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Genetic analysis of European common ash (*Fraxinus excelsior* L.) populations affected by ash dieback

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Abstract

European common ash is an important component of mixed forest ecosystems in Bavaria and is considered a valuable tree species under climate change. The first aim of the present study was to assess the genetic diversity within and between ash populations in Bavaria in comparison with neighboring regions. Because ash stands have been heavily attacked by ash shoot disease in the last few years, the second aim of the study was to detect genetic differences between susceptible and less susceptible trees (trees with defoliation up to 30%) within populations. Altogether 41 populations were investigated using nuclear and chloroplast microsatellites. The results showed high genetic variation within and high genetic differentiation between ash populations. Higher chloroplast microsatellite variation was detected instead populations from south-eastern Bavaria compared to other regions. The comparison of susceptible and less susceptible groups of individuals within each population

revealed medium to high genetic differences in some cases. For the observed heterozygosity, higher values were found for the group of less susceptible trees compared to the group of all trees or to the group of susceptible trees within populations. This could be a first indication that individuals with a higher heterozygosity might be able to withstand ash dieback in a better way compared to homozygotic individuals. Within the group of less susceptible trees a relatively wide genetic base exists whereupon a future breeding programme can be built. Thus it is of utmost importance for the species to maintain less affected trees in order to keep the genetic potential for future adaptation processes within ash populations, seed production and regeneration.

Key words: *Fraxinus excelsior*, genetic diversity, *Hymenosyphus fraxineus*, nuclear microsatellites, chloroplast microsatellites.

Introduction

European common ash (*Fraxinus excelsior* L.) is an important component of mixed forest ecosystems in Bavaria, representing a valuable native European noble hardwood species. Ash is less competitive compared to other broadleaves (e.g. beech) and can become a domi-

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nant species on sites connected to high groundwater levels and/or a high soil base saturation (KÖLLING and WALENTOWSKI, 2002). In contrast to beech, ash has less dense crowns which allows other shade-tolerant species to form a more structured forest. Ash is considered a valuable tree species under climate change, because it can tolerate warm and dry conditions (KÖLLING, 2007). Currently there are 155 certified seed stands for European common ash in Bavaria from which seeds were harvested regularly until the outbreak of ash dieback a few years ago, comparable to the dutch elm disease. Forestry now runs the risk of losing this economically and ecologically valuable tree species for mixed forests due to the increasingly severe ash dieback.

European ash (*Fraxinus excelsior* L.) is threatened by ash dieback, a disease caused by the fungus *Hymenoscyphus fraxineus* (BARAL et al., 2014), occurring first in Poland and meanwhile in most parts of Europe (PUKACKI and PRZYBYL, 2005; BAKYS et al., 2009a; WOODWARD and BOA, 2012; VASAITIS et al., 2013). The origin of the fungus was unknown until recently when it was proved to originate from East Asia, where it is a saprophytic fungus and plays a similar ecological role on *F. manshurica* as *H. albidus* on *F. excelsior* (VASAITIS et al., 2013; ZHAO et al., 2012). As a consequence of the increasing damage the research dealing with ash has also increased. The causative agent was identified in 2009 (KOWALSKI and HOLDENRIEDER, 2009; BAKYS et al., 2009b; QUELOZ et al., 2011) and methods to describe the disease stages in ash were developed later on (MCKINNEY et al., 2011a; LENZ et al., 2012a; METZLER et al., 2013). LENZ et al. (2012a) described the status and the distribution of the disease for Bavaria. Due to its successful dispersal and infectious strategy, *H. fraxineus* was able to rapidly establish itself on various sites. In 2009 on permanent study plots of the Bavarian State Institute of Forestry (LWF) only 9% of pole stage and 1% of mature forests were dead. In 2011 20% dead trees in pole stage forests and 2% of dead trees in mature forests were recorded (LENZ et al., 2012a).

After observing that some individuals were less affected by the disease than others systematic investigations on the resistance against the disease were started (MCKINNEY et al., 2011a; PLIURA et al., 2011; KJÆR et al., 2011; STENER, 2013). Such investigations include the question whether the lower susceptibility of certain individuals might be in relation to genetics. Several studies in ash exist using molecular markers on genetic structure (e.g. HÖLTKEN et al., 2003; MORAND et al., 2002; HEUERTZ et al., 2001, 2003, 2004a, 2004b, 2006; HEBEL et al., 2006a, 2006b; FERRAZZINI et al., 2007; BACLES and ENNOS, 2008; SUTHERLAND et al., 2010; GÖMÖRY et al., 2012), others studies are dealing with gene flow and hybridization (e.g. FERNÁNDEZ-MANJARRÉS et al., 2006; THOMASSET et al., 2013, 2014; GÉRARD et al., 2013). In Southern Germany one study on genetic variation of European ash focus on provenance regions (HEBEL et al., 2006b). The results generally revealed a high genetic variation within the analysed populations.

In Bavaria detailed information on genetic diversity of ash populations based on molecular markers have been lacking until now. The present paper assess the genetic

diversity within and between ash populations from Bavaria (South Germany) in comparison to ash populations from other European countries with special emphasis on certified seed stands. Another aim of this study is to evaluate genetic differences between susceptible and less susceptible trees within populations, an aspect which had been completely overlooked. Due to the lack of resistance related markers for ash, selectively neutral ones (nuclear and chloroplast microsatellites) were used in the present study, as for other similar studies in the past (e.g. HÖLTKEN et al., 2003; HEUERTZ et al., 2004a, 2004b; HEBEL et al., 2006a, 2006b; FERRAZZINI et al., 2007; BACLES and ENNOS, 2008; GÖMÖRY et al., 2012).

Material and Methods

Plant material

The investigated ash populations comprise fourteen registered seed stands from Germany (German provenance regions 02, 04, 06, 07, 08), Austria and Slovenia, seventeen provenances from Germany, Switzerland and Romania in the provenance trial “Marxheim” (Bavaria) established in 1988 (Kleinschmit et al. 2002) and ten permanent study plots installed in 2010 by the Bavarian State Institute of Forestry (LWF) (Table 1). From the German and Austrian seed stands mixed seed samples were available and 96 seeds per provenance were analysed. From other populations, buds or cambium from individual trees were collected. The number of sampled trees is given in Table 2. Altogether the number of analyzed samples per population ranged from 20–100 for nuclear microsatellites (Table 2) and from 13–97 for chloroplast microsatellites (Table 3).

DNA isolation

DNA was extracted for all samples using the ATMAP method (DUMOLIN et al., 1995). Before extraction, embryos from seeds, buds (1 bud per tree) and cambium (tissue of 1 cm diameter) were lyophilized for 24 hours. DNA extracts were fluorometrically quantified (Gene Quant Pro, Amersham Bioscience) and adjusted to 20 ng/μl.

PCR amplification

All populations were analysed at nuclear and chloroplast microsatellites using the Qiagen-multiplex-Kit for PCR.

nSSR amplification

Altogether 2358 individuals were genotyped at eight highly polymorphic nuclear microsatellite loci (LEFORT et al., 1999; BRACHET et al., 1999) combined into three multiplex PCRs (fluorescent labels of primers in brackets).

multiplex A: Femsatl10 (Cy5) + M2-30 (Dy751), concentration in primermix: 2 μM each

multiplex B: Femsatl11 (Cy5) + Femsatl12bis (Dy751) + Femsatl19 (IRD700), concentration in primermix: 2 μM each

multiplex C: Femsatl4 (Cy5) + Femsatl8 (Dy751) + Femsatl16 (IRD700), concentration in primermix: 1 μM + 2 μM + 2 μM, respectively.

Multiplex PCRs were performed using fluorescent labelled primers (concentrations of primers are given above) in a mixture of 10 µl total volume containing 1 X reaction buffer (Qiagen) and about 20 ng template DNA. The PCR program started with initial denaturation at 95°C for 15 min, followed by 25 cycles of 94°C for 30 sec, 55°C for 90 sec, 72°C for 30 sec and a final elongation step at 60°C for 30 min. The length of the PCR fragments was determined by using an automated sequencer (CEQ8000 Beckman-Coulter) and analysed by using an internal size standard. Fragment length determination and allele assignment were carried out using the fragment analysis tool of CEQ8000 (Beckman-Coulter).

cpSSR amplification

Altogether 2146 individuals were genotyped at five chloroplast microsatellite loci (WEISING and GARDNER, 1999) combined into one multiplex PCR.

multiplex D: ccmp2 (Cy5) + ccmp4 (Cy5) + ccmp6 (IRD700) + ccmp7 (Cy5) + ccmp10 (Cy5), concentration in primermix: 2 µM + 1 µM + 2 µM + 8 µM + 2 µM, respectively.

The multiplex PCR was performed using fluorescent labelled primers (concentrations of primers are given above) in a mixture of 10 µl total volume containing 1 X reaction buffer (Qiagen) and about 20 ng template DNA. The PCR program started with initial denaturation at 95°C for 15 min, followed by 25 cycles of 94°C for 30 sec, 57°C for 90 sec, 72°C for 30 sec and a final elongation step at 60°C for 30 min. Fragment analysis was performed as for nuclear microsatellites.

Data analysis

Data analysis was performed separately for the three types of investigated populations (seed stands, provenance trial "Marxheim", permanent study plots (LWF)), because of different establishment histories of the populations (e.g. type of material used for the provenance trial).

nSSR

For nuclear microsatellites GenAlEx 6.41 (PEAKALL and SMOUSE, 2006) was used to calculate the genetic parameters for each population: N_A for the mean number of alleles, N_R for the mean number of rare alleles per population with frequencies below 5%, N_p for the mean number of private alleles occurring only in one of the populations, genetic diversity N_E for the effective number of alleles (BROWN and WEIR, 1983), observed heterozygosity H_O (HARTL and CLARK, 1997) and expected heterozygosity H_E (HARTL and CLARK, 1997). In order to compare the number of alleles independent of sample size, allelic richness (A) was calculated with rarefaction using the software FSTAT (GOUDET, 1995), thereby the calculation is based on the smallest number of individuals typed in a population and given as the mean over all loci.

The software GenAlEx 6.41 (PEAKALL and SMOUSE, 2006) was also used to estimate the pairwise genetic distance between all provenances following NEI (1972). The

software FSTAT (GOUDET, 1995) was used to calculate overall F_{IS} and genetic differentiation by overall and pairwise F_{ST} values (Weir and Cockerham, 1984). Additionally a Bayesian approach (STRUCTURE, FALUSH et al., 2003) was used to identify structuring for the fourteen seed harvesting stands and the seventeen provenances of the provenance trial. The analysis parameters were set to 20.000 runs in the burn-in period, then 20.000 iterations, under the admixture model. K was set to 1–15 for the seed harvesting stand analysis and to 1–20 for the provenance trial analysis with 10 iterations per K for both. The highest "delta K" corresponds to the uppermost level of hierarchy among runs with K = 1–15 and K = 1–20 based on the rate of change between successive K values (EVANNO et al., 2005). The means of the probabilities (Ln(K)) of the genetic data over the 10 replicated runs and their standard deviations plotted against the number of estimated clusters were used in order to choose the most likely number of clusters. Thereby the K-value with the highest mean value together with the lowest standard deviation was selected.

cpSSR

For chloroplast microsatellites, again GenAlEx 6.41 (PEAKALL and SMOUSE, 2006) was used to calculate genetic parameters. Based on haplotypes defined for each sample the following parameters of genetic variation were calculated: N_A for the mean number of variants, genetic diversity N_E for the number of effective alleles (BROWN and WEIR, 1983) and the haploid genetic diversity h for the probability that two individuals are different (SCHNEIDER et al., 1997).

Genetic differentiation between populations was again calculated with GenAlEx 6.1 (PEAKALL and SMOUSE, 2006) using Nei's genetic distance (NEI, 1972) and visualized for the seed stands by constructing an unrooted Neighbor-Joining tree using PHYLIP (FELSENSTEIN, 2004).

Comparison of susceptible versus less susceptible trees

A comparison of susceptible versus less susceptible trees in relation to *H. fraxineus* attack was performed within the provenance trial and the permanent study plots. The goal was to see if the used neutral markers were able to detect genetic differences between the damaged and the undamaged subpopulation within a provenance/plot, but also to verify if the surviving individuals are genetically constricted or not. This is important for future breeding strategies.

Damage was recorded for the sampled trees following the classification scale elaborated by LENZ et al. (2012b). Each tree was classified into one of six classes starting from class 0 for trees with no symptoms to class 5 with 100% damage. Classes 0–1 (trees with no symptoms or with little damage and up to 30% lack of crown foliage) were then combined into one group called "less susceptible". Classes 2–5 were combined into another group called "susceptible". In a first step genetic parameters were compared between all trees per population and the less susceptible trees. The rationale behind this was to estimate/predict changes in genetic parameters of future

ash populations. These calculations should give a picture of populations affected by ash shoot disease before and after the selection of the trees through the disease. The results should give an impression of the genetic parameters of the remaining populations after the susceptible trees have died and are not available for future reproduction. In a second step susceptible trees were compared to less susceptible ones for each population in order to detect the genetic difference between the two collectives.

The software GenAlEx 6.41 (PEAKALL and SMOUSE, 2006) was used to calculate N_A for the mean number of alleles, genetic diversity N_E for the effective number of alleles (BROWN and WEIR, 1983) and observed (H_O) and expected (H_E) heterozygosity (HARTL and CLARK, 1997) for each group. Additionally allelic richness (A , values given after rarefaction) and heterozygote deficit (F_{IS}) was calculated in FSTAT (GOUDET, 1995). Relatedness of individuals for both groups (susceptible vs. less susceptible) within populations was calculated according to QUELLER and GOODNIGHT (1989) in GenAlEx 6.41 (PEAKALL and SMOUSE, 2006). Because some studies found high heritabilities of resistance against ash shoot disease (e.g. STENER et al., 2013), we wanted to find out, if relatedness between less susceptible trees within populations was higher compared to the relatedness between susceptible trees. The mean of the pairwise relatedness values for each group is given. For each value the differences between susceptible and less susceptible groups were tested for significance using a Mann-Whitney Test in R (R CORE TEAM, 2013).

Genetic differentiation between groups within populations was quantified using pairwise Nei's genetic distances (NEI, 1972) in GenAlEx 6.41 (PEAKALL and SMOUSE, 2006) and pairwise F_{ST} -values (tested for significance) in FSTAT (GOUDET, 1995).

Finally all susceptible versus all less susceptible individuals were pooled into two groups and differences in genetic diversity parameters (allelic richness A , observed heterozygosity H_O , genetic diversity H_S , heterozygote deficit within (F_{IS}) and among (F_{ST}) populations) including tests of significance were calculated in FSTAT (GOUDET, 1995).

Results

Genetic diversity

Results based on nSSR-markers

Altogether 338 alleles were detected at eight nuclear microsatellite loci. The lowest variation was shown by the locus Femsat16 with 15 alleles, whereas locus Femsat10 exhibited the highest variation with 73 alleles. Distribution of alleles within populations varied considerably with often 2-3 highly frequent alleles (>20%–40%). Within seed stands Slovenian populations (SLO-Brezje, SLO-Konjiska gora) and one Austrian population (AUT-Karnerviertel) had low numbers of rare alleles (72, 48 and 80 respectively), whereas other populations (e.g. D-Osthofstein_02, D-Plantage_04, D-Hochstift_04, and AUT-Ettenau) carry between 137 and 146 rare alleles (data not shown).

Because of different establishment histories of the three types of investigated populations the results were compared separately for seed stands, the provenance trial "Marxheim" and the permanent study plots (LWF) presented in *Table 2*. For seed stands, the mean number of alleles per locus (N_A) varied between 11.4 (SLO-Konjiska gora) and 24.1 (AUT-Ettenau). Similar large differences between populations were found for the mean values of rare alleles (N_R) varying from 6.1 (SLO-Konjiska gora) to 18.3 (AUT-Ettenau). For 12 out of 14 stands unique alleles (N_P) were detected which are missing in all other populations. The highest values for such private alleles were found in D-Kempton_07 (mean of 0.8). Genetic diversity (N_E) varied between 5.4 (AUT-Karnerviertel) and 10.9 (AUT-Ettenau), allelic richness (A) was between 9.5 (AUT-Karnerviertel) and 14.1 (AUT-Ettenau). Values for the observed heterozygosity (H_O) was found in the medium range (0.694–0.768), values for expected heterozygosity (H_E) were slightly higher (0.750–0.856).

The number of alleles (N_A) of the populations within the provenance trial varied between 11.5 (D-Lichtenfels) and 16.8 (D-Scheßlitz). Both the differences and the values are thereby slightly lower compared to the seed harvesting stands. A similar trend was found for the number of rare alleles (N_R). Private alleles (N_P) were found in eight out of seventeen analysed populations, pointing to the lower sample number compared to the seed stands and to the fact that for a provenance trial only a fraction of the natural population is present. Noteworthy is the high proportion of private alleles (N_P) in the Romanian provenance "RO-Botosani" (mean of 0.8) of only 28 analysed individuals. This refers to a considerable difference of the eastern-European provenance compared to the Central European ones. The genetic diversity (N_E) ranged between 5.4 (D-Kelheim) and 7.9 (D-Scheßlitz). Thereby the provenances exhibit relatively consistent values without pronounced extremes. The allelic richness (A) ranged from 9.1 (D-Lichtenfels, D-Kelheim) to 12.9 (D-Scheßlitz). The observed heterozygosity (H_O) values were found in the medium range (0.687–0.806), whereas the values for the expected heterozygosity (H_E) were slightly higher, but less variable (0.771–0.844).

Within the ten permanent study plots the number of alleles (N_A) ranged from 6.3 (D-Freising-I) to 22.3 (D-Freising-II). The differences between certain populations were high and partly dependent on the different sample numbers. Remarkably low values were found for the stand "D-Freising-I". Only one stand (D-Coburg) reached the number of alleles (N_A) of the seed stands. On seven out of ten plots we found private alleles (N_P). Genetic diversity (N_E) varied between 3.8 (D-Freising-I) and 9.0 (D-Freising-III). Allelic richness (A) ranged from 5.4 (D-Freising-I) to 14.6 (D-Töging-I). The observed heterozygosity (H_O) was again in the medium range (0.644–0.796). Values for the expected heterozygosity (H_E) varied between 0.657 (D-Freising-I) and 0.853 (D-Freising-III).

Over all populations no regional patterns regarding the magnitude of the genetic variation were found.

Table 1. – Description of 41 populations of European ash (*Fraxinus excelsior*) including the type of plant material sampled. Numbers following the population names of the seed stands correspond to the provenance region for common ash in Germany.

Population	Country	Type of investigated populations	Plant material
D-Adelsheim_07	Germany	Seed stands	Mixed seeds
D-Heuweg_06	Germany		Mixed seeds
D-Kempten_07	Germany		Mixed seeds
D-Kempten_08	Germany		Mixed seeds
D-Landsberg_07	Germany		Mixed seeds
D-Traunstein_08	Germany		Mixed seeds
D-Weißenhorn_07	Germany		Mixed seeds
D-Ostholstein_02	Germany		Mixed seeds
D-Plantage_04	Germany		Mixed seeds
D-Hochstift_04	Germany		Mixed seeds
AUT-Ettenau	Austria		Mixed seeds
AUT-Karnerviertel	Austria		Mixed seeds
SLO-Brezje	Slovenia		Buds
SLO-Konjska gora	Slovenia		Buds
D-Wertheim	Germany	Provenance trial “Marxheim”	Buds and cambium
D-Dillingen	Germany		
D-Feuchtwangen	Germany		
D-Kelheim	Germany		
D-Lichtenfels	Germany		
D-Mellrichstadt	Germany		
D-Scheßlitz	Germany		
D-Treuchtlingen	Germany		
D-Weißenhorn	Germany		
D-Sinntal	Germany		
D-Bovenden	Germany		
D-Walkenried	Germany		
D-Wienhausen	Germany		
D-Lehnsahn	Germany		
CH-Aargau-Möhlhlin	Switzerland	Permanent study plots (LWF)	Buds and cambium
CH-Aargau-Muri	Switzerland		
RO-Botosani	Romania		
D-Coburg	Germany		
D-Freising-I			
D-Freising-II			
D-Freising-III			
D-Röthelbach			
D-Schweinfurt-I			
D-Schweinfurt-II			
D-Töging-I			
D-Töging-II			
D-Töging-III			

Results based on cpSSR markers (haplotypic diversity)

The average number of alleles within populations (N_A) ranged from 1.0 (many populations) to 2.0 (D-Freising-II), with the mean of 1.2 (*Table 3*). Genetic diversity (N_E) has comparable values between 1.0 (several populations) and 1.7 (D-Töging-I). Moreover, the values for haploid genetic diversity (h) varied between 0 and 0.321 (D-Töging-I), with a mean of 0.054.

A clear relationship between certain regions and diversity parameters was not detected. Sixteen populations over all three types of investigated populations were monomorphic for the analysed chloroplast microsatellite loci. Few populations stood out with high

diversity values (e.g. haploid diversity), among them D-Töging-I and II, D-Freising-II, D-Scheßlitz and one Austrian population (AUT-Ettenau).

Genetic differentiation

Based on nSSR markers

For the seed stands the genetic distance (NEI , 1972) varied between 0.10 (D-Traunstein_08 to AUT-Ettenau) and 0.35 (D-Hochstift_04 to AUT-Karnerviertel). Within the provenance trial the genetic distance values between provenances were higher with values between 0.18 (CH-Aargau-Muri to D-Dillingen) and 0.71 (D-Sinntal to D-Kelheim). For permanent study plots, values between

0.14 (D-Töging-I to D-Töging-II) and 0.63 (D-Röthelbach to D-Freising-I) were calculated (data not shown).

Overall F_{IS} -values were 0.108 for all 41 populations and 0.112 for the German populations. Differentiation (overall F_{ST}) between all 41 populations was similar to the differentiation between all 34 German populations ($F_{ST}=0.046$, $p<0.01$ and $F_{ST}=0.045$, $p<0.001$, respectively). Pairwise F_{ST} values ranged from 0,005 (D-Töging-I to D-Schweinfurt-I) to 0,145 (D-Freising-I to

D-Röthelbach) for the permanent study plots. In the provenance trial the F_{ST} -values varied between 0,025 (D-Scheßlitz to D-Treuchtlingen) and 0,117 (D-Sinntal to D-Kelheim). The ash seed stands showed values from 0,008 (AUT-Ettenau to SLO-Brezje) to 0,072 (AUT-Karnerviertel to SLO-Konjiska gora) (data not shown).

Bayesian statistics revealed that for the seed harvesting stands the highest peak for delta K was found at $K=2$, delineating only one population from Southern

Table 2. – Genetic parameters for 41 populations of *Fraxinus excelsior* based on eight nuclear microsatellite loci.

Population	N	N_A	N_R	N_P	N_E	A	H_O	H_E
D-Adelsheim_07	96	21.1	15.5	0.0	8.7	12.4	0.727	0.835
D-Hcuweg_06	96	20.6	14.8	0.1	9.0	12.5	0.716	0.827
D-Kempten_07	95	20.6	14.5	0.8	8.3	11.9	0.696	0.818
D-Kempten_08	93	19.0	12.5	0.1	8.4	12.0	0.723	0.804
D-Landsberg_07	96	22.4	16.4	0.3	9.4	12.6	0.748	0.840
D-Traunstein_08	95	20.4	14.4	0.3	8.4	12.0	0.736	0.835
D-Weißenhorn_07	96	20.8	15.1	0.1	8.0	12.1	0.732	0.831
D-Ostholstein_02	96	23.6	17.5	0.3	10.7	13.9	0.715	0.856
D-Plantage_04	96	22.4	17.1	0.3	8.2	12.5	0.695	0.843
D-Hochstift_04	96	22.9	17.1	0.1	8.6	12.7	0.765	0.842
AUT-Ettenau	96	24.1	18.3	0.4	10.9	14.1	0.768	0.850
AUT-Karnerviertel	94	15.3	10.0	0.0	5.4	9.5	0.694	0.750
SLO-Brezje	32	15.8	9.6	0.1	8.7	13.1	0.750	0.838
SLO-Konjiska gora	31	11.4	6.1	0.1	6.3	9.6	0.743	0.769
D-Wertheim	38	13.5	7.3	0.1	6.4	10.2	0.687	0.784
D-Dillingen	37	14.5	7.8	0.0	7.5	11.4	0.690	0.778
D-Feuchtwangen	33	13.0	7.6	0.3	6.5	10.6	0.744	0.803
D-Kelheim	44	11.9	6.4	0.1	5.4	9.1	0.725	0.774
D-Lichtenfels	40	11.5	5.4	0.0	5.9	9.1	0.726	0.787
D-Mellrichstadt	31	12.6	6.9	0.0	7.5	10.5	0.806	0.844
D-Scheßlitz	36	16.8	11.0	0.1	7.9	12.9	0.703	0.816
D-Treuchtlingen	38	13.9	7.5	0.0	7.5	10.8	0.746	0.830
D-Weißenhorn	42	12.5	7.1	0.0	5.8	9.7	0.763	0.810
D-Sinntal	35	13.8	8.9	0.0	5.8	10.6	0.715	0.771
D-Bovenden	41	15.4	10.0	0.1	6.2	11.0	0.742	0.783
D-Walkenried	39	13.1	7.5	0.0	5.9	9.8	0.771	0.798
D-Wienhausen	39	11.8	5.8	0.0	6.3	9.5	0.746	0.818
D-Lehnsahn	39	13.1	6.5	0.0	6.6	10.3	0.703	0.814
CH-Aargau-Möhlin	40	13.9	7.1	0.1	7.3	10.7	0.757	0.802
CH-Aargau-Muri	43	15.3	10.0	0.1	7.4	11.5	0.772	0.782
RO-Botosani	28	12.9	6.8	0.8	6.9	11.0	0.702	0.795
D-Coburg	99	21.5	18.1	0.4	4.6	10.5	0.737	0.721
D-Freising-I	37	6.3	2.0	0.0	3.8	5.4	0.796	0.657
D-Freising-II	100	22.3	16.9	0.5	7.6	12.1	0.728	0.807
D-Freising-III	76	20.4	14.5	0.3	9.0	12.7	0.724	0.853
D-Röthelbach	51	13.5	8.3	0.1	5.4	9.7	0.701	0.789
D-Schweinfurt-I	24	15.1	10.4	0.3	7.8	13.6	0.719	0.832
D-Schweinfurt-II	31	14.5	8.1	0.0	7.6	12.2	0.644	0.797
D-Töging-I	20	15.3	7.3	0.1	8.8	14.6	0.790	0.846
D-Töging-II	49	18.3	12.8	0.1	7.5	12.3	0.735	0.813
D-Töging-III	20	12.3	4.0	0.0	7.8	11.9	0.783	0.819
Mean	57.5	16.3	10.5	6.4	7.4	14.6	0.733	0.806

N, number of individuals; N_A , mean number of alleles; N_R , rare alleles (frequency <5%); N_P , private alleles; N_E , genetic diversity as the number of effective alleles; (A), allelic richness after rarefaction (rarefaction size = 18); H_O , observed heterozygosity; H_E , expected heterozygosity.

Table 3. – Genetic parameters for 41 European ash populations based on five chloroplast microsatellite loci.

Population	N	N _A	N _E	h
D-Adelsheim_07	82	1.2	1.0	0.035
D-Heuweg_07	93	1.6	1.2	0.138
D-Kempten_07	96	1.0	1.0	0.000
D-Kempten_08	85	1.2	1.0	0.005
D-Landsberg_07	94	1.2	1.0	0.012
D-Traunstein_08	92	1.0	1.0	0.000
D-Weißenhorn_07	87	1.0	1.0	0.000
D-Ostholstein_02	96	1.4	1.1	0.060
D-Plantage_04	92	1.0	1.0	0.000
D-Hochstift_04	95	1.0	1.0	0.000
AUT-Ettenau	43	1.6	1.6	0.287
AUT-Karnerviertel	13	1.0	1.0	0.000
SLO-Brezje	30	1.2	1.0	0.013
SLO-Konjiska gora	29	1.0	1.0	0.000
D-Wertheim	37	1.0	1.0	0.000
D-Dillingen	35	1.0	1.0	0.000
D-Feuchtwangen	31	1.0	1.0	0.000
D-Kelheim	43	1.6	1.1	0.053
D-Lichtenfels	37	1.2	1.0	0.020
D-Mellrichstadt	29	1.2	1.0	0.037
D-Scheßlitz	36	1.8	1.4	0.235
D-Treuchtlingen	38	1.2	1.0	0.011
D-Weißenhorn	41	1.6	1.0	0.030
D-Sinntal	35	1.0	1.0	0.000
D-Bovenden	42	1.2	1.0	0.009
D-Walkenried	34	1.2	1.0	0.034
D-Wienhausen	39	1.0	1.0	0.000
D-Lehnsahn	38	1.2	1.0	0.029
CH-Aargau-Möhl	40	1.0	1.0	0.000
CH-Aargau-Muri	37	1.2	1.0	0.011
RO-Botosani	28	1.0	1.0	0.000
D-Coburg	97	1.2	1.0	0.020
D-Freising-I	38	1.0	1.0	0.000
D-Freising-II	88	2.0	1.4	0.229
D-Freising-III	78	1.2	1.2	0.099
D-Röthelbach	52	1.0	1.0	0.000
D-Schweinfurt-I	25	1.4	1.0	0.033
D-Schweinfurt-II	33	1.4	1.4	0.194
D-Töging-I	19	1.8	1.7	0.321
D-Töging-II	49	1.8	1.4	0.238
D-Töging-III	20	1.2	1.1	0.064
Mean	52.3	1.2	1.1	0.054

N, number of individuals; N_A, mean number of alleles; N_E, genetic diversity as the number of effective alleles; h, haploid genetic diversity.

Austria (AUT-Karnerviertel) from the rest. Therefore for further interpretations the second most likely number of clusters (K=13) (*Figure 1a*) was considered. For the provenance trial the optimal number of genetic clusters was found to be 17 (*Figure 1b*). Also the means of the probabilities of the genetic data supported the selection of the mentioned clusters, because they were highest for

the chosen K-values and had low standard deviation (*Fig. 1c* and *1d*).

Subsequently clusters at the population level were visualised for the seed stands and provenance trial separately (*Figure 2* and *3*, respectively). Populations showed strong differences in proportions of each cluster referring to differences between populations. Notewor-

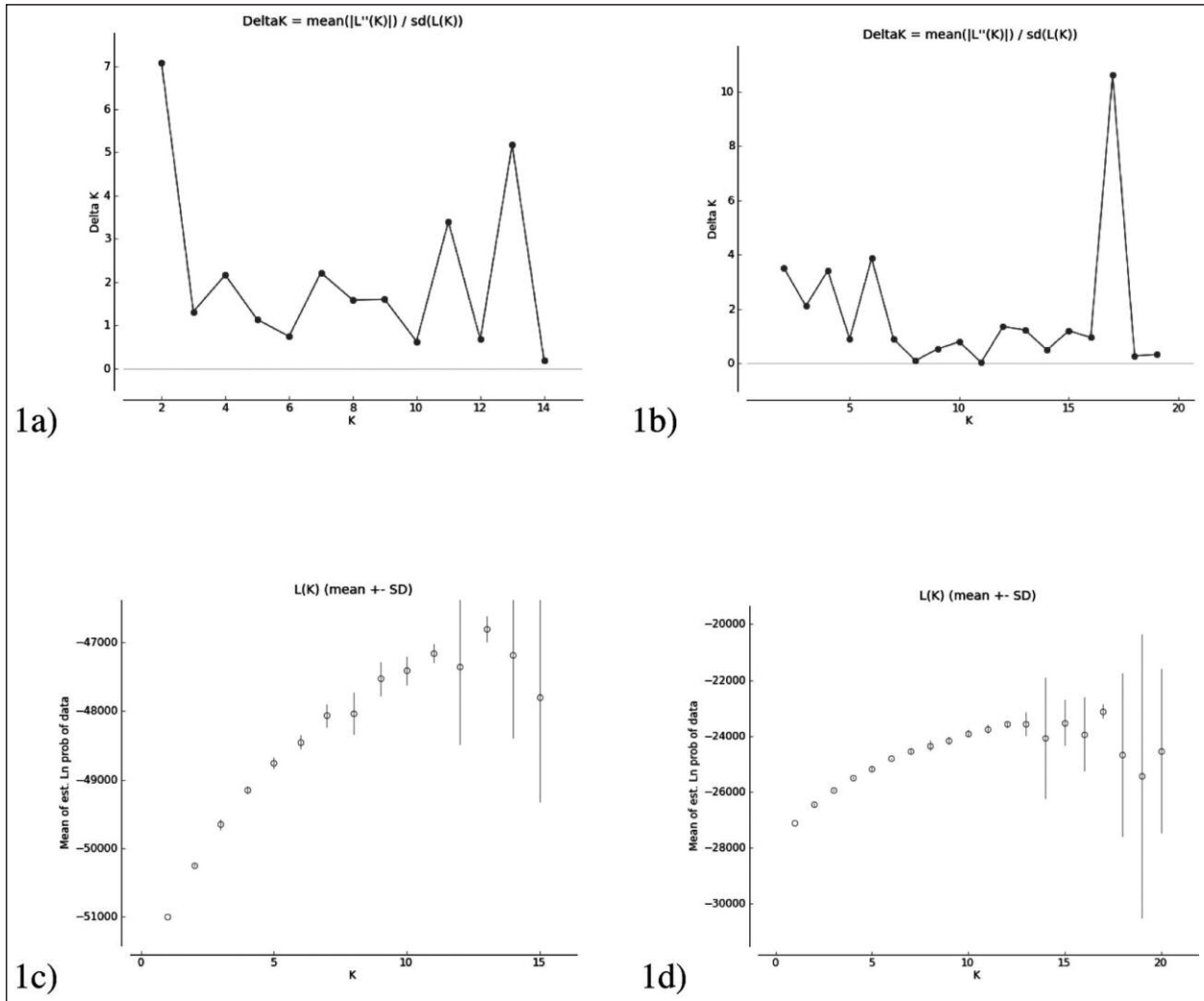


Figure 1. – Values for delta K 1a) for the seed harvesting stands and b) the provenance trial; means of the probabilities of the genetic data over the 10 replicated runs and their standard deviations plotted against the number of estimated clusters K for the analyses of 1c) 14 ash seed harvesting stands and 1d) 17 provenances of the provenance trial "Marxheim" based on nuclear microsatellite data.

thy, in most of the cases each population had a specific high proportion cluster differing between populations.

Based on cpSSR

Because of the more conservative behaviour of the chloroplast markers, they are more likely to be suited for detecting large-scale regional patterns. As an example in Figure 4 the relationship of populations was visualized for the seed harvesting stands in a dendrogram. One group of populations was clearly distinguishable in the western part of Bavaria comprising the populations D-Kempton_07, D-Kempton_08, D-Weißenhorn_07 and D-Landsberg_07. More eastern distributed populations with D-Heuweg_06, D-Traunstein_08 and AUT-Ettenau formed a second group, which carried the allele "98" at locus "cemp10". More southerly distributed populations from Austria and Slovenia (AUT-Karnerviertel, SLO-Brezje and SLO-Konjska gora) were again distinguished from the mentioned groups, but all other Ger-

man populations resided within this group as well. Thus based on the analysed chloroplast markers, differentiation was detected for populations from Southwest Germany, but clear differences between populations from north and south of the Alps were not found.

Genetic variation of susceptible versus less susceptible tree collectives

These calculations were performed for populations of the provenance trial and the permanent study plots.

In a first step a comparison of the genetic variation between all trees and the less susceptible trees within populations was made (for sample numbers of each group see Table 2 and 4, respectively).

The mean number of alleles (N_A) ranged from 6.3 (D-Freising-I) to 22.3 (D-Freising-II) considering all trees per population (Table 2) and from 4.6 (D-Freising-I) to 19.4 (D-Coburg) in the group of less susceptible trees

(data not shown). The values were lower in all populations in the group of less susceptible trees (Mann-Whitney-test; $p < 0.01$).

Genetic diversity (N_E) ranged from 3.8 (D-Freising-I) to 9.0 (D-Freising-III) in all trees per population and from 3.4 (D-Freising-I) to 7.6 (D-Schweinfurt-I) in the

group of less susceptible trees (Figure 5) and decreased in 22 out of 26 populations (data not shown) (Mann-Whitney-test; $p < 0.01$).

The calculation of allelic richness was based on 5 samples and ranged from 3.9 to 6.7 considering the whole populations and from 3.9 to 6.3 in the fraction of less

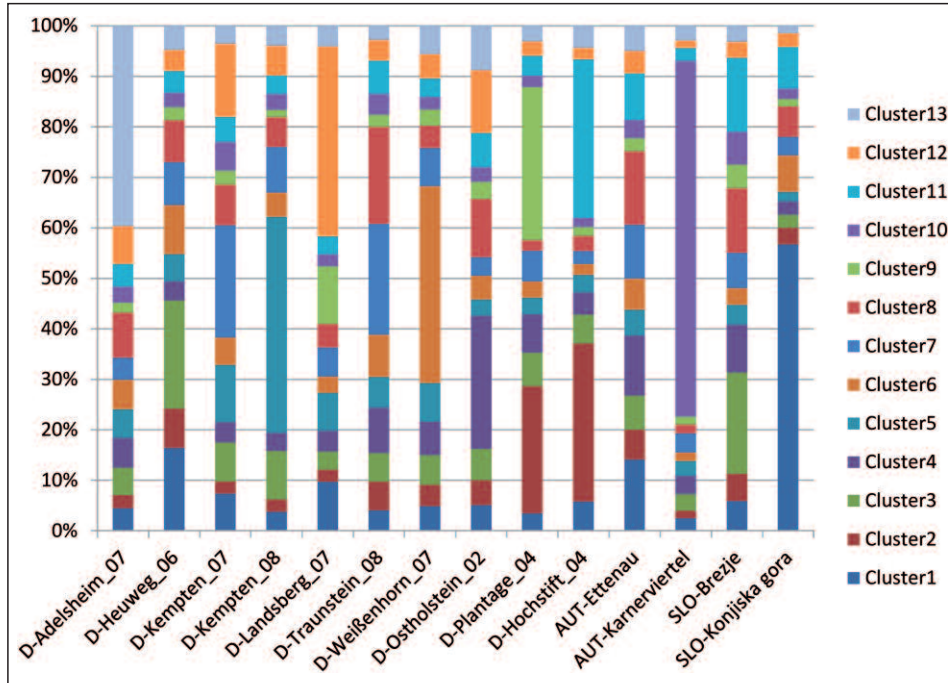


Figure 2. – Frequency diagram of clusters of 14 ash seed harvesting stands based on nuclear microsatellite data; bars representing the 14 populations and colors representing 13 clusters.

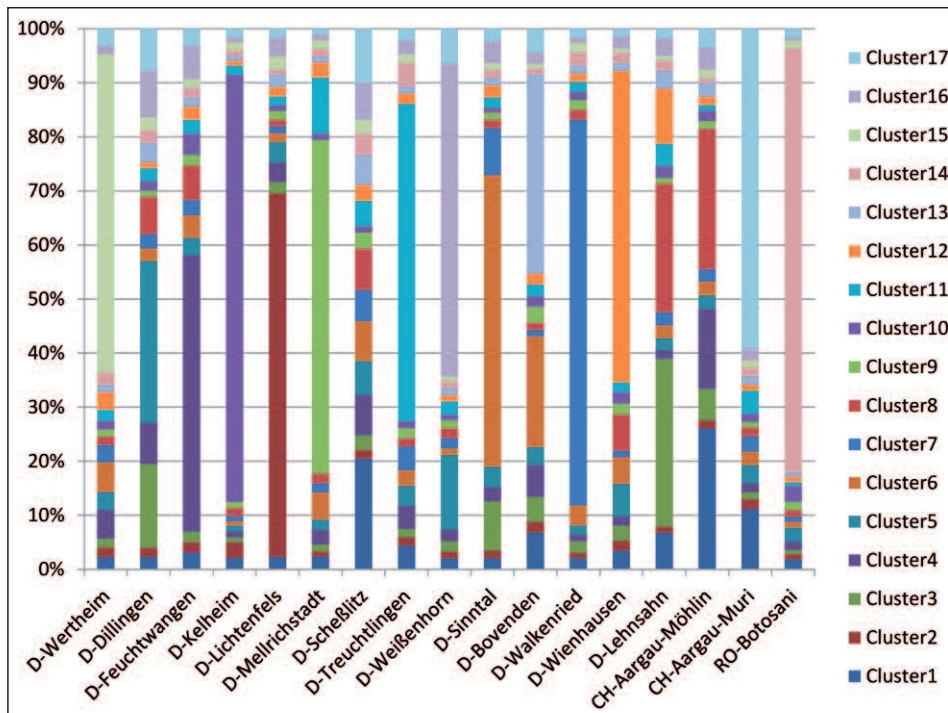


Figure 3. – Frequency diagram of clusters of 17 provenances of the provenance trial "Marxheim" based on nuclear microsatellite data; bars representing the 17 provenances and colors representing 17 clusters.

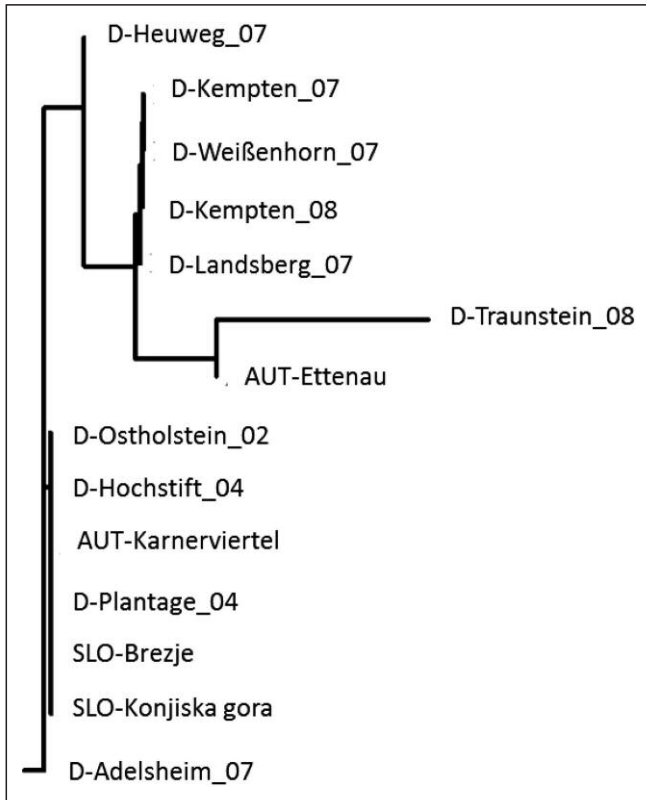


Figure 4. – Clustering of seed harvesting stands (Neighbor-Joining tree) based on the genetic distance following Nei (1972) using chloroplast microsatellites.

susceptible trees. The differences between all trees and less susceptible trees per population were not significant (Mann-Whitney-test, $p=0.6$).

When comparing observed heterozygosity (H_o) between all trees per population (H_o total) and the less susceptible trees per population (H_o less susceptible) the values increased slightly in two thirds of the prove-

nances (Figure 6). The results were not significant (Mann-Whitney-test, $p=0.8$) but a trend was visible.

In a second step genetic differences between susceptible and less susceptible individuals were evaluated by calculating several genetic diversity parameters (Table 4). Population "D-Schweinfurt-I" was omitted in this step, because all observed individuals were classified as "less susceptible", as ash dieback was not detected in the year of assessment.

Genetic diversity ranged from 3.66 (D-Freising-I) to 9.04 (D-Freising-III) for the susceptible groups and from 3.41 (D-Freising-I) to 7.68 (D-Töging-II) for the less susceptible group. Thirteen populations showed higher values for genetic diversity in the less susceptible compared to the susceptible groups and for the other 13 populations the situation was the opposite.

Allelic richness varied between 3.95 (D-Freising-I) and 5.91 (D-Töging-I) for the susceptible groups and between 3.63 (D-Freising-I) and 5.5 (D-Freising-II) for the less susceptible group. In 15 populations allelic richness was higher in the less susceptible groups, in 11 populations the group of susceptible trees showed higher values for allelic richness.

Observed heterozygosity ranged from 0.643 (D-Scheßlitz) to 0.810 (D-Melrichstadt) for the groups of susceptible trees and from 0.643 (D-Schweinfurt-II) to 0.847 (D-Treuchtlingen) for the less susceptible ones. Eighteen populations showed higher values for the less susceptible trees.

Expected heterozygosity varied between 0.647 (D-Freising-I) and 0.850 (D-Freising-III) in the susceptible groups and between 0.636 (D-Freising-I) and 0.830 (D-Töging-II) in the less susceptible groups. This value was higher in 11 populations and lower in 15 populations for the less susceptible compared to the susceptible groups.

F_{IS} -values ranged from -0.201 (D-Freising-I) to 0.229 (D-Scheßlitz) in the groups of susceptible trees and from -0.229 (D-Freising-I) to 0.206 (D-Schweinfurt-II)

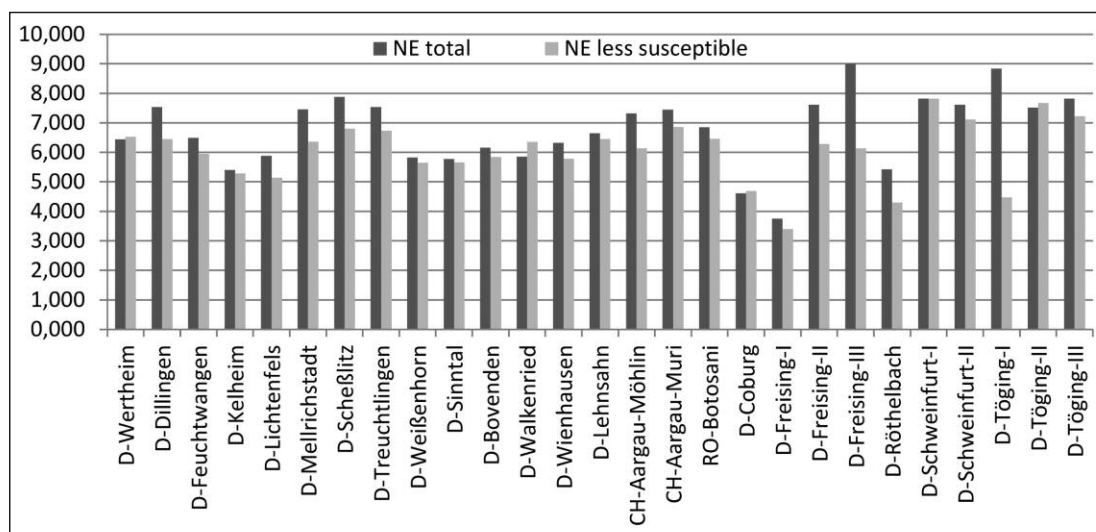


Figure 5. – Genetic diversity (N_E) for all trees per population (N_E total) compared to the less susceptible trees per population (N_E less susceptible).

in the groups of less susceptible trees. Here in 12 populations the F_{IS} -values were higher and in 14 populations lower for the groups of less susceptible trees compared

to the groups of susceptible trees. Relatedness of individuals (r) were compared for the two groups (susceptible vs. less susceptible) within populations. The values

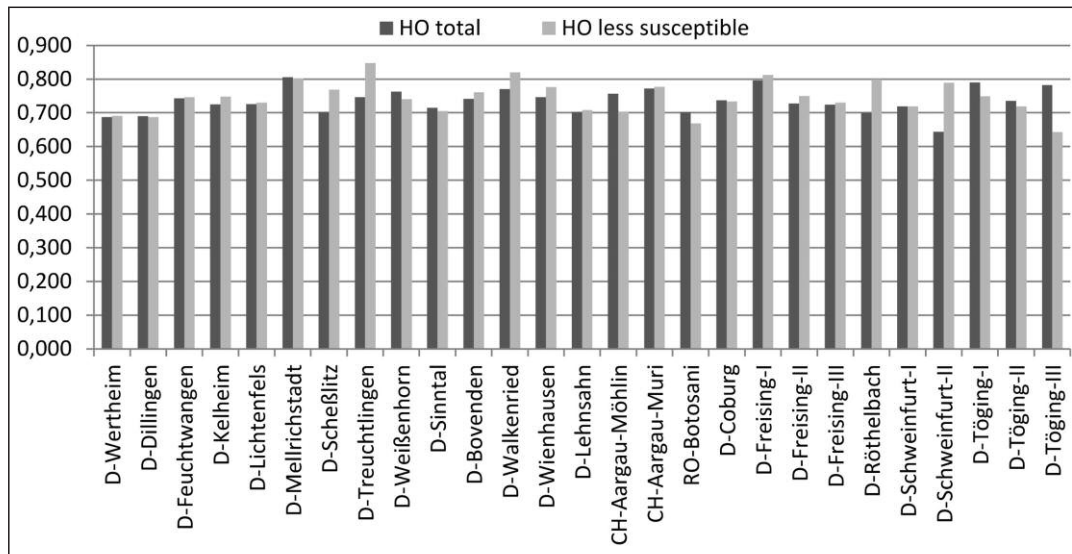


Figure 6. – Observed heterozygosity (H_0) for all trees per population (H_0 total) compared to the less susceptible trees per population (H_0 less susceptible).

Table 4. – Diversity parameters, relatedness, Nei's genetic distance and pairwise F_{ST} -values for susceptible and less susceptible trees within populations of the provenance trial and the permanent study plots based on nuclear microsatellites.

Population	n		N_E		A_4		H_0		H_E		F_{IS}		r		d	F_{ST}
	s	ls	s	ls	s	ls	s	ls	s	ls	s	ls	s	ls		
D-Wertheim	19	19	5,29	6,53	4,49	5,00	0,684	0,691	0,734	0,800	0,095	0,163	0,177	0,077	0,15	0,015ns
D-Dillingen	17	20	6,57	6,45	4,93	5,11	0,691	0,687	0,744	0,780	0,101	0,145	0,131	0,076	0,13	0,006*
D-Feuchtwangen	22	11	5,96	5,95	4,95	5,11	0,742	0,747	0,789	0,784	0,083	0,096	0,093	0,050	0,18	0,006ns
D-Kelheim	20	24	5,12	5,29	4,64	4,59	0,696	0,748	0,764	0,769	0,116	0,049	0,179	0,187	0,06	-0,008ns
D-Lichtenfels	24	16	5,93	5,14	4,84	4,58	0,724	0,730	0,793	0,753	0,109	0,063	0,093	0,146	0,09	-0,003ns
D-Mellrichstadt	14	17	6,83	6,37	5,29	5,07	0,810	0,801	0,831	0,823	0,062	0,057	0,047	0,063	0,22	0,006ns
D-Scheßlitz	19	17	7,17	6,81	5,31	5,23	0,643	0,769	0,807	0,795	0,229	0,063	0,038	0,048	0,16	0,004ns
D-Treuchtlingen	29	9	7,02	6,73	5,18	5,49	0,715	0,847	0,825	0,809	0,151	0,011	0,042	0,017	0,14	-0,012ns
D-Weißenhorn	20	22	5,38	5,65	4,90	4,87	0,788	0,741	0,805	0,796	0,047	0,092	0,107	0,094	0,11	-0,001ns
D-Sinntal	13	22	4,74	5,66	4,60	4,74	0,731	0,706	0,757	0,749	0,075	0,081	0,137	0,146	0,18	0,020ns
D-Bovenden	26	15	5,92	5,84	4,94	5,00	0,731	0,761	0,776	0,773	0,078	0,05	0,111	0,092	0,08	-0,008ns
D-Walkenried	23	16	4,99	6,35	4,49	5,13	0,737	0,820	0,772	0,810	0,068	0,02	0,150	0,082	0,11	-0,001ns
D-Wienhausen	23	16	6,01	5,79	4,88	4,96	0,726	0,777	0,811	0,805	0,128	0,068	0,070	0,071	0,11	-0,006ns
D-Lehnsahn	15	23	6,06	6,45	4,96	5,08	0,685	0,708	0,788	0,814	0,166	0,152	0,069	0,050	0,12	-0,005ns
CH-Aargau-Möhl	21	19	7,19	6,14	5,21	4,95	0,805	0,704	0,809	0,763	0,03	0,104	0,055	0,109	0,15	0,011ns
CH-Aargau-Muri	23	20	6,60	6,86	5,07	5,11	0,768	0,777	0,780	0,763	0,037	0,009	0,119	0,136	0,09	0,000ns
RO-Botosani	11	17	5,99	6,46	5,05	5,12	0,750	0,668	0,762	0,794	0,064	0,189	0,141	0,093	0,14	-0,007ns
D-Coburg	20	79	4,18	4,69	4,29	4,40	0,751	0,734	0,705	0,721	-0,037	-0,012	0,237	0,222	0,04	-0,002ns
D-Freising-I	27	10	3,66	3,41	3,59	3,63	0,789	0,813	0,647	0,636	-0,201	-0,229	0,121	0,140	0,09	0,020ns
D-Freising-II	86	8	7,32	6,29	5,15	5,50	0,722	0,750	0,800	0,803	0,103	0,132	0,044	0,056	0,18	0,002ns
D-Freising-III	61	8	9,04	6,14	5,52	5,39	0,725	0,730	0,850	0,804	0,156	0,159	0,023	0,011	0,24	0,003ns
D-Röthelbach	37	8	5,40	4,30	4,76	4,62	0,689	0,749	0,783	0,742	0,134	0,066	0,187	0,029	0,18	0,003ns
D-Schweinfurt-II	4	21	4,66	7,12	5,50	5,28	0,750	0,643	0,723	0,786	0,106	0,206	0,009	0,036	0,39	0,010ns
D-Töging-I	13	5	8,21	4,47	5,91	5,13	0,774	0,800	0,841	0,758	0,12	0,055	0,407	0,388	0,43	0,006ns
D-Töging-II	19	30	5,08	7,68	4,64	5,40	0,705	0,753	0,730	0,830	0,061	0,109	0,091	0,009	0,20	0,031**
D-Töging-III	7	13	5,82	7,22	5,34	5,40	0,768	0,789	0,789	0,794	0,107	0,047	0,012	0,021	0,33	0,009ns

n, number of individuals; N_E , genetic diversity; A_4 , allelic richness after rarefaction (rarefaction size = 4); H_0 , observed heterozygosity; H_E , expected heterozygosity; F_{IS} , fixation index; r, relatedness of individuals within groups; d, Nei's genetic distance; F_{ST} , pairwise F_{ST} -value; s, susceptible trees; ls, less susceptible trees; significance levels for pairwise F_{ST} -values: ns, non-significant; * $p < 0,05$; ** $p < 0,01$; all other tested values were non-significant and therefore not mentioned in the table.

ranged from 0.009 (D-Schweinfurt-II) to 0.407 (D-Töging-I) for the groups of susceptible trees and from 0.009 (D-Töging-II) to 0.388 (D-Töging-I). In 13 populations the values were higher and in 13 populations they were lower in the groups of less susceptible trees compared to the groups of susceptible ones. In single cases differences between susceptible and less susceptible groups were high, but no significant differences were found for any of the values.

Genetic distances (NEI, 1972) between the two groups in each population ranged from 0.06 (D-Kelheim) to 0.22 (D-Mellrichstadt) for provenances of the provenance trial. Within the four provenances D-Kelheim, D-Lichtentfels, D-Bovenden, CH-Aargau-Muri the genetic distance between groups was low. For the other provenances it was at medium level. For the permanent study plots the range of genetic distances was larger, with values between 0.04 (D-Coburg) and 0.43 (D-Töging-I). Pairwise F_{ST} -values ranged from -0.012 (D-Treuchtlingen) to 0.020 (D-Sinntal) for the provenance trial and from -0.002 (D-Coburg) to 0.031 (D-Töging-II) for the permanent study plots. In single cases differences between susceptible and less susceptible groups were high, but only two were significant (D-Dillingen, D-Töging-II, Table 4).

When pooling all susceptible and all less susceptible individuals into two groups slight but non-significant differences were found for A (4.9 vs 5.0), H_O (0.727 vs 0.741), H_E (0.800 vs 0.798), F_{IS} (0.092 vs 0.072) and F_{ST} (0.066 vs 0.063) (data not shown).

Discussion

Genetic variation of ash populations

Our investigation shows high genetic variation within and high genetic differentiation between ash populations. Differentiation was substantially higher than for other tree species as *Fagus sylvatica* (for 10 German populations Nei's genetic distance was between 0.05 and 0.21 based on ten nuclear microsatellite markers, own unpublished results) and *Prunus avium* (DE ROGATIS et al., 2013). High genetic variation within stands was found for ash in similar studies (e.g. in Italy FERRAZZINI et al., 2007). Pairwise population differentiation in the present study ($F_{ST}=0.005-0.145$) was slightly higher compared to Italian ($F_{ST}=0.001-0.104$, FERRAZZINI et al., 2007) and British ash populations ($F_{ST}=0.008-0.111$, SUTHERLAND et al., 2010). However overall differentiation ($F_{ST}=0.046$) was lower compared to populations from Bulgaria ($F_{ST}=0.087$, HEUERTZ et al., 2001). F_{IS} -values ($F_{IS}=0.108$ and $F_{IS}=0.112$, overall and for German populations, respectively) were higher compared to other studies (e.g. $F_{IS}=0.030$ in HEUERTZ et al., 2004b).

Ash is a species which often regenerates naturally, but which has also been introduced artificially by planting. For the investigated populations we have no information on the history or the intensity of human impact. Both factors, natural and artificial regeneration, can influence genetic structures especially on a microgeographic scale. In addition recolonization history and gene flow by pollen and seed are important factors influ-

encing the genetic variation pattern of ash. Strong differentiation among populations was found using chloroplast markers ($G_{ST}=0.89$) reflecting low mixing of recolonizing lineages in Europe, which indicates that historical effective seed dispersal occurred mainly over short distances (HEUERTZ et al., 2004a). Therefore pollen dispersal might be more effective, since the dispersion of pollen by wind is typical for *F. excelsior*. Nevertheless the data on pollen dispersal is contradictory. Very limited pollen dispersal has been reported in *F. excelsior* 50% and 95% of pollen dispersed at less than 10 m and 50 m, respectively, (ALTMAN and DITTMER, 1964 in HEUERTZ et al., 2001). DOUGLAS (2006) also mentioned that pollen flow and seed dispersal in ash is generally restricted within the stand. These findings could explain the high genetic variation observed within populations and also the high genetic differentiation between populations found in the present study. Moreover, the movement of reproductive material during artificial regeneration with often differing genetic variation depending e.g. on the number of seed trees might also play a role. However a study in Scotland indicated that pollen gene flow into each of three fragmented populations was extensive (46–95%) and that the pollen dispersal had two components: a very localized and restricted one and a second one stretching over long distances with dispersal occurring over several kilometers (BACLES et al., 2005). Effective dispersal distances (average 328 m) were greater for fragmented populations than values reported for contiguous populations (BACLES et al., 2005).

No clear geographic pattern was found for the analysed populations based on nuclear microsatellites using allele frequencies. Each population seems to be genetically unique. However, certain characteristics were detected, especially regarding the number of rare alleles, which suggest large scale genetic differences. The two Slovenian populations and the region south of the Alps adjacent to Slovenia (AUT-Karnerviertel) showed the lowest values of rare alleles. This is a strong indication that different genetic structures exist in the south of the Alps compared to populations from the north.

The more conservative chloroplast markers suggest an east-west division of ash in Bavaria. Thereby the eastern part is clearly more heterogeneous. The altogether lower variation found in chloroplast markers conforms to findings within other studies for ash described in the literature (HEUERTZ et al., 2004a; HEBEL et al., 2006b). However, based on chloroplast markers, differences between the populations were found in the present study. Several populations were monomorphic regarding the chloroplast markers, i.e. the variation introduced via the maternal line was low. Other populations showed remarkably high values for the variation in chloroplast types. Historical contact of different chloroplast lineages (ash survived the last glacial maximum in Iberia, Italy, the eastern Alps and the Balkan Peninsula, HEUERTZ et al., 2004a) could be an explanation for the high chloroplast variation in populations from south-eastern Bavaria D-Töging-I and II, D-Freising-II and one neighboring population from Austria (AUT-Ettenau). Such high variation in the region of south-eastern Bavaria is

congruent with earlier findings by HEUERTZ et al. (2004a) who have detected four chloroplast types in this region. Interestingly in the same region (south-eastern Bavaria) sycamore maple (*Acer pseudoplatanus*) showed a higher chloroplast variation compared to other regions in Germany (Konnert, unpublished results). Even for silver fir (*Abies alba*) this region was a contact zone for western and eastern recolonization routes (KONNERT and BERGMANN, 1995). For the population D-Scheßlitz from northern Bavaria artificial introduction might be a more likely reason for the observed high haploid diversity.

Consequently the present research showed that ash populations from southern Germany were genetically very different. However, most of the studied populations displayed symptoms of ash dieback, although in different intensity, except for population D-Schweinfurt-I, where ash dieback has not been detected in the year of assessment. Nevertheless, resistance on the population level seems rather unlikely, because population D-Schweinfurt-I did not reveal any specifics based on the analysed markers that could explain such resistance. More likely is that *H. fraxineus* has not arrived in that population. From own field observations and studies of clonal trials (e.g. MCKINNEY et al., 2011a) the resistance against ash dieback seems to be on an individual rather than on a population level.

Genetic differences between susceptible and less susceptible trees

For the provenance trial and the permanent study plots differences between susceptible and less susceptible trees within each of the populations were detected. In some cases the genetic distance can be considered medium to high with significant F_{ST} -values in two populations (D-Dillingen, D-Töging-II, *Table 4*). With regard to genetic variation and diversity between susceptible and less susceptible trees the trend was not consistent. However, genetic variation (number of alleles and genetic diversity) decreased when comparing all trees to the less susceptible ones within each population. According to theoretical predictions, genetically less diverse populations have lower adaptability (YOUNG et al., 1996; WILLI et al., 2006).

Our results slightly indicate that heterozygotic individuals might be able to withstand ash dieback in a better way in comparison to homozygotic individuals, because the degree of heterozygotic individuals (observed heterozygosity, H_o) was higher in the less susceptible groups compared to the susceptible groups. This means a higher variability on the individual level for heterozygotic individuals and those might therefore have higher plasticity and reaction capacity against the disease. NAMKOONG et al. (1998) and TESSIER DU CROS et al. (1999) earlier suggested that heterozygotic individuals could be more resistant to environmental stresses.

A general trend for higher relatedness of individuals within the groups of less susceptible trees was not found. Both groups exhibited strong and weak relationship between individuals and some populations showed high, others low genetic difference between the two groups.

The remaining level of genetic diversity within the group of less susceptible individuals gives reason for hope that it will be possible to select diverse individuals and merge them into a breeding population. This is important for breeding programs to ensure a wide genetic base, particularly since analyses based on microsatellites revealed genetic variation in *H. fraxineus* populations (BENGTSSON et al., 2012; FUSSI et al., 2012; GHERGHEL et al., 2013) and genotype dependent differences in the pathogen – tree interaction might be expected.

At the moment contrasting opinions on the genetic control of resistance against ash dieback exist. Within clonal trials in Sweden (STENER, 2013) and Denmark (MCKINNEY et al., 2011a; KJÆR et al., 2011) strong genetic control of resistance against ash dieback was found. This was based upon the observation of artificially or naturally infected trees in common garden experiments that have shown clonal differences in infection intensity (MCKINNEY et al., 2011b). On the other hand doubts exist about the degree of resistance of individuals without symptoms (ENDERLE et al., 2013), since the mechanisms preventing those individuals from becoming infected is still largely unknown. Nevertheless it is of utmost importance to identify and preserve less susceptible trees for gene conservation and future breeding programs. Results of the present study have shown that within the group of less susceptible trees a wide genetic base exists whereupon a future breeding program can be built. Detailed recommendations for future germplasm collection can be found in PAUTASSO et al. (2013). Different management procedures of affected common ash stands, from clear-cutting to removal of single heavy diseased trees, are already ongoing. As an example SANDER (2014) describes a scenario in one forest district and recommends that less affected individuals should not be removed, which is important for the species in order to maintain the genetic potential for future seed production and regeneration.

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