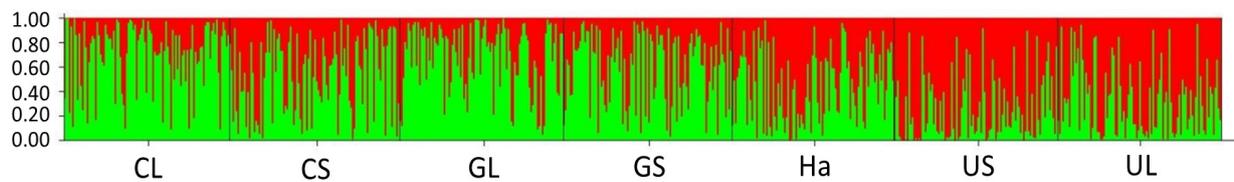


Fig. 6

Clustering of individuals of the different adult provenances assuming $K = 2$. Vertical bars indicate the assignment probability of each individual to the two inferred clusters

affecting the seedlings is unknown. Further, no significant correlations were found between the adaptive traits and the environmental conditions on the original provenance sites. Likely, more provenances would be necessary to conduct more robust correlation analyses.

Interestingly, neighboring provenances did not present more similar adaptive trait variation than distant provenances. For instance, the provenance GS showed a significantly larger height, height increment, and an earlier bud burst (Müller et al. 2015) than the neighboring provenance GL, which grows only 5 km apart. Also the mortality rate between the two provenances was significantly different one year after planting. In the second year there was still a difference of 11.3 % between GS and GL, albeit statistically not significant. These results may be explained by different soil properties of the provenances, at least for height and height increment, since there was a trend of better growth of provenances from sandy soils than for loamy soils (Figs. 2 and 3). Nevertheless, for the mortality rate this trend was not observed and other studies that analyzed the same provenances found only minor soil influence on several traits such as fine root biomass, total fine root surface area, fine root production, forest net primary production, wood production, leaf production, fruit mass production, mean annual ring width, or mean sensitivity of growth (Hertel et al. 2013, Müller-Haubold et al. 2013, Knutzen et al. 2017). Flowering conditions, stand density or weather conditions can influence the genetic structure of seeds due to alterations of the mating system, which may lead to differences in the genetic composition of seeds among years (Konnert and Behm 1999). Hence, different genetic structures of neighboring adult provenances during their establishment could be another explanation for the observed differences.

There are some practical limitations using field experiments. For instance, it was not possible to conduct height measurements as precisely as under controlled conditions. Different planting depths of the seedlings and irregularities of the soil surface might have caused some variations. Nevertheless, the high number of seedlings and the block design of the experiment should have effectively mitigated these inaccuracies. The results of the first year measurements might have been influenced by a planting shock. Later measurements however, should show genotypic differences among provenances. It is known that stress resistance increases with ontogeny (Niinemets 2010) and that the increment of beech differs between juvenile age stages (Lieseback 2012). The mortality rate increased with the duration of the common garden experiment from

2011 to 2013, most likely due to an accumulation of stress over time affecting not adapted seedlings. Thus, long-term observations of the common garden experiment would provide more insights into the development of the seedlings from the different provenances. Nevertheless, despite their long lifetime, the most vulnerable phase of trees remains their seedling and sapling stage (Gray et al. 2011). Hence, information about the performance of seedlings from different provenances in this early life stage is of great importance.

Genetic diversity, differentiation and population structure

All analyzed provenances in this study exhibited a high genetic variation within provenances (mean H_o : 0.606; mean H_e : 0.618), with no statistically significant differences between adult provenances and seedlings. High genetic diversity based on SSRs was also revealed by other studies of beech (Vornam et al. 2004, H_e : 0.765; Oddou-Muratorio et al. 2011, H_e : 0.72; Bilela et al. 2012, H_e : 0.777; Rajendra et al. 2014, H_e : 0.622), and can be expected for outcrossing, wind-pollinated (tree) species (Hamrick et al. 1992, Hamrick and Godt 1996, Petit and Hampe 2006). A high genetic diversity is a good basis for adaptation. Beech forests with a wide genetic basis are more likely to be able to cope with warmer and drier conditions, and thus, to adapt to new environmental conditions (Bilela et al. 2012). However, in light of rapid climate change, gene flow from better adapted populations or the use of assisted migration might be necessary (Jump and Peñuelas 2005, Jump et al. 2006, Gray et al. 2011). All juvenile provenances grouped to the corresponding source provenances in the UPGMA dendrogram supported by high bootstrap values. These results suggest a good sampling design and no influence of the treatment of the seedlings in the greenhouse on their genetic structure. Nevertheless, adult provenances were used to get a precise impression of population structure and differentiation among the analyzed provenances in Northern Germany. The neutral genetic structure among provenances was low, albeit statistically significant (mean G_{ST} 0.010, $p < 0.01$). Only the provenances US and UL were slightly higher differentiated from the others as referred by the STRUCTURE analysis (Fig. 6). A low population structure of beech in Central Europe was also detected by other studies (e.g., (Demesure et al. 1996, Magri et al. 2006, Lalagüe et al. 2014, Rajendra et al. 2014). Departures from the model assumptions in the STRUCTURE software (Hardy-Weinberg equilibrium within populations and linkage equilibrium

between loci within populations (Pritchard et al. 2000)) can lead to an overestimation of K . Since only 8.3 % of all SSR markers were in LD over all adult provenances and only two adult provenances showed deviations from Hardy Weinberg equilibrium, a reliable analysis of population structure is expected. The estimated null allele frequency for the investigated SSR loci was 3.6 % for the juvenile and 3.7 % for the adult provenances. Population genetic parameters seem to be mostly unbiased for null-allele frequencies ranging between 5 % and 8 % on average across loci (Oddou-Muratorio et al. 2009). Thus, although some loci may be affected by null alleles, a reliable population genetic analysis can be assumed in our study.

Conclusions

This study aimed to determine adaptive trait and genetic variation of beech provenances on a regional scale in Northern Germany. Significant differences in the performance of the provenances were detected for all analyzed traits. No influence of precipitation at provenance origins was detected on the performance of the provenances at the common garden site located near the lower precipitation limit of beech. Furthermore, even neighboring provenances presented more different adaptive trait variation than distant provenances. Indeed, a long-term study would be required to assess the future development of the provenances. A high genetic diversity within and only minor population structure among provenances was detected, which can be assumed to be a good basis for adaptation. Nevertheless, these results are based on neutral genetic markers, and future studies should also take adaptive genetic variation into account.

Acknowledgement

We thank the late Dr. Sarah Seifert for providing SSR data of the adult beech populations, and her scientific support during the study. We thank A. Dolynska, C. Radler, G. Dinkel and A. Capelle for their technical assistance as well as all persons who helped us with the field work. We thank C Döring for the regionalization of the precipitation and temperature data. The study was supported by the Ministry for Science and Culture of Lower Saxony within the network KLIF – climate impact and adaptation research in Lower Saxony.

References

- Asuka Y, Tani N, Tsumura Y, Tomaru N (2004) Development and characterization of microsatellite markers for *Fagus crenata* Blume. *Mol Ecol Notes* 4 (1):101-103. <https://doi.org/10.1046/j.1471-8286.2003.00583.x>
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67 (1):48. <https://doi.org/10.18637/jss.v067.i01>
- Bilela S, Dounavi A, Fussi B, Konnert M, Holst J, Mayer H, Renneberg H, Simon J (2012) Natural regeneration of *Fagus sylvatica* L. adapts with maturation to warmer and drier microclimatic conditions. *For Ecol Manag* 275:60-67. <https://doi.org/10.1016/j.foreco.2012.03.009>
- Charru M, Seynave I, Morneau F, Bontemps JD (2010) Recent changes in forest productivity: An analysis of national forest inventory data for common beech (*Fagus sylvatica* L.) in north-eastern France. *For Ecol Manag* 260 (5):864-874. <https://doi.org/10.1016/j.foreco.2010.06.005>
- Demesure B, Comps B, Petit RJ (1996) Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. *Evolution* 50 (6):2515-2520. <https://doi.org/10.2307/2410719>
- Dounavi A, Netzer F, Celepirovic N, Ivanković M, Burger J, Figueroa AG, Schön S, Simon J, Cremer E, Fussi B, Konnert M, Renneberg H (2016) Genetic and physiological differences of European beech provenances (*F. sylvatica* L.) exposed to drought stress. *For Ecol Manag* 361:226-236. <https://doi.org/10.1016/j.foreco.2015.11.014>
- Durand J, Bodenes C, Chancerel E, Frigerio JM, Vendramin G, Sebastiani F, Buonamici A, Gailing O, Koelewijn HP, Villani F, Mattioni C, Cherubini M, Goicoechea PG, Herran A, Ikarán Z, Cabane C, Ueno S, Alberto F, Dumoulin PY, Guichoux E, de Daruvar A, Kremer A, Plomion C (2010) A fast and cost-effective approach to develop and map EST-SSR markers: oak as a case study. *BMC Genomics* 11:570. <https://doi.org/10.1186/1471-2164-11-570>
- Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4 (2):359-361. <https://doi.org/10.1007/s12686-011-9548-7>
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14 (8):2611-2620. <https://doi.org/10.1111/j.1365-294x.2005.02553.x>
- Gray LK, Gylander T, Mbogga MS, Chen PY, Hamann A (2011) Assisted migration to address climate change: recommendations for aspen reforestation in western Canada. *Ecol Appl* 21 (5):1591-1603. <https://doi.org/10.1890/10-1054.1>
- Hamrick JL, Godt MJW, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. *New Forest* 6 (1-4):95-124 <https://doi.org/10.1007/bf00120641>
- Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. *Philos T Roy Soc B* 351 (1345):1291-1298. <https://doi.org/10.1098/rstb.1996.0112>
- Hanewinkel M, Cullmann DA, Schelhaas M-J, Nabuurs G-J, Zimmermann NE (2013) Climate change may cause severe loss in the economic value of European forest land. *Nat Clim Change* 3 (3):203-207. <https://doi.org/10.1038/nclimate1687>
- Hertel D, Strecker T, Müller-Haubold H, Leuschner C, Guo D (2013) Fine root biomass and dynamics in beech forests across a precipitation gradient - is optimal resource partitioning theory applicable to water-limited mature trees? *J Ecol* 101 (5):1183-1200. <https://doi.org/10.1111/1365-2745.12124>
- Hofmann M, Durka W, Liesebach M, Bruehlheide H (2015) Intraspecific variability in frost hardiness of *Fagus sylvatica* L. *Eur J For Res* 134 (3):433-441. <https://doi.org/10.1007/s10342-015-0862-6>
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical J* 50 (3):346-363. <https://doi.org/10.1002/bimj.200810425>
- Jump AS, Peñuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. *Ecol Lett* 8 (9):1010-1020. <https://doi.org/10.1111/j.1461-0248.2005.00796.x>
- Jump AS, Hunt JM, Martínez-Izquierdo JA, Peñuelas J (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. *Mol Ecol* 15 (11):3469-3480. <https://doi.org/10.1111/j.1365-294x.2006.03027.x>
- Knapp HD, Emde F-A, Engels B, Lehrke S, Hendrischke O, Klein M, Kluttig H, Krug A, Schäfer H-J, Scherfose V, Schröder E, Schweppe-Kraft B (2008) Naturerbe Buchenwälder: Situationsanalyse und Handlungserfordernisse. Bonn, Vilm: Bundesamt für Naturschutz
- Knutzen F, Dulamsuren C, Meier IC, Leuschner C (2017) Recent climate warming-related growth decline impairs European beech in the center of its distribution range. *Ecosystems*. <https://doi.org/10.1007/s10021-017-0128-x>
- Konnert M, Behm A (1999) Genetische Strukturen einer Saatgutpartie - Einflussfaktoren und Einflussmöglichkeiten. *Beiträge für Forstwirtschaft und Landschaftsökologie* 33:152-156
- Lalagüe H, Csilléry K, Oddou-Muratorio S, Safrana J, de Quattro C, Fady B, González-Martínez SC, Vendramin GG (2014) Nucleotide diversity and linkage disequilibrium at 58 stress response and phenology candidate genes in a European beech (*Fagus sylvatica* L.) population from southeastern France. *Tree Genet Genomes* 10 (1):15. <https://doi.org/10.1007/s11295-013-0658-0>
- Langella O (1999) Populations version 1.2.32 [online]. To be found in <http://www.bioinformatics.org/project/?group_id=84>

- Liesebach M (2012) Wachstum und phänotypische Variation von sechs Herkünften der Rot-Buche (*Fagus sylvatica* L.) an einem Standort in Schleswig-Holstein. *Appl Agric Forestry Res* 62:179-192
- Magri D, Vendramin GG, Comps B, Dupanloup I, Geburek T, Gömöry D, Latalowa M, Litt T, Paule L, Roure JM, Tantau I, van der Knaap WO, Petit RJ, de Beaulieu JL (2006) A new scenario for the quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytol* 171 (1):199-221. <https://doi.org/10.1111/j.1469-8137.2006.01740.x>
- Meier ES, Edwards Jr TC, Kienast F, Dobbertin M, Zimmermann NE (2011) Co-occurrence patterns of trees along macro-climatic gradients and their potential influence on the present and future distribution of *Fagus sylvatica* L. *J Biogeogr* 38 (2):371-382. <https://doi.org/10.1111/j.1365-2699.2010.02405.x>
- Meirmans PG, Hedrick PW (2011) Assessing population structure: F(ST) and related measures. *Mol Ecol Resour* 11 (1):5-18. <https://doi.org/10.1111/j.1755-0998.2010.02927.x>
- Müller-Haubold H, Hertel D, Seidel D, Knutzen F, Leuschner C (2013) Climate responses of aboveground productivity and allocation in *Fagus sylvatica*: a transect study in mature forests. *Ecosystems* 16 (8):1498-1516. <https://doi.org/10.1007/s10021-013-9698-4>
- Müller M, Seifert S, Finkeldey R (2015) A candidate gene-based association study reveals SNPs significantly associated with bud burst in European beech (*Fagus sylvatica* L.). *Tree Genet Genomes* 11 (6):116. <https://doi.org/10.1007/s11295-015-0943-1>
- Nei M (1972) Genetic Distance between Populations. *Am Nat* 106 (949):283-292. <https://doi.org/10.1086/282771>
- Nei M (1987) *Molecular Evolutionary Genetics*. New York: Columbia University Press. [https://doi.org/10.1016/0047-2484\(89\)90093-6](https://doi.org/10.1016/0047-2484(89)90093-6)
- Nguyen QN, Polle A, Pena R (2017) Intraspecific variations in drought response and fitness traits of beech (*Fagus sylvatica* L.) seedlings from three provenances differing in annual precipitation. *Trees*. <https://doi.org/10.1007/s00468-017-1539-1>
- Niinemets Ü (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. *For Ecol Manag* 260 (10):1623-1639. <https://doi.org/10.1016/j.foreco.2010.07.054>
- Oddou-Muratorio S, Vendramin GG, Buiteveld J, Fady B (2009) Population estimators or progeny tests: what is the best method to assess null allele frequencies at SSR loci? *Conserv Genet* 10 (5):1343-1347. <https://doi.org/10.1007/s10592-008-9648-4>
- Oddou-Muratorio S, Klein EK, Vendramin GG, Fady B (2011) Spatial vs. temporal effects on demographic and genetic structures: the roles of dispersal, mating and differential mortality on patterns of recruitment in *Fagus sylvatica*. *Mol Ecol* 20 (9):1997-2010. <https://doi.org/10.1111/j.1365-294x.2011.05039.x>
- Page RD (1996) TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 12 (4):357-358. <https://doi.org/10.1093/bioinformatics/12.4.357>
- Pastorelli R, Smulders MJM, Van't Westende WPC, Vosman B, Giannini R, Vettori C, Vendramin GG (2003) Characterization of microsatellite markers in *Fagus sylvatica* L. and *Fagus orientalis* Lipsky. *Mol Ecol Notes* 3 (1):76-78. <https://doi.org/10.1046/j.1471-8286.2003.00355.x>
- Peakall R, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6 (1):288-295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peakall R, Smouse PE (2012) GenAlix 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics* 28 (19):2537-2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Peñuelas J, Boada M (2003) A global change-induced biome shift in the Montseny mountains (NE Spain). *Glob Change Biol* 9 (2):131-140. <https://doi.org/10.1046/j.1365-2486.2003.00566.x>
- Petit RJ, Hampe A (2006) Some evolutionary consequences of being a tree. *Annu Rev Ecol Evol S* 37 (1):187-214. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110215>
- Pluess AR, Weber P (2012) Drought-adaptation potential in *Fagus sylvatica*: linking moisture availability with genetic diversity and dendrochronology. *PLoS One* 7 (3):e33636. <https://doi.org/10.1371/journal.pone.0033636>
- Pluess AR, Frank A, Heiri C, Lalague H, Vendramin GG, Oddou-Muratorio S (2016) Genome-environment association study suggests local adaptation to climate at the regional scale in *Fagus sylvatica*. *New Phytol* 210 (2):589-601. <https://doi.org/10.1111/nph.13809>
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155 (2):945-959
- R Core Team (2016) R: A language and environment for statistical computing. [online]. To be found in <<http://www.R-project.org/>>
- Rajendra KC, Seifert S, Prinz K, Gailing O, Finkeldey R (2014) Subtle human impacts on neutral genetic diversity and spatial patterns of genetic variation in European beech (*Fagus sylvatica*). *For Ecol Manag* 319:138-149. <https://doi.org/10.1016/j.foreco.2014.02.003>
- Robson TM, Sánchez-Gómez D, Cano FJ, Aranda I (2012) Variation in functional leaf traits among beech provenances during a Spanish summer reflects the differences in their origin. *Tree Genet Genomes* 8 (5):1111-1121. <https://doi.org/10.1007/s11295-012-0496-5>
- Rose L, Leuschner C, Köckemann B, Buschmann H (2009) Are marginal beech (*Fagus sylvatica* L.) provenances a source for drought tolerant ecotypes? *Eur J For Res* 128 (4):335-343. <https://doi.org/10.1007/s10342-009-0268-4>
- Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour* 8 (1):103-106. <https://doi.org/10.1111/j.1471-8286.2007.01931.x>
- Scharnweber T, Manthey M, Criegee C, Bauwe A, Schröder C, Wilmking M (2011) Drought matters – Declining precipitation influences growth of *Fagus sylvatica* L. and *Quercus robur* L. in north-eastern Germany. *For Ecol Manag* 262 (6):947-961. <https://doi.org/10.1016/j.foreco.2011.05.026>
- Seifert S (2012) Variation of candidate genes related to climate change in European beech (*Fagus sylvatica* L.). Göttingen, 134 p, University of Göttingen
- Seifert S, Vornam B, Finkeldey R (2012) DNA sequence variation and development of SNP markers in beech (*Fagus sylvatica* L.). *Eur J For Res* 131 (6):1761-1770. <https://doi.org/10.1007/s10342-012-0630-9>
- Shorthouse D (2010) SimpleMapp, an online tool to produce publication-quality point maps [online]. To be found in <<http://www.simplemapp.net>>
- Tarp P, Helles F, Holten-Andersen P, Larsen JB, Strange N (2000) Modelling near-natural silvicultural regimes for beech - an economic sensitivity analysis. *For Ecol Manag* 130 (1-3):187-198. [https://doi.org/10.1016/S0378-1127\(99\)00190-5](https://doi.org/10.1016/S0378-1127(99)00190-5)
- Višnjić Č, Dohrenbusch A (2004) Frost resistance and phenology of European beech provenances (*Fagus sylvatica* L.). *Allg Forst Jagdztg* 175:101-108
- von Wühlisch G, Krusche D, Muhs HJ (1995) Variation in temperature sum requirement for flushing of beech provenances. *Silvae Genet* 44:343-346
- von Wühlisch G, Hansen JK, Mertens P, Liesebach M, Meierjohann E, Muhs H-J, Teissier du Cros E, de Vries S, Terazawa K, Madsen P, Sagheb-Talebi K (eds) Variation among *Fagus sylvatica* and *Fagus orientalis* provenances in young international field trials: The organizing committee of the 8th IUFRO international beech symposium c/o Hokkaido Forestry Research Institute Bibai, Hokkaido, Japan
- Vornam B, Decarli N, Gailing O (2004) Spatial distribution of genetic variation in a natural beech stand (*Fagus sylvatica* L.) based on microsatellite markers. *Conserv Genet* 5 (4):561-570. <https://doi.org/10.1023/b:coge.0000041025.82917.ac>