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Western and eastern post-glacial migration pathways shape the genetic structure of sycamore maple (*Acer pseudoplatanus* L.) in Germany

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ABSTRACT

Sycamore maple (*Acer pseudoplatanus* L.) is a broadleaved forest tree species mainly occurring in mountainous areas of Europe, preferring fresh to moist, deep, calcareous soils. As was the case with other important forest tree species, sycamore maple was absent from the area north of the Alps during the last ice age. Therefore, we investigated the origin of sycamore maple in this area and its genetic variation across the landscape as the result of its postglacial migration into this area. We compared our results with the current delineation of sycamore maple into regions of provenance in Germany. Altogether 1043 trees from Germany, but also, in few cases, from bordering countries, were analysed using 11 nuclear and one chloroplast microsatellite (SSR) markers. Both the analysis of chloroplast (cpDNA) haplotypes and the Bayesian cluster analysis based on nuclear SSR data revealed distinct genetic structures of sycamore maple in Germany, suggesting more than one origin. The spatial distribution of a particular cpDNA haplotype (haplotype '105') might be the result of a migration event with an eastern origin, probably along the Eastern Alps, which could have occurred during the Holocene. The other haplotype (haplotype '102') might be of (south-) western origin, representing a migration pathway along the Western Alps. This hypothesis is further supported by a Bayesian cluster analysis based on nuclear SSRs. We identified two clusters, one distributed in the western part of the study area and one in the eastern. Between them, a zone of genetic introgression occurs, where populations exhibit a mixed membership to both clusters. This zone of genetic admixture is narrower in the south. An analysis of the landscape shape confirms that average genetic differentiation per distance unit is higher in the south. This is also in agreement with higher genetic distances between regions of provenance in the south. The mountainous landscape of this region could have contributed to this higher genetic differentiation, by posing geographic barriers to gene flow. On the contrary, wide-scale seed movement, also beyond the limit of native range, as well as a flat relief could have resulted in a wider zone of admixture in the north. Based on our results, we argue that the current delineation of provenance regions for sycamore maple in Germany fits to the inferred patterns of genetic differentiation.

1. Introduction

The genetic variation of tree species in Central European temperate forests has been shaped by post-glacial migration (Magri, 2008; Petit et al., 2002; Taberlet et al., 1998; Tollefsrud et al., 2008), adaptation (Rellstab et al., 2016; Savolainen et al., 2007) and human activities (Bradshaw, 2004; Neophytou et al., 2015). The effects of all three factors differ among species. For instance, the ancestors of Central European white oaks (*Quercus* spp.) arose from refugial areas in Iberian, Italian and Balkan peninsulas, whereas intensive gene flow has led to a continuous gene pool within each species (no or loose genetic structure; Kremer et al., 2002; Petit et al., 2002; Neophytou et al., 2015). A different pattern can be observed in the case of silver fir (*Abies alba* L.), which is associated with montane forests. The post-glacial migration of this species followed mountain ranges, while clinal variation can be observed along migration pathways or in introgression zones (Konnert and Bergmann, 1995; Liepelt et al., 2009).

Sycamore maple (*Acer pseudoplatanus* L.) is a broadleaved forest tree species with a wide natural distribution area in Central Europe (Pasta et al., 2016), which has rarely been in the focus of population genetic studies. Within its native range, it commonly grows on moist, cool and nutrient-rich sites, often on calcareous geological substrate, and can establish itself on steep rocky slopes, screes and ravines (Ellenberg and Leuschner, 2010). Late-frost tolerance and a robust root system are

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among the properties which apparently increase its competition ability on such sites (Schmidt and Roloff, 2009). In general, it is a rather minor tree species, mostly occurring in mixed broadleaved or conifer-broadleaved stands, whereas its abundance tends to increase at higher altitudes (Hein et al., 2008; Schmidt and Roloff, 2009). There, it forms late successional subalpine forests together with the common beech (*Fagus sylvatica* L.), particularly under oceanic influence (e.g. Vosges Mountains, Black Forest; Ellenberg and Leuschner 2010).

Despite its preference for cool, mountainous sites, sycamore maple must have reached its current native range relatively early during the Holocene. Macrofossil samples have provided evidence about its widespread presence in Southwestern Germany during the Atlantic period, about 8.000–5.000 years before present (Firbas, 1949). However, a refinement of its post-glacial history based on palynological studies is hindered (1) by low pollen production resulting in low occurrence of pollen in lake or peat bog sediments and, therefore, in the pollen records and (2) by the fact that sycamore maple pollen is not distinguishable from other maple species represented in Europe (Huntley and Birks, 1983). Thus, conclusions based on this approach can be drawn only at the genus level. As shown by palynological studies, the first considerable amount of *Acer* sp. pollen in records north of the Alps was found about 10.000 years ago with a northward spread in the following 2.000–3.000 years (Brewer et al., 2017).

More details about the origin of sycamore maple in Europe have been revealed by phylogenetic studies. Sycamore maple displays a higher number of different chloroplast DNA (cpDNA) haplotypes and a higher genetic differentiation in southern Europe (Aguinagalde et al., 2005; Bittkau, 2002; Petit et al., 2003). Four refugia were proposed in Southern Europe: one on the Balkan peninsula, one in Northern Greece, one in southern Italy and one in Corsica, each representing a different maternal lineage (Bittkau, 2002). On the contrary, a single, separate maternal lineage is widespread in Central Europe (Germany and Eastern France) including the northern, western and southern sides of the Alps, suggesting (1) a common origin of these populations from a refugial site in or near the Alps and (2) that the aforementioned southern European refugial populations did not contribute to the recolonization of Central Europe except for the area southeast of the Western Carpathian Mountains which might have been colonized by a Balkan origin (Bittkau, 2002).

After recolonizing its current natural distribution, sycamore maple adapted to the local climatic conditions, which has been shown by provenance and progeny tests (Neophytou et al., 2017; Weiser, 1996). In addition, it was actively introduced by humans beyond the northern limit of its native range and is now naturalized, for instance, in Sweden and Britain (Pasta et al., 2016). Currently, it shows a tendency to further spread into sites with nutrient-rich soils (especially with high nitrogen content), former riparian hardwood forests (after flood regulation has led to soil drainage), as well as disturbed (e.g. ruderal) sites (Binggeli, 1992; Collet et al., 2008; Jensen et al., 2008; Reif et al., 2016). Given (1) its good performance over a wide range of site conditions, (2) the ease of silvicultural treatment, (3) desirable wood properties, and (4) its potential to replace common ash (*Fraxinus excelsior* L.), whose populations have been decimated due to ash dieback on some sites, there is a growing interest for using sycamore maple in forestry. Thus, breeding programs have been initiated (Jánosi et al., 2017; Krabel and Wolf, 2013). In this context, the question of population genetic structure is gaining in importance. This applies both to procurement of provenance-certified forest reproductive material and breeding (Morgenstern, 2011).

Here, we take advantage of a wide-range selection of plus-trees in the frame of a German nationwide tree breeding programme (Jánosi et al., 2017), in order to investigate the spatial distribution of the species' genetic variation. The selected population covers a large part of the native range in Central Europe (mainly Germany), as well as areas where sycamore maple has been presumably introduced. First, we address the question of the origin of sycamore maple in a potential crossroads of its post-glacial migration. Second, we assess genetic variation across the landscape, as a result of its post-glacial recolonization history. Third, we compare our results with the current delineation of sycamore maple into regions of provenance in Germany.

2. Materials and methods

2.1. Sampled trees

Our sample consisted of 1043 plus trees. These included (1) newly selected plus trees in the field, (2) superior individuals selected within half-sib families or provenances in progeny tests or provenance trials, respectively, and (3) clones of plus trees from seed orchards, which had been selected previously. All trees were georeferenced. In cases (2) and (3) the geographic coordinates of the origin (not of the field trial) were assigned to the trees. 1031 of the plus trees were from Germany, while 12 trees belonged to half-sib families of trees from Austria (4 trees), Czechia (4 trees), Denmark (3 trees) and Switzerland (1 tree). Buds were collected from all trees and sent to the laboratory for DNA isolation.

2.2. Laboratory procedures and marker scoring

We extracted DNA using an ATMAB (alkyltrimethylammonium bromide) extraction protocol described in Dumolin et al. (1995). After DNA isolation, we carried out multiplex or simplex polymerase chain reactions (PCR) to amplify one chloroplast (cpSSR) and 11 nuclear microsatellite (nSSR) loci. Five of the nSSR loci have been developed specifically for *A. pseudoplatanus*, six were transferred from other *Acer* species (Table 1). For each PCR reaction we used fluorescent labelled primers in a mixture of 10 µl total volume containing 1 X reaction buffer (Qiagen), locus-specific primer concentration (Table 1) and about 20 ng template DNA. The amplified loci, PCR-multiplex combination, as well as concentration and fluorescent dyes of the primers are presented in Table 1. PCR programs included following steps: initial denaturation at 95 °C for 15 min, 30 cycles with denaturation at 94 °C for 30 s, followed by primer annealing at 58 °C for 90 s and then extension at 72 °C for 30 s, and ending with a final extension step at 60 °C for 30 min. We determined the length of the PCR fragments by using an automated sequencer (CEQ8000 Beckman Coulter) based on an internal size standard. We carried out fragment length determination and allele assignment using the fragment analysis tool of CEQ8000 (Beckman-Coulter).

Given that sycamore maple is tetraploid (Darlington and Wylie, 1955; Pandey et al., 2004), we expected a tetrasomic inheritance pattern, i.e. up to 4 alleles per locus within each individual. However, only 5 out of 11 loci displayed this inheritance pattern. At the remaining loci, a disomic inheritance pattern was observed (i.e. up to two alleles per locus for each individual). After scoring, we exported the genotype tables and proceeded with population genetic analysis. For loci with tetrasomic inheritance, we reported presence-absence of each allele at this stage. The maximum number of alleles per individual and locus and the allele length range for each locus are presented in Table 1.

2.3. Data analysis: origin and population structure

In order to investigate the origin of the sycamore maple across Germany we plotted on a map: (1) the haplotype frequencies at the chloroplast DNA locus ccmp10 and (2) the proportion of membership to each of the clusters defined with use of a Bayesian cluster analysis based on the 11 genotyped nuclear microsatellites. In order to prepare the input file, we used the package POLYSAT in R using the function write.structure (Clark and Jaseniuk, 2017).

We performed the Bayesian clustering applying the STRUCTURE method (Pritchard et al., 2000; Falush et al., 2007). We used the program STRAUTO v1.0 which allows automation and parallelization of