Genetic effects of the introduction of *Quercus rubra* to Germany

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Abstract

Northern red oak (*Quercus rubra*) was introduced to Europe in the late 17th century and is today the most important deciduous foreign tree species in Germany. Despite its importance little is known about the origin of local red oak stands and patterns of genetic variation in German red oak stands. To be able to make recommendations regarding the adaptive potential of red oak and the selection of provenances with respect to climate change, a better understanding of the genetic diversity and structure of German red oak stands is needed. Therefore, individuals from 62 stands in Germany and North America were genotyped at five chloroplast microsatellite loci to characterize chloroplast haplotype diversity and geographical structure. Compared to reference stands from the natural distribution range, German stands demonstrated a relatively low genetic differentiation among populations and represented only a fraction of the haplotype diversity found in North America. For several stands located in the south of Germany a considerably higher haplotype diversity was found. Therefore, we conclude that German stands originated from a limited geographic range within the natural distribution, which was possibly located in the northern part of the native distribution range. While most German stands showed signatures of founder effects due to originating from a small number of individuals within a limited range, particularly stands in the south of Germany could have benefited from admixture and multiple introductions of different North American provenances.

Keywords Chloroplast microsatellites, *Quercus rubra*, Haplotype diversity, Origin, Provenances

Introduction

Since the rediscovery of the Americas in the end of the 15th century, thriving trade routes connecting the ports of the old and new worlds also transported alien plants and animals. While numbers of established alien species were relatively low until 1800, new introductions increased significantly at the turn of the century (Hulme 2009). Introduced populations of non-native species often contain only a fraction of the genetic information compared to the population they were originating from (Nei et al. 1975; Barrett and Husband 1990). This founder effect, thus, could result in the establishment of new populations with their own characteristic gene pool. Founder populations are usually small and, thus, more affected by random drift (Graw 2005). As a consequence, the adaptive potential of founder populations is limited. However, multiple introductions seem to increase diversity over a longer period (Dlugosch and Parker 2008). Suarez and Tsutsui (2008) even suggested that multiple introductions can enhance the adaptive potential by providing a source of variation important for adaptation and, thus, can be critical for the success of establishment and spread. A different situation arises when harvest of seeding material is only conducted in a limited area within a species' natural range, and then brought to the area of introduction. After going through this genetic bottleneck due to insufficient sampling, the newly founded population cannot represent the full range of the species' variation.

Northern red oak was first introduced to Europe at the end of the 17th century. While it was planted for ornamental reasons in parks and botanical gardens until the middle of the 18th century (Bauer 1951; Nagel 2015), it is now the most important foreign deciduous tree species in Germany (Bundesministerium für Ernährung und Landwirtschaft (BMEL) 2014). Albeit characterized by different properties compared to native white oak species, such as a ring-porous (permeable) wood, inoperative for the production of wine barrels, northern red oak delivers valuable wood and in a shorter rotation period of only 80-120 years compared to the native oak species with rotation periods of > 140 years. Other positive aspects are its comparably low demand for water and nutrient availability (Nagel 2015). As *Quercus rubra* belongs to the section *Lobatae*, which is restricted to North America, it does not hybridise with native white oak species of the section *Quercus* (Magni Diaz 2004; Nagel 2015). *Q. rubra* is considered invasive in some European countries (Riepšas and Straigytė 2008; Chmura 2013; Möllerová 2005). A species is regarded as invasive when it is established, spreads on its own, dominates over native species and negatively affects ecosystem or human (Reichard and White

2001). Impacts of invasive species are often difficult to evaluate and depend on the point of view (Simberloff et al. 2013). They can range from beneficial (e.g., when allowing a rare bird species to nest in an alien tree species' crown) to adverse (e.g., when changing community structure and reducing species' richness) (Powell et al. 2011; Simberloff et al. 2013). However, forestry does not consider *Q. rubra* invasive in Germany, because it is inferior to native shade-tolerant tree species, subject to heavy browsing, and can be easily controlled by tending measures (Nagel 2015).

With regard to climate change it can be expected that *Q. rubra* will be able to increase forest adaptability and productivity on particular sites in Germany, if the choice of the right provenances can be accomplished (Roloff and Grundmann 2008; Bolte et al. 2009). Previous studies suggestesd that local populations of red oak were established with seeding material originating from the northern parts of the natural distribution range (Bauer 1954; Magni Diaz 2004; Nagel 2015; Merceron et al. 2017). In order to better evaluate the adaptive potential of German red oak stands, it is thus necessary to characterise and eventually narrow down the geographic origin of the provenances used for the establishment of German red oak stands. This is especially important for stands that will be a part of future breeding strategies and serve as seed orchards.

By comparing haplotype diversity and structure in German stands and North American reference populations, we aim to assess to what extend the haplotype diversity found in the natural range is represented in Germany. In the present study, chloroplast microsatellite (cpSSR) markers were used to analyse haplotype diversity. Chloroplasts are cell organelles of prokaryotic origin, they are haploid and their structure and gene order highly conserved across and within taxa (Knippers 2006; Alexander and Woeste 2014). Due to its maternal mode of inheritance and lack of recombination, cpDNA haplotypes usually have relatively low variation within populations (Zhang et al. 2015). However, being haploid and uniparenterally inherited cpDNA is more than nuclear DNA affected by stochastic processes, such as genetic drift and founder events (Alexander and Woeste 2014). Therefore,, haplotype differentiation within one species can be high across populations and regions (Zhang et al. 2015). Thus, cpDNA markers have been successfully used in the past to trace post-glacial recolonization routes of white oak species in Europe (Petit et al. 2002) or to reveal the haplotype composition of autochthonous stands (Gailing et al. 2009). As a consequence of cpDNA being maternally inherited, using cpDNA markers for one species over a wide geographic area can allow to trace back routes of

historical seed dispersal. Identified haplotypes and haplotype variation can be compared with reference populations within the natural range and, thus, reveal information of their origin.

While the analysis of genetic variation patterns in northern red oak stands within their natural range was subject of several studies in the past (Daubree and Kremer 1993; Romero-Severson et al. 2003; Magni et al. 2005; Zhang et al. 2015; Borkowski et al. 2017), it has rarely been studied in Europe (Magni Diaz 2004). It was found that within the natural range of red oak genetic differentiation increased towards the north (Borkowsky et al. 2017). In contrast, there was no geographic pattern found in Europe (Magni Diaz 2004). This observation was explained by multiple introductions and admixture of material within Europe (Magni Diaz 2004), a suggestion partly shared also by Daubree and Kremer (1993). Although to identify the region of origin of introduced stands was one of his objectives, Magni Diaz (2004) did not succeed this goal due to the absence of a clear phylogeographic structure within the natural range. Merceron et al. (2017) studied SNP markers in both North American and European red oak stands with focus on France and found three main genetic clusters in the red oaks' natural range, while only two of these clusters were observed in Europe, likely suggesting that European stands originated from the northern parts of the natural range. Further, Merceron et al. (2017) reported a continuous, predominantly latitudinal, geographic gradient and only a weak phylogeographic structure in the natural range. Our study aimed at complementing these findings by providing insight in what ways the genetic variation of German red oak populations differs from the variation found in different parts of the natural range by using the cpSSR markers. In this study they are used for the first time to analyse and compare chloroplast haplotype diversity and structure in *Q. rubra* plantations across both Germany stands and North American reference populations. Our objectives are (i) to assess to what extend the haplotype diversity found in the natural range is represented in Germany and, if possible, (ii) to identify a high haplotype diversity region in Germany, which could reflect admixture of material from different regions of origin and potentially adaptive potential.

We hypothesize that 1) the introduction of *Q. rubra* to Germany resulted in a reduced overall haplotype diversity (due to sampling / bottleneck effects), and 2) haplotype diversity varies across Germany as the result of different introduction histories in the many principalities in the 19th century.

Material and Methods

Plant Material

Samples were collected as buds or as green leaves. In total 432 trees were sampled: 385 trees from 39 stands in Germany of unknown origin (8-10 samples per stand, Supplementary Table 1S and Fig. 1S) plus material from 47 trees of 8 stands of known North American origin (5-7 samples per stand, Supplementary Fig. 2S), represented in a provenance trial in Northern Germany (Supplementary Table 2S) (Liesebach and Schneck 2011). Additionally, 12 natural populations (8 samples per population) from the northern distribution range of the species were included as references (Supplementary Table 3S and Fig. 2S) (Lind and Gailing 2013; Lind-Riehl et al. 2014). Within each stand, samples were taken randomly.

To cover a wide geographic range, sample stands were selected in 5 different federal states of Germany: 10 stands in Lower Saxony, 10 in North-Rhine Westphalia, 6 in Brandenburg, 7 in Thuringia and 6 in Baden-Wuerttemberg. Sample stands were chosen to match the following criteria: (1) they should possibly be pure *Q. rubra* stands, (2) mature, i.e. 50-80 year old, (3) feature a rectangular shape, and (4) should be in locations, where they will be cultivated in the future as well (according to the forest administration), as not only the present but the expected future climate conditions would match their autecological properties.

DNA isolation

DNA was extracted from about 1 cm^2 leaf tissue per tree with the DNeasy™ 96 Plant Kit from Qiagen (Hilden, Germany). Depending on the availability we used either a small piece of the fresh leaf or 1-2 whole buds from a fresh twig.

Chloroplast microsatellites

Eight different universal cpSSR markers for angiosperms (*ccmp1*, *ccmp2*, *ccmp3*, *ccmp4*, *ccmp5*, *ccmp6*, *ccmp7*, *ccmp10*) (Weising and Gardner 1999) and three cpSSRs developed for oaks (*ucd4*, *udt1*, *udt4*) (Deguilloux et al. 2003) were tested for amplification and variation in 43 red oak samples from 11 different stands (Supplementary Table 4S). Five of these cpSSR markers showed variation and were thus used in the study: two universal (*ccmp2* and *ccmp4*) and three oak-specific markers (*udt1*, *udt4* and *ucd4*, see Table 4S for further information). In comparison to Zhang et al. (2015), we included two additional SSRs (*ucd4* and *udt1*), which allowed a better resolution of haplotypes.

We used one touchdown PCR program for all markers in a Biometra TProfessional thermocycler (Jena, Germany). The PCR protocol started with 15 min for initial denaturation at 95 °C, followed by 10 cycles of 1 min denaturation at 94 °C, 1 min annealing at 60 °C (-1 °C per cycle) and 1 min extension at 72 °C. This first set of cycles is then followed by another 25 cycles of 1 min denaturation at 94 °C, 1 min annealing at 50 °C and 1 min extension at 72 °C. The PCR ended with a final 20 min extension step.

For each single primer pair, PCRs were conducted in a 14 µl volume containing 1 µl of genomic DNA (about 0.6 ng/µl), 6.8 µl ddH_2O, 1.5 µl PCR buffer (containing 0.8 M Tris-HCl and 0.2 M $(NH_4)_2SO_4$), 1.5 µl $MgCl_2$ (25 mM), 1 µl of each dNTP (2.5 mM), 1 µl primer (forward, 5 pM/µl), 1 µl primer (reverse, 5 pM/µl) and 1 U HOT FIREPol® Taq-Polymerase from Solis BioDyne (Tartu, Estonia). For multiplexing of markers *ccmp2* and *ccmp4*, we used the following PCR mix: 1 µl of genomic DNA (about 0.6 ng/µl), 4.8 µl ddH_2O, 1.5 µl PCR buffer (containing 0.8 M Tris-HCl and 0.2 M $(NH_4)_2SO_4$), 1.5 µl $MgCl_2$ (25 mM), 1 µl of each dNTP (2.5 mM), 2 x 1 µl primer (forward, 5 pM/µl), 2 x 1 µl primer (reverse, 5 pM/µl) and 1 U HOT FIREPol® Taq-Polymerase (Solis BioDyne; Tartu, Estonia).

After PCR we performed an agarose gel electrophoresis to determine the ideal dilution ratio for the capillary electrophoresis. The gel electrophoresis was carried out at 90 v for 20 min on a 1.5 % agarose gel with TAE as running buffer (1X working solution). The DNA was stained with Roti-Safe GelStain from Roth (Karlsruhe, Germany). Then, the samples were diluted according to the intensity of amplification products on the gel.

For the separation of the cpDNA fragments we performed a capillary electrophoresis on an ABI Prism Genetic Analyzer 3130xl (Applied Biosystems). The fragments were scored using the software package ‘GeneMapper version 3.7’ (Applied Biosystems).

Data analyses

Chloroplast DNA haplotypes were based on all genotyped cpSSR markers. The software PermutCpSSR (https://www6.bordeaux-aquitaine.inra.fr/biogeco/Production-scientifique/Logiciels/Contrib-Permut/Permut; Pons and Petit 1996; Burban et al. 1999) was used to determine the total haplotypic diversity H_T, the average expected within-population haplotypic diversity H_S, the genetic differentiation among populations G_{ST} and the genetic differentiation while taking mutational differences into account (R_{ST}). The haplotype network

was created with Arlequin version 3.5 (Excoffier and Lischer 2010). The software computes a matrix of pairwise distances between all haplotypes, using the sum of squared size differences.

The software package GENECLASS2 (Piry et al. 2004), was used to tentatively assign German red oak populations to North American reference populations. It uses a Bayesian method introduced by Rannala and Mountain (1997).

To analyse a possible correlation between genetic and geographic distances, Mantel tests were performed separately for German populations and North American reference populations. First, pairwise genetic distances were calculated between all populations (Bruvo et al. 2004), and, then, the distances between each population pair were summed up and divided by the number of pairwise distances between two populations to weigh them by the number of individuals in each population. Second, a matrix of the geographic distance (in km) was computed. Finally, the software package GeneAlEx 6.5 (Peakall and Smouse 2006, 2012) was used to perform both a Mantel test and a Principle Coordinates Analysis (PCoA).

Results

Chloroplast haplotype distribution

Across all analysed populations we found 13 different chloroplast haplotypes. Five of them (D, E, F, M and O) were found only in Germany, while 5 others (G, H, I, K and L) were found solely in North America (Table 1). Most of these haplotypes were rare. However, haplotype K from the upper Midwest had a frequency of 16.8 % in North America and was absent in Germany. It occurs both in *Q. rubra* and *Q. ellipsoidalis* stands, two interfertile and hybridising red oak species. Though haplotype A was dominant in both North America and Germany, it showed a much higher relative frequency in Germany (80.3 %) as compared to North America (50.3 %). The next three frequent haplotypes together made up only 18.7 % in German stands, but 48.6 % in North American reference populations (Table 1). Haplotype B was the next most frequent haplotype in Germany (12.0 %) and had a similar frequency (10.8 %) also in all North American samples. Interestingly, this haplotype is different from haplotype A in each of the five loci (Fig. 1). Although it was found in Germany in each of the five federal states but in North American samples, it occurred mainly in the centre and north of the natural distribution range (Fig. 2). Haplotype C, which is closely related to haplotype A, was found only in the north of the range (on the tip of the Keweenaw Peninsula and in Ontario). In German stands, it was usually found at low frequency, only in single samples, except Baden-Wuerttemberg,

where it was found at relatively high frequencies composing 57 % of all haplotypes C in Germany (although only 6 stands were sampled there). Haplotypes K and L were restricted to the area of the Great Lakes and found there in considerable numbers, but was not found in our samples of German stands.

Haplotype diversity and structure

The minimum spanning tree in Fig. 1 shows the presence of three main lineages or clusters consisted of haplotypes related to haplotypes A, B and H, respectively. In our limited samples in North America, lineage "A" was found in all regions, but predominantly in the north and north-east; "B" was found in one stand in the south, but also in the north-central and the north-west of the species' natural distribution; "H" was mainly found in the south.

Due to the fixation for the same one haplotype , haplotype diversity within many North American populations was low (H_S = 0.173), while the total haplotype diversity was high (H_T = 0.692) accounting for a relatively high number of different haplotypes (Table 2). Unlike study by Zhang et al. (2015) based on a more restricted geographic range, high genetic differentiation was found among all North American populations (G_{ST} = 0.750, R_{ST} = 0.755). Generally, the haplotype diversity within populations, as well as the total haplotype diversity, were moderate in Germany (H_S = 0.291, H_T = 0.337). However, haplotype diversity within populations was on average higher in Germany (H_S = 0.291) than in North America (H_S = 0.173) likely due to a high degree of admixture in populations from Southwest Germany (Fig. 3). There, the haplotypic diversity was considerably higher than in all other regions (H_S = 0.537, H_T = 0.655 for Baden-Wuerttemberg). Apart from the high haplotype diversity, Baden-Wuerttemberg featured two of five private haplotypes and the greatest share of haplotype C among all regions analysed. As a consequence of the frequent occurrence of haplotype A in most German stands, genetic differentiation among all populations was relatively low (G_{ST} = 0.137, R_{ST} = 0.047).

Relationship between German and North American populations

In the Principal Coordinate Analysis (PCoA), 68.1 % of the variation was explained by the first principal coordinate (Coord. 1 in Fig. 4), while 8.77 % was explained by the second coordinate (Coord. 2 in Fig. 4). The PCoA showed that all German stands densely cluster with reference populations from most areas of North America (Fig. 4). Within this larger group, there is further grouping for stands from North-Rhine Westphalia, Lower Saxony and Brandenburg. Stands

from Thuringia and Baden-Wuerttemberg were linearly distributed along the Coord. 1 in Supplementary Fig. 3S. Overall, the North American reference populations were more differentiated than the German populations. Stands outside the main cluster were the North American populations NQ-E, NQ-R and Nantahala (USA-4), which all contained rare haplotypes not occurring in Germany. MTU and Mine consisted mainly of haplotype B, while BR1 and Keweenaw on the upper end of the distribution mainly contained haplotype C. The PCoA and GENECLASS2 analyses showed that some German stands were similar in haplotype composition to North American stands and were consequently assigned to these reference populations (Supplementary Table 5S). However, the absence of a correlation between genetic and geographic distances makes a reliable assignment difficult (see Mantel tests discussed below in the next section).

Relationship between genetic and geographic distance

While the Mantel test showed no correlation ($R_{XY} = 0.148$, $P = 0.161$) between genetic and geographic distances for North American reference populations (Supplementary Fig. 4S), it displays a slight positive correlation ($R_{XY} = 0.284$, $p = 0.001$) for the stands in Germany (Supplementary Fig. 5S). Also, while still very low, the R^2 value in Germany is four times higher ($R^2 = 0.08$) than for North American reference populations ($R^2 = 0.02$).

Discussion

German plantations originate from a limited geographic range in North America

Northern red oak populations in Germany seem to originate from a geographically restricted region in North America, covering two lineages, one of which is found all over North America, but mainly in the north and north-east (lineage 1) and one that was found mainly in the north-central and north-western parts (lineage 2, see Fig. 1). The low haplotype diversity and differentiation among German populations is for example shown in the multivariate PCoA analysis where populations from North America are widely scattered and German populations form a compact group with definite borders.

While North American populations are, due to their low haplotypic diversity within populations and high total haplotypic diversity, highly distinguishable from one another, German populations show only low differentiation among populations and an overrepresentation of the most common haplotype A. With regard to haplotype diversity, German stands represent only

a fraction of the diversity found in North America. Likewise, the low differentiation among populations (G_{ST} = 0.137) suggest that German populations originate from a restricted geographic range. Accordingly, genetic differentiation at cpDNA markers among *Q. rubra* populations from a restricted region in the Great Lakes region was considerably lower (G_{ST} = 0.206, Zhang et al. 2015) as compared to differentiation described in studies that covered a wider range (G_{ST} = 0.75; this study; G_{ST} = 0.58, Birchenko et al. 2009; G_{ST} = 0.46, Magni et al. 2005). Moreover, the absence of haplotypes G, K and L (27.0 % in North America), which were only found in five out of 20 populations in North America and with high frequencies in some individual populations, support the conclusion, that German *Q. rubra* stands originate from a limited geographic range in North America. In line with previous findings by Magni et al. (2005), no clear phylogeographic structure of haplotypes was found within the natural distribution area of *Q. rubra*. Furthermore, the absence of a correlation between geographic and genetic distance in North America impedes the identification of the geographic origin of German plantations. Although their exact origin cannot be determined, the presence of haplotype B with restricted distribution in North America (two populations on the Upper Peninsula of Michigan, one population in Indiana) is in accordance with an origin of German populations from the northern part of the natural distribution range. A range-wide high density characterization of haplotypes in North American *Q. rubra* populations as it was done for European white oak species (Petit et al. 2002) might allow to narrow down the origin of German red oak stands. Our findings conform with a recent study analysing SNP markers in North American red oak stands as well as in stands, that are located mainly in France: Merceron et al. (2017) found, that from three identified genetic clusters (G1, G2, G3) within the natural range, only two could be recovered in Europe. From these three clusters, one occurs mainly in the south (G2), one mainly in the north-east (G1) and the third cluster (G3), which supposedly diverged from the first two clusters, mainly in the north-central and north-west of the natural distribution (Merceron et al. 2017). The latter is reported to occur more evenly distributed over all regions. In their study, the genetic cluster mainly found in the south of the natural distribution could have been largely extirpated in or have never been introduced to Europe in the first place. Merceron et al. (2017) state that this could be due to the source populations for the European gene pool being located in the Northern part of the range. Likewise, an early study addressing the taxonomy of *Q. rubra* variants by means of phenotypic traits came to the conclusion, that German red oak stands comprise mainly *Q. rubra* var. *rubra*, a variety that is

characterised by a shallower cupule and bigger acorns and is predominant in the North of the natural distribution area (Bauer 1954).

Although our sampling in North America was limited and not designed to analyse haplotype diversity across the whole natural distribution area, the geographic distribution of the cpDNA lineages found in this study seem to match the distribution of genetic clusters derived from SNP markers found by Merceron et al. (2017). By comparing the present study with the one by Merceron et al. (2017) the occurrence of three cpDNA lineages in North America and three clusters derived from nuclear SNP markers is striking. According to both studies only the two northern lineages or clusters were recovered in Europe, one that occurs mainly in the north-central / north-west (G3 or lineage 2) and one that occurs mainly in the north (lineage 1) / north-east (G1) of the natural distribution area. In addition, haplotype B from lineage 2 was found in all regions in Germany, but was present only in the north and central part of the natural distribution range in North America.

When a species migrates to an unoccupied geographic area, especially if it is introduced on purpose or by accident, its gene pool hardly represents its full range of varieties. Merely a fraction of the species' variation provides the foundation for the future population, being challenged not only by new but also constantly changing environmental conditions (Meimberg et al. 2006; Mayr 1954). Northern red oak seems to find itself in exactly this position in Germany: Sampling from a limited geographic range has limited the genetic diversity at chloroplast markers in most German stands, reflecting founder events far away from the original distribution area. As they are the cause for reduced genetic diversity, sampling effects should also be visible at nuclear microsatellite markers. Although *Q. rubra* is currently well adapted to conditions in Germany (Roloff and Grundmann 2008), it might still have a lower capacity to react to changing environmental conditions in the future. To better describe the adaptive potential of red oak populations in Germany, future studies will focus on the assessment of genetic diversity at nuclear SSRs and genic EST-SSRs in representative samples of selected German and North American reference populations.

Admixed material at least for plantations from Southwest Germany

While stands in Germany generally show only low overall haplotype diversity and low genetic differentiation among populations, a different pattern is found for the stands probed in Baden-Wuerttemberg. Here, higher haplotype diversity and number of private haplotypes compared to all the other examined regions of Germany (e.g. the more frequent occurrence of haplotype

C in Baden-Wuerttemberg, while this haplotype is rare in other federal states) suggest that the imported seeding material for the establishment of the stands was admixed consisting of material from different regions within the natural distribution range (e.g. due to multiple introductions). In fact, northern red oak went through several periods of cultivation since its first introduction at the end of the 17th century. After first unsuccessful trials in the middle of the 18th century, there were intensified efforts to establish *Q. rubra* in the second half of the 19th century and in the middle of the 20th century (Nagel 2015). The material for the establishment of the new trials could partly have been collected from red oak stands of the first two generations, but, as France and the Netherlands were also making efforts to establish northern red oak (Nagel 2015), the material could also have been obtained from there. Also, additional material could have been brought directly from stands within the natural distribution area. In either case the positive correlation between the genetic and geographic distance for German red oak stands found by the Mantel-test (Supplementary Fig. 5S) also suggests differentiation between regions in Germany, which is caused by the introduction of different gene pools.

Potential for invasiveness

An invasive species can be described as a species, that is introduced intentionally or accidentally, able to spread and maintain reproduction on its own and negatively affects ecosystem or human (Simberloff et al. 2013; Pyšek et al. 2008). While a newly introduced species spreads easily and quickly mainly due to the lack of serious competition, it can - and in many cases did - result in the loss of native species' diversity (Ledig 1992). Thus, it is crucial to further monitor the impact of introduced species on native forest vegetation. In Europe, *Q. rubra* is, among others, considered invasive in Belgium, Poland, Czech Republic and Lithuania. Reasons for this judgement are its high spreading ability on less fertile soil and negative impacts on the amount and diversity of microorganisms and below-canopy grass species (Möllerová 2005; Branquart et al. 2007; Riepšas and Straigytė 2008; Chmura 2013).

In Germany, however, northern red oak is not considered as invasive (Vor 2005), although, it can outcompete native oak species due to its higher shade tolerance and competitiveness at all development stages (Stratmann and Warth 1987; Vor and Lüpke 2004; Kuehne et al. 2014). At early stages red oak is even able to overgrow dominant native tree species such as *Fagus sylvatica*, if light availability is above 50 % of open field conditions (Vor and Lüpke 2004).

Ineffective vectors for seed dispersal, heavy browsing and the lack of vegetative reproduction diminish its relatively high reproductive potential (Nagel 2015). Even in places where *Fagus sylvatica* is not dominating the potential natural vegetation, red oak's ability to spread depends on the distance of the seed to the propagule source, the main tree species of the stand and light availability and can thus be used to prevent further expansion (Jagodziński et al. 2018).

Future perspective

Our study revealed that although chloroplast haplotype variation of red oak in Germany is relatively low, evidence for admixture and multiple introductions of this species are reflected in higher haplotypic diversity and genetic differentiation in stands in Baden-Wuerttemberg. The presence of haplotype B in all regions in Germany, unlike North America where it has a limited geographic distribution, mainly in the northern part of the natural distribution range, suggests an origin of German populations from the northern part of the natural distribution range. These results support the similar conclusion drawn by Merceron et al. (2017) that red oak stands in Europe originate from the north of the natural distribution area. Further analyses at microsatellite and SNP markers in adaptive genes should be performed to confirm the genetic divergence in two lineages within Europe. If the number of sampled stands in both North America and Germany and the number of cpDNA and other markers are increased, the origin of German red oak stands in the north of the natural distribution area could potentially be narrowed down further based on the occurrence of rare and geographically restricted variants. The level of genetic variation and adaptive potential of the species should also be characterized at nuclear markers including candidate genes with potential role in local adaptation.

Tables and Figures

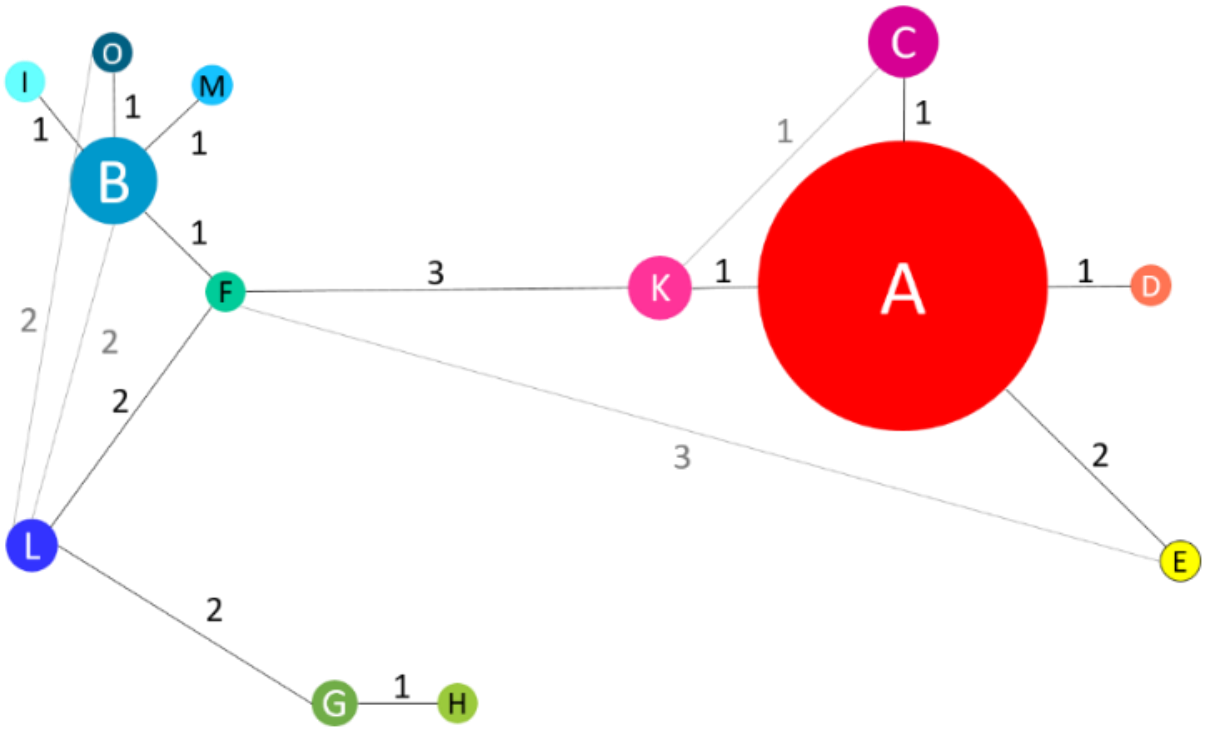

Fig. 1 Minimum spanning tree representing the chloroplast haplotype network (Excoffier and Lischer 2010) of *Quercus rubra* stands in Germany. Numbers next to the lines indicate the number of markers which differ between two haplotypes.

A

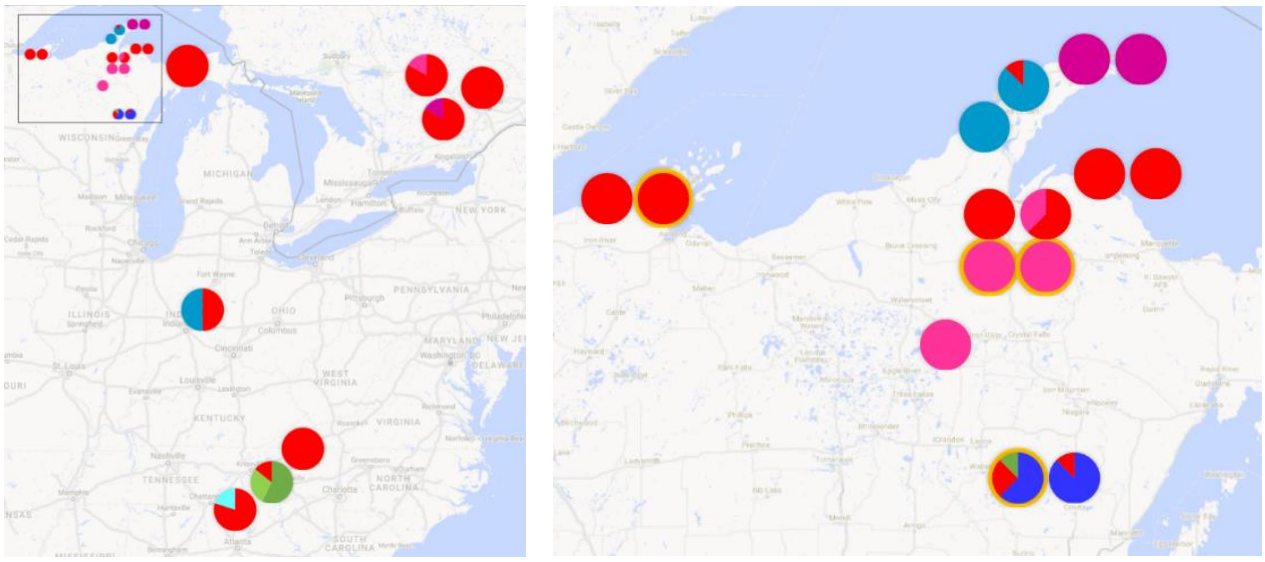

Fig. 2 A: Distribution of *Quercus rubra* chloroplast haplotypes in North America. Samples were partly obtained from a provenance trial in Lübeck, Germany (Google Maps 2017c; Liesebach and Schneck 2011). **B**: Stands zoomed out from the box inside **A** that represent chloroplast haplotype distribution south of the great lakes. Populations were sampled and genotyped in earlier studies. Yellow rings mark populations of closely related *Quercus ellipsoidalis* (Google Maps 2017b; Lind and Gailing 2013; Lind-Riehl et al. 2014)

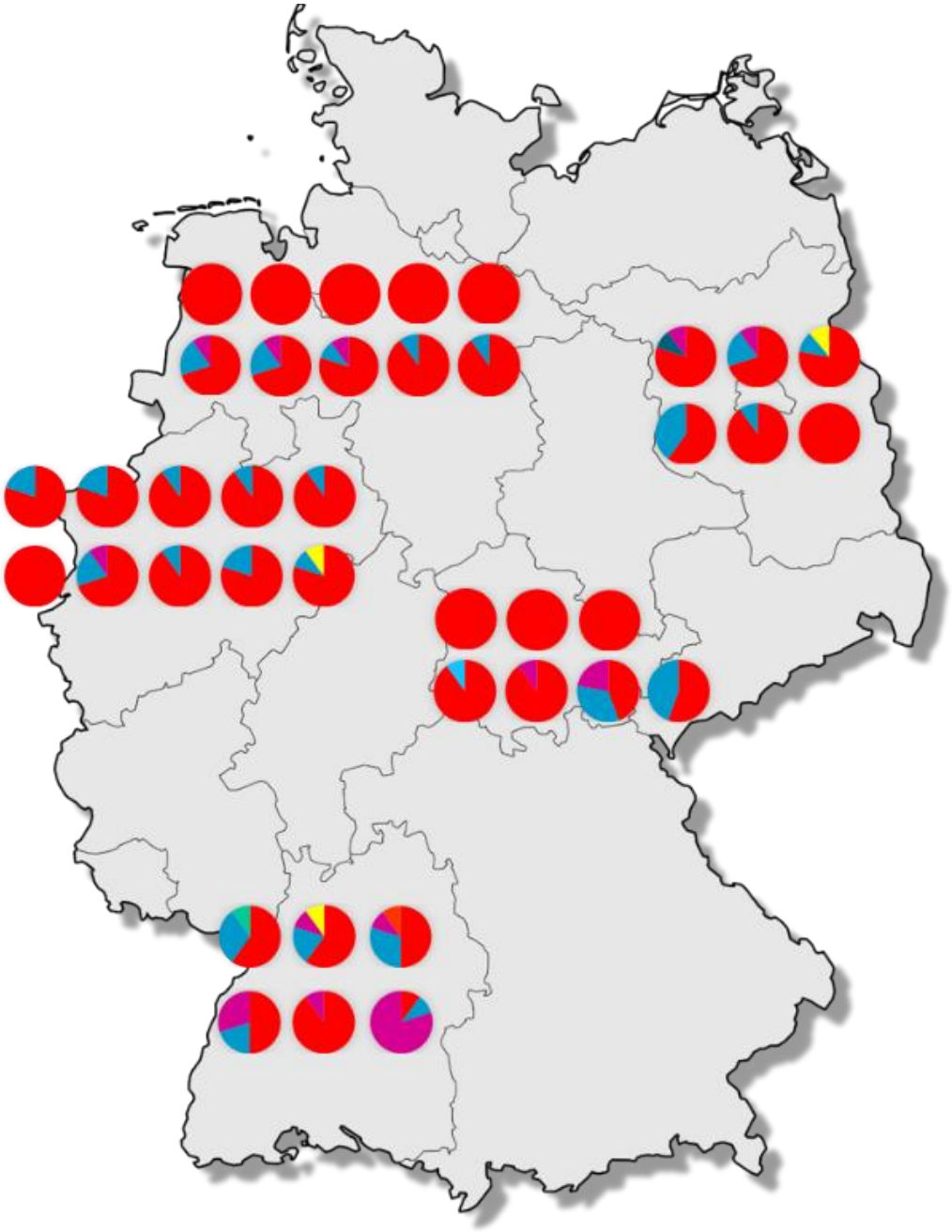

Fig. 3 Distribution of *Quercus rubra* chloroplast haplotypes in Germany (map of Germany by David Liuzzo 2007)

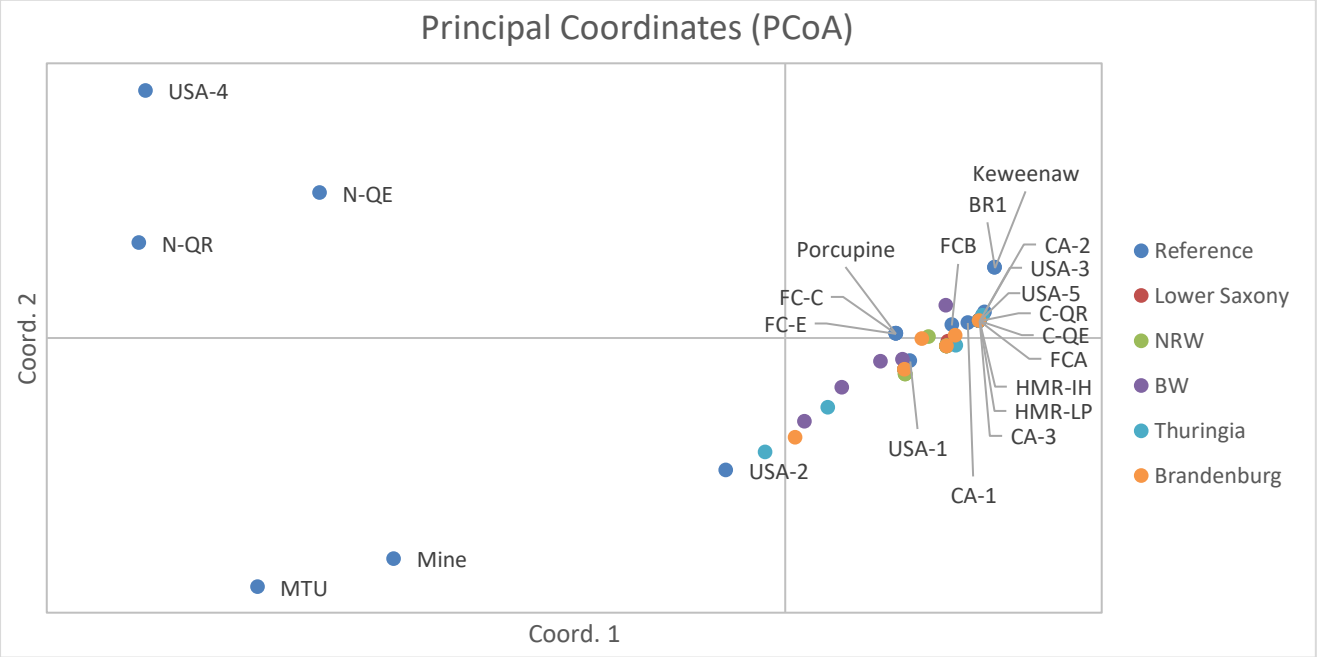

Fig. 4 Principal Coordinate Analysis (PCoA) based on *Quercus rubra* chloroplast markers for all populations (reference populations are labelled)

Table 1 Description of *Quercus rubra* chloroplast haplotypes (A-O) and their frequencies

Haplotype	Five cpSSR markers and their allele fragment sizes, bp					Germany		Reference		Overall	
	ccmp2	*ccmp4*	*ucd4*	*udt1*	*udt4*	*f*	*f* (%)	*f*	*f* (%)	*f*	*f* (%)
A	228	116	99	86	145	309	80.3	84	50.3	393	71.2
B	227	115	98	85	146	46	12.0	18	10.8	64	11.6
C	228	116	99	87	145	23	6.0	17	10.2	40	7.3
D	228	117	99	86	145	1	0.3			1	0.2
E	228	116	97	86	146	3	0.8			3	0.5
F	227	116	98	85	146	1	0.3			1	0.2
G	226	117	97	85	146			5	3.0	5	0.9
H	226	117	97	84	146			2	1.2	2	0.4
I	227	115	98	84	146			1	0.6	1	0.2
K	228	116	99	85	145			28	16.8	28	5.1
L	226	118	98	85	146			12	7.2	12	2.2
M	227	115	98	85	145	1	0.3			1	0.2
O	228	115	98	85	146	1	0.3			1	0.2

Table 2 *Quercus rubra* chloroplast haplotype diversity within populations (H_S), total haplotype diversity (H_T), differentiation among populations (G_{ST}), the genetic differentiation while taking mutational differences into account (R_{ST}), the number of haplotype (N_a) and the number of private haplotypes (N_p)

Data	H_S	H_T	G_{ST}	R_{ST}	N_a	N_p
Germany	0.291	0.337	0.137	0.047	8	5
Lower Saxony	0.180	0.187	0.036	-0.020	3	0
North-Rhine Westphalia	0.260	0.246	-0.056	-0.071	3	0
Baden-Wuerttemberg	0.537	0.655	0.181	0.097	6	2
Thuringia	0.240	0.313	0.234	0.190	4	1
Brandenburg	0.340	0.354	0.040	0.039	5	1
North America	0.173	0.692	0.750	0.755	8	5
North America (Great Lakes) (Zhang et al. 2015)	0.346	0.436	0.206	0.253		

Supplementary Tables and Figures

Fig. 1S Study sites in Germany (Google Maps 2017a)

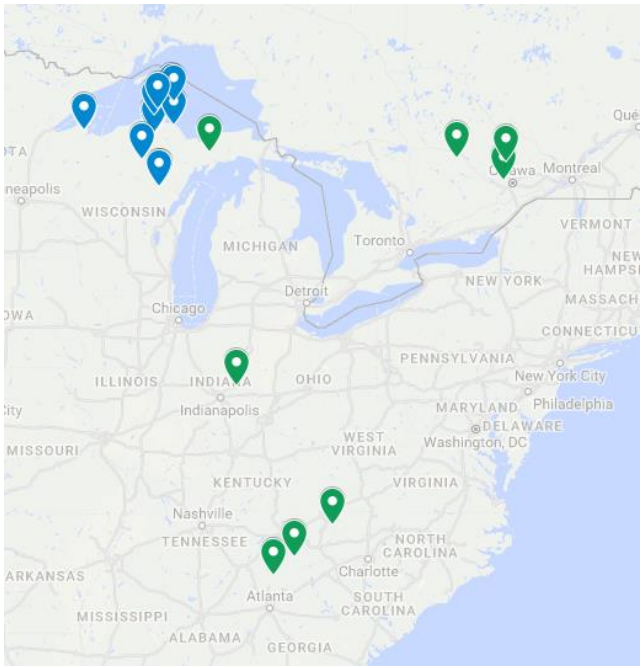

Fig. 2S Reference stands in North America (green and blue sites are listed in Tables 2S and 3S, respectively) (Google Maps 2018)

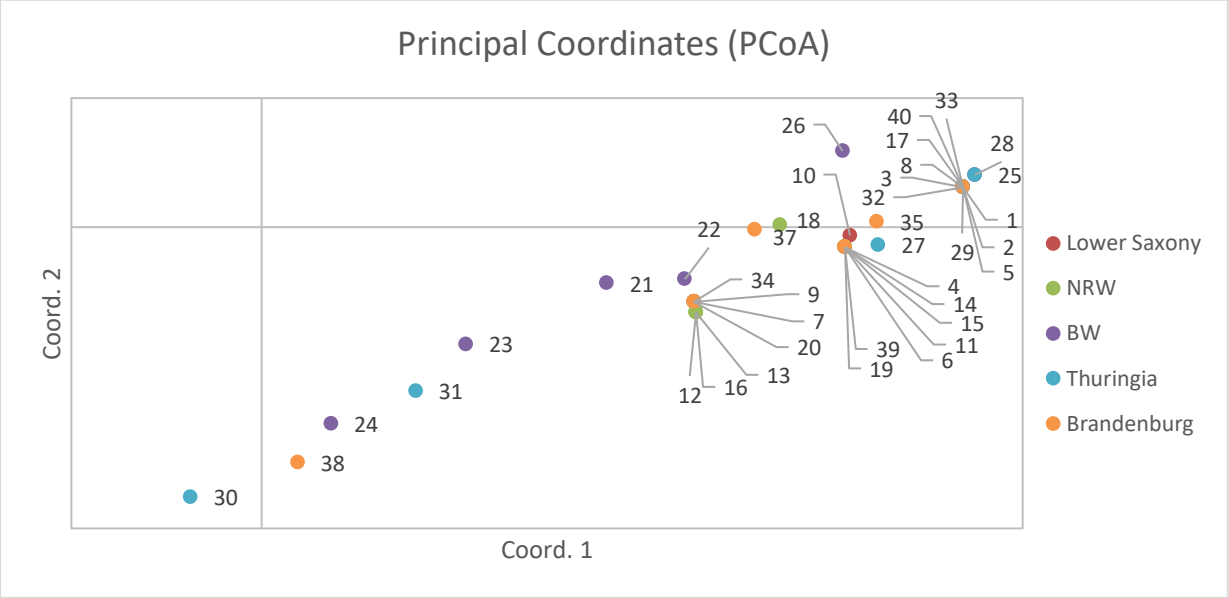

Fig. 3S Principal Coordinate Analysis (PCoA) of German populations based on *Quercus rubra* chloroplast markers

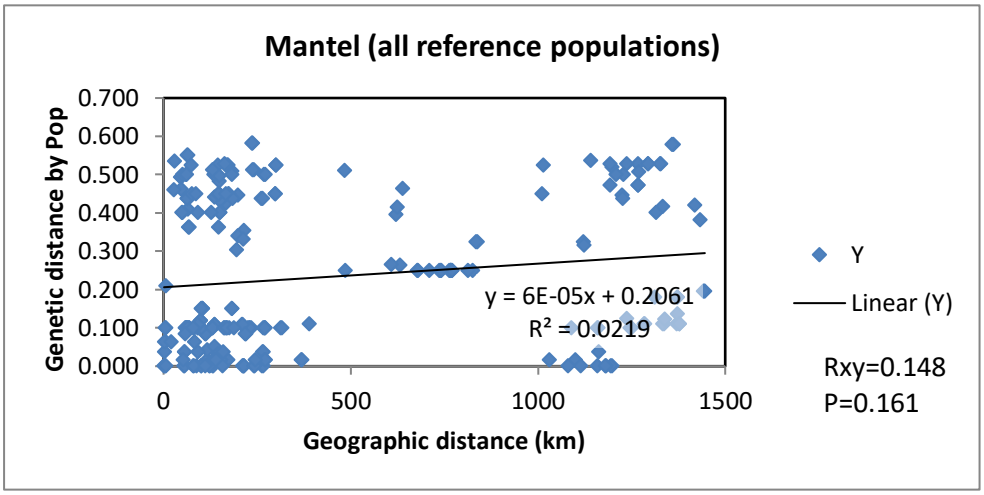

Fig. 4S Mantel test for all reference populations of *Quercus rubra*

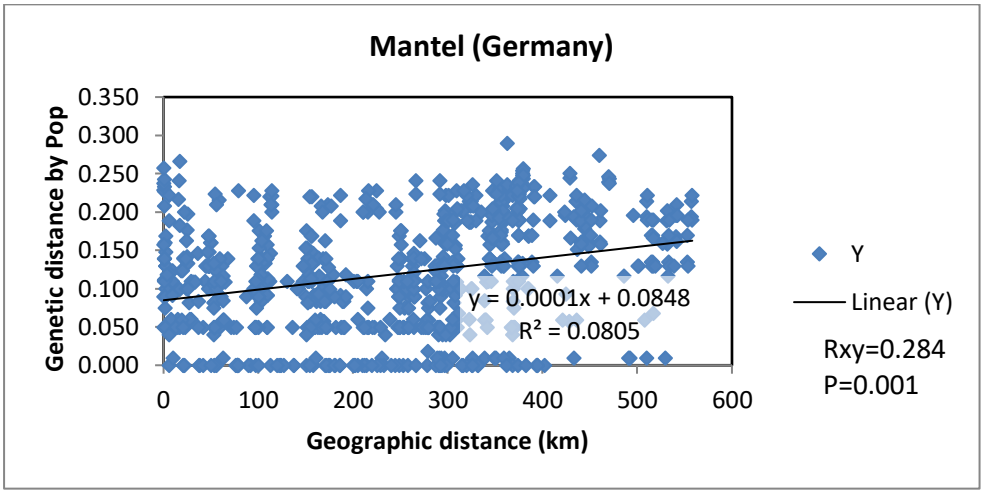

Fig. 5S Mantel test for German populations of *Quercus rubra*

Table 1S Study sites in Germany

#	Region	District	N	Latitude	Longitude	Altitude
1	Dassel	Sievershausen	10	51.77971	9.58899	524 m
2	Rotenburg	Spange 1	10	53.00454	9.34475	84 m
3	Rotenburg	Spange 2	10	53.00273	9.26215	80 m
4	Fuhrberg	Beerbusch	10	52.40619	9.83055	73 m
5	Rotenburg	Thörenwald	10	53.34354	9.35411	57 m
6	Rotenburg	Luhne	10	53.14049	9.37014	54 m
7	Rotenburg	Diensthoop	10	52.94624	9.36675	69 m
8	Rotenburg	Hepstedt 1	8	53.18048	9.18389	58 m
9	Göhrde	Zienitz	10	53.10025	10.8545	94 m
10	Rotenburg	Hepstedt 2	10	53.24972	9.18638	53 m
11	Rhein-Sieg-Erft	Knechtstenden	10	51.03911	6.80227	72 m
12	Niederrhein	Schwalm-Nette 1	10	51.15004	6.19473	104 m
13	Niederrhein	Schwalm-Nette 2	10	51.16385	6.18172	97 m
14	Niederrhein	Leucht 1	10	51.54538	6.48695	53 m
15	Niederrhein	Leucht 2	10	51.53801	6.50687	52 m
16	Niederrhein	Leucht 3	10	51.55844,	6.50824	73 m
17	Niederrhein	Leucht 4	10	51.53854	6.51056	57 m
18	Niederrhein	Leucht 5	10	51.53741	6.49099	68 m
19	Niederrhein	Leucht 6	10	51.54422	6.48661	56 m
20	Niederrhein	Leucht 7	10	51.53946	6.49804	72 m
21	Offenburg	Schutterwald 1	10	48.45871	7.86786	158 m
22	Offenburg	Schutterwald 2	10	48.45346	7.85918	148 m
23	Offenburg	Schutterwald 3	10	48.46007	7.86184	152 m
24	Offenburg	Schutterwald 4	10	48.45299	7.85969	148 m
25	Achern	Großweiher	10	48.65421	8.03769	143 m
26	Oberkirch	Renchen	10	48.57306	7.99869	171 m
27	Weida	Treben 1	10	51.06314	12.42265	166 m
28	Weida	Leina	10	50.97307	12.54363	222 m
29	Sonderhausen	Volkenroda	10	51.29994	10.66687	315 m
30	Neustadt	Strößwitz 1	9	50.76691	11.72482	382 m
31	Neustadt	Strößwitz 2	9	50.76442	11.7242	392 m
32	Jena	Holzland	10	50.77549	11.64021	372 m
33	Weida	Treben 2	10	51.05847	12.50526	190 m
34	Wünsdorf	Großbeeren	10	52.36366	13.35163	46 m
35	Potsdam	Güterfelde 1	10	52.45505	13.0795	47 m
37	Rathenow	Kater	9	52.55401	12.2304	44 m
38	Potsdam	Güterfelde 3	10	52.38356	13.00608	38 m
39	Lehnin	Brandenburg	10	52.41631	12.46474	37 m
40	Baruth	Merzdorf	10	51.98894	13.37158	143 m

Table 2S Study sites in North America for samples collected from a provenance trial in Lübeck, Germany (Liesebach and Schneck 2011)

Stand	Abbr.	Country	State	Location	N
Atomic Energy	CA-1	Canada	Ontario	Renfrew, Atomic Energy, Chalk River	6
Constance Bay	CA-2	Canada	Ontario	Ottawa, Constance Bay	6
Pl. de Kazabazua	CA-3	Canada	Ontario	Gatineau, Plaines de Kazabazua, Basse-Lièvre	6
Chattahoochee	USA-1	USA	Georgia	Fannin, Chattahoochee, Toccoa	5
Anderson	USA-2	USA	Indiana	Madison, Anderson	6
Hiawatha	USA-3	USA	Michigan	Chippewa, Hiawatha, Soo	6
Nantahala	USA-4	USA	North Carolina	Clay, Nantahala, Tusquitee	7
Cherokee	USA-5	USA	Tennessee	Sullivan, Cherokee, Watauga	5

The origin of these populations in North America is shown in Fig. 2.

Table 3S Study sites in North America

Abbreviation	Region	N	Latitude	Longitude	Altitude
FC-A[1]	Ford Forestry Center, MI	8	46.65261	-88.50193	415 m
FC-B[1]	Ford Forestry Center, MI	8	46.67442	-88.52427	393 m
FC-C[1]*	Ford Forestry Center, MI	8	46,65401	-88,59044	401 m
FC-E[1]*	Ford Forestry Center, MI	8	46,66555	-88,55531	406 m
BR1[1]	Brockway Mountain, MI	8	47.46616	-87.91671	355 m
MTU[1]	Michigan Tech Trails, MI	8	47.10055	-88.5475	270 m
HMR-IH[1]	Huron Mountain Res, MI	8	46.85357	-87.84522	256 m
HMR-LP[1]	Huron Mountain Res, MI	8	46.84994	-87.83022	289 m
N-QE[2]*	Nicolet NF, WI	8	45.32194	-88.33138	304 m
N-QR[2]	Nicolet NF, WI	8	45.34805	-88.38805	345 m
Keweenaw[3]	Brockway Mountain, MI	8	47.4401	-87.85658	321 m
Mine[3]	Calumet Township, MI	8	47.25398	-88.42676	372 m
Porcupine[3]	Kentuck Lake, WI	8	46.00003	-88.9996	532 m
C-QR[2]	Chequamegon NF, WI	8	46.715	-91.03555	328 m
C-QE[2]*	Chequamegon NF, WI	8	46.74527	-91.07222	384 m

MI - Michigan,; WI – Wisconsin; [1]Lind and Gailing 2013; [2]Lind-Riehl et al. 2014; [3]unpublished. The origin of these population in North America is shown in Figs 2 and 3.
*Populations were genetically identified as *Q. ellipsoidalis* (Lind and Gailing 2013; Lind-Riehl et al. 2014), which is closely related to *Q. rubra*. Both species are interfertile and hybridize with each other in contact zones.

Table 4S Chloroplast microsatellite markers (cpSSRs) used in this study

cpSSR	Dye colour	Direction	Primer sequences (5'-3')	Repeat motif	Size, bp	n_a	Location
ccmp2[1]	Blue	Forward	GATCCCGGACGTAATCCTG	$(A)_{11}$	233-234	2	5' to *trnS*
		Reverse	ATCGTACCGAGGGGTTCGAAT				
ccmp4[1]	Blue	Forward	AATGCTGAATCGAYGACCTA	$(T)_{13}$	126	3	*atpF* intron
		Reverse	CCAAAATATTBGGAGGACTCT				
ucd4[2]	Blue	Forward	TTATTTGTTTTTGGTTTCACC	$(T)_{12}$	97	3	intergenic
		Reverse	TTTCCCATAGAGAGTCTGTAT				ycf6-psbM
udt1[2]	Blue	Forward	ATCTTACACTAAGCTCGGAA	$(A)_{11}$	86	3	intergenic
		Reverse	TTCAATAACTTGTTGATCCC				trnE-trnT
udt4[2]	Blue	Forward	GATAATATAAAGAGTCAAAT	$(A)_9$	147	3	Intergenic
		Reverse	CCGAAAGGTCCTATACCTCG				trnE-trnT

[1]Weising and Gardner 1999; [2]Deguilloux et al. 2003

Table 5S Populations assignment with GENECLASS2, Bayesian method used (Rannala and Mountain 1997). Assignments to the south (red), centre (yellow) and north (green) of the natural distribution area

			Rank 1	Score	Rank 2	Score	Rank 3	Score
Assigned sample			Assigned Ref	%	Assigned Ref	%	Assigned Ref	%
1	Dassel	Sievershausen	Hiawatha	12.0	C-QR	12.0	C-QE	12.0
2	Rotenburg	Spange 1	Hiawatha	12.0	C-QR	12.0	C-QE	12.0
3	Rotenburg	Spange 2	Hiawatha	12.0	C-QR	12.0	C-QE	12.0
4	Fuhrberg	Beerbusch	Chattahoochee	36.8	Atomic Energy	17.1	HMR-LP	5.3
5	Rotenburg	Thörenwald	Hiawatha	12.0	C-QR	12.0	C-QE	12.0
6	Rotenburg	Luhne	Chattahoochee	36.8	Atomic Energy	17.1	HMR-LP	5.3
7	Rotenburg	Diensthoop	Chattahoochee	96.0	Anderson	4.0	Atomic Energy	0.0
8	Rotenburg	Hepstedt 1	Hiawatha	11.8	C-QR	11.8	C-QE	11.8
9	Göhrde	Zienitz	Chattahoochee	96.0	Anderson	4.0	Atomic Energy	0.0
10	Rotenburg	Hepstedt 2	Chattahoochee	34.4	Atomic Energy	15.3	Constance Bay	15.3
11	Rhein-Sieg-Erft	Knechtstenden	Chattahoochee	36.8	Atomic Energy	17.1	HMR-LP	5.3
12	Niederrhein	Schwalm-Nette 1	Chattahoochee	97.5	Anderson	2.5	Atomic Energy	0.0
13	Niederrhein	Schwalm-Nette 2	Chattahoochee	97.5	Anderson	2.5	Atomic Energy	0.0
14	Niederrhein	Leucht 1	Chattahoochee	36.8	Atomic Energy	17.1	HMR-LP	5.3
15	Niederrhein	Leucht 2	Chattahoochee	36.8	Atomic Energy	17.1	HMR-LP	5.3
16	Niederrhein	Leucht 3	Chattahoochee	97.5	Anderson	2.5	Atomic Energy	0.0
17	Niederrhein	Leucht 4	Hiawatha	12.0	C-QR	12.0	C-QE	12.0
18	Niederrhein	Leucht 5	Chattahoochee	94.8	Atomic Energy	1.4	HMR-LP	0.4
19	Niederrhein	Leucht 6	Chattahoochee	36.8	Atomic Energy	17.1	HMR-LP	5.3
20	Niederrhein	Leucht 7	Chattahoochee	96.0	Anderson	4.0	Atomic Energy	0.0
21	Offenburg	Schutterwald 1	Chattahoochee	78.9	Anderson	21.1	Atomic Energy	0.0
22	Offenburg	Schutterwald 2	Chattahoochee	90.3	Anderson	9.7	Constance Bay	0.0
23	Offenburg	Schutterwald 3	Anderson	99.9	Chattahoochee	0.1	FC-B	0.0
24	Offenburg	Schutterwald 4	Anderson	100.0	Chattahoochee	0.0	Mine	0.0
25	Achern	Großweiher	Constance Bay	28.2	HMR-IH	8.7	HMR-LP	8.7
26	Oberkirch	Renchen	Keweenaw	50.0	BR1	50.0	Constance Bay	0.0
27	Weida	Treben 1	Atomic Energy	26.4	HMR-LP	8.2	Hiawatha	8.2
28	Weida	Leina	Constance Bay	28.2	HMR-IH	8.7	HMR-LP	8.7
29	Sonderhausen	Volkenroda	Hiawatha	12.0	C-QR	12.0	C-QE	12.0
30	Neustadt	Strößwitz 1	Anderson	100.0	Chattahoochee	0.0	Mine	0.0
31	Neustadt	Strößwitz 2	Anderson	100.0	Chattahoochee	0.0	FC-B	0.0
32	Jena	Holzland	Hiawatha	12.0	C-QR	12.0	C-QE	12.0
33	Weida	Treben 2	Hiawatha	12.0	C-QR	12.0	C-QE	12.0
34	Wünsdorf	Großbeeren	Chattahoochee	96.0	Anderson	4.0	Atomic Energy	0.0
35	Potsdam	Güterfelde 1	Atomic Energy	22.8	Constance Bay	22.8	HMR-LP	5.9
37	Rathenow	Kater	Chattahoochee	98.1	Atomic Energy	0.6	HMR-LP	0.1
38	Potsdam	Güterfelde 3	Anderson	100.0	Chattahoochee	0.0	Mine	0.0
39	Lehnin	Brandenburg	Chattahoochee	36.8	Atomic Energy	17.1	HMR-LP	5.3
40	Baruth	Merzdorf	Hiawatha	12.0	C-QR	12.0	C-QE	12.0

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