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Upper Rhine Valley: A migration crossroads of middle European oaks

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ABSTRACT

The indigenous oak species (*Quercus* spp.) of the Upper Rhine Valley have migrated to their current distribution range in the area after the transition to the Holocene interglacial. Since post-glacial recolonization, they have been subjected to ecological changes and human impact. By using chloroplast microsatellite markers (cpSSRs), we provide detailed phylogeographic information and we address the contribution of natural and human-related factors to the current pattern of chloroplast DNA (cpDNA) variation. 626 individual trees from 86 oak stands including all three indigenous oak species of the region were sampled. In order to verify the refugial origin, reference samples from refugial areas and DNA samples from previous studies with known cpDNA haplotypes (chlorotypes) were used. Chlorotypes belonging to three different maternal lineages, corresponding to the three main glacial refugia, were found in the area. These were spatially structured and highly introgressed among species, reflecting past hybridization which involved all three indigenous oak species. Site condition heterogeneity was found among groups of populations which differed in terms of cpDNA variation. This suggests that different biogeographic subregions within the Upper Rhine Valley were colonized during separate post-glacial migration waves. Genetic variation was higher in *Quercus robur* than in *Quercus petraea*, which is probably due to more efficient seed dispersal and the more pronounced pioneer character of the former species. Finally, stands of *Q. robur* established in the last 70 years were significantly more diverse, which can be explained by the improved transportation ability of seeds and seedlings for artificial regeneration of stands during this period.

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1. Introduction

The Upper Rhine Valley is one of the major geographic features in the western part of Central Europe. As a biogeographical region, it displays a relatively wide variety of ecological conditions, hosting diverse vegetation communities, which include several types of broadleaved forests (Oberdorfer, 1992). Due to favorable climate and topography, human settlement in the area began early in the Holocene and land use dates back to at least 7500 years before present (BP; Lang et al., 2003; Zolitschka et al., 2003; Houben et al., 2006). Oaks (*Quercus* spp.) have been both an important member of the natural vegetation communities and a source of timber and other non-wood products for humans during much of the Holocene (since 10,500 years BP). The genus *Quercus* is represented by three species of white oaks (section *Quercus* based on recent taxonomic studies; Denk and Grimm, 2010) in the area. *Quercus robur*, being able to tolerate waterlogging, mainly occupies lowland areas flanking the river banks (Aas, 2008a). On the other hand, *Quercus petraea* requires well drained soils and thus is more abundant in hilly areas like the piedmont of the Vosges Mountains (Aas, 2008b). The third species, *Quercus pubescens*, is a submediterranean vegetation element. In the Upper Rhine Valley, it has a marginal distribution occurring in exceptionally dry sites with alkalic soils (Oberdorfer, 1992; Bussotti, 2008).

As many arboreal elements of the regional flora, oaks migrated to the Upper Rhine Valley soon after the end of the last ice age, during the Preboreal period (first occurrence about 9500–9000 years BP), originating from refugial areas around the Mediterranean Sea (Brewer et al., 2002). Genetic evidence about the refugial origin and post-ice-age recolonization routes of Central European oaks has been provided by a large number of studies conducted in the last two decades, mostly based on chloroplast DNA (e.g. Petit et al., 1993, 2002a; Deguilloux et al., 2004). In general, it has been shown that oak populations in Central Europe share their chloroplast DNA haplotypes (chlorotypes) with regions around the Mediterranean, which hosted refugial populations during the last glacial. The genetic differentiation among refugial sites agrees with the hypothesis that the main maternal lineages of cpDNA arose there during the last glacial (or even earlier) under the effects of genetic drift and mutation (Kremer and Petit, 1993; Petit et al., 2002a). The longitudinal distribution of the different maternal lineages reflects a general south-to-north direction of post-glacial recolonization pathways (Petit et al., 2002a; Slade et al., 2008).

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Moreover, the general lack of further genetic variants confined to non-refugial regions supports that no significant genetic differentiation has happened during the current interglacial (Petit et al., 2002a).

Post-ice-age migration history has largely determined regional cpDNA diversity levels of the Central European oaks. High cpDNA diversity arose where different recolonization routes met (Csaikl et al., 2002; Petit et al., 2002b). On the other hand, seed mediated gene flow, being restricted by the barochoric seed dispersal of oak acorns, contributed to a limited within-population differentiation, but strong phylogeographic structure in terms of cpDNA (Ennos, 1994; Petit et al., 2004). An additional major factor affecting cpDNA variation in oaks is hybridization. Due to linkage equilibrium, chlorotypes can be exchanged among interfertile species after an initial hybridization event and a number of successive backcrossings, (Kremer and Petit, 1993). A wide scale sharing of cpDNA geographic patterns supports historical interbreeding among members of the European white oaks (Petit et al., 2002b). Finally, human management may have also had important consequences on cpDNA variation and spatial distribution. Through seed transfer and creation of artificial stands, human has intervened into seed mediated cpDNA gene flow, by increasing it and probably by contributing to a decrease of differentiation among populations (König et al., 2002; Gailing et al., 2007). This human impact is expected to be strong in areas with an early human settlement and intensive land use, like the Upper Rhine Valley.

In the present study, we focus on the phylogeography of the indigenous oaks in the Upper Rhine Valley based on chloroplast DNA markers. First, we investigate the refugial origin of the stands and the spatial distribution of the different genetic variants in a region with complex migration history. Second, we analyze the genetic variation and its distribution within and among populations and we compare its patterns between *Q. robur* and *Q. petraea*, taking their ecological and physiological differences into account. Third, we address the human influence on the cpDNA distribution of the oaks, by putting a special emphasis on the consequences of recent forest management.

2. Materials and methods

2.1. Study area and sample collections

The study area comprises the Upper Rhine Valley, delimited by the Vosges Mountains to the west, the Jura Mountains to the south and the Black Forest to the east. The northern limit of the study area was put along the border between Alsace (France) and Rhineland-Palatinate (Germany), along the Rhine (separating Rhineland-Palatinate from Baden-Württemberg) and between Baden-Württemberg and Hessen. The sampled forest stands mostly occurred in lowland sites and to a lesser extent in the foothill zone. A total of 626 trees from 86 oak stands were sampled. The sample was representative of the whole geographic region and covered the whole amplitude of site conditions. Individuals of all three indigenous oak species of the area – *Q. robur*, *Q. petraea* and *Q. pubescens* – were included. Classification into species groups or intermediates was made based on morphological (field characterization) and genotypic data (Bayesian clustering analyses based on 21 nuclear microsatellite loci; results not shown here). In 13 cases, individuals of more than one species were sampled from the same stand. Due to the fact that *Q. robur* is more widespread in the area, it was represented by a higher number of individuals and stands. In total, 314 individuals of *Q. robur* were collected from 63 stands compared to 214 *Q. petraea* individuals from 39 stands and 90 *Q. pubescens* individuals from six stands. In addition, sampling included 8 hybrid forms found in seven different stands. Leaf material or buds were collected from each tree. Information about the age of the stands was gathered based on forest local inventory data.

With respect to their site and climatic conditions, the sampled stands were subdivided into three biogeographical subregions (Table 1). A further categorization was made concerning the age of the stands. Again three categories were defined: (1) Stands of age up to 70 years, which have originated after the Second World War, (2) stands of age from 100 to 130 years, due to the fact that seed transportation by rail in Central Europe reached a maximum in this period (König et al., 2002; Gailing et al., 2007), and (3) stands older than 130 years. Stands with an age between 70 and 100 years were not represented in our sample, probably due to reduced plantation activity between the First and the Second World War. Details about the sample stands are included in the supplementary material (Supplementary data in electronic version; Table S1).

In addition to the sampled stands in the Upper Rhine Valley, 71 trees from 11 oak stands in the Iberian Peninsula (Navarra and Basque Country), Italy (Sardinia and Central Italy) and the Balkan Peninsula (Central Bulgaria and Northern Greece), representing refugial sites, were included in the sample. Finally, seven DNA reference samples representing the most common central European haplotypes of white oaks (section *Quercus* according to a recent study of Denk and Grimm (2010)), kindly provided by the INRA Bordeaux, were used as a comparison. These samples possessed known haplotypes, previously described in Dumolin-Lapègue et al. (1997) and Petit et al. (2002a).

2.2. Laboratory procedures

After sample collections, plant material was transferred to the laboratory and frozen at −80 °C. Subsequently, it was freeze-dried in vacuum and DNA was extracted using the DNeasy 96 extraction kit (Qiagen, Hilden, Germany). Polymerase Chain Reactions (PCR) were carried out for the amplification of 10 chloroplast DNA microsatellites (cpSSRs). These were chosen according to a previous phylogenetic study in oaks (Deguilloux et al., 2004). The analyzed loci included ccmp2, ccmp6 and ccmp10, designed on *Nicotiana tabacum* (Weising et al., 1999), and μcd4, μcd5, μdt1, μdt3, μdt4, μkk3 and μkk4, designed on *Q. petraea* and *Q. robur* (Deguilloux et al., 2003). Primers from these loci were fluorescently labeled with blue (FAM; loci ccmp10, μdt1 and μcd4), green (HEX; loci ccmp2, ccmp6, μdt3 and μdt4) or yellow color (Atto550; μcd5, μkk3 and μkk4) and were mixed in one multiplex. For the PCR reaction, the SuperHot Mastermix (Genaxxon, Biberach, Germany) a premixed mastermix including all PCR components except DNA template and primers was used. Reaction volume was set to 10 μl, comprised of 5 μl reaction mastermix (2×), 1 μl of primer mix (0.2 μM of each primer pair in the mastermix), 2 μl water and 1 μl diluted DNA (ca. 4 ng/μl). The PCR-programme included following steps: (1) denaturation at 95 °C for 15 min; (2) 30 cycles with a denaturation step at 94 °C for 30 s, primer annealing at 46 °C for 1 min and 30 s and an elongation step at 72 °C for 30 s; (3) Elongation at 72 °C for 10 min and (4) a final step at 60 °C for 30 min. Allele scoring was performed by means of a capillary electrophoresis using an ABI Prism 3100 genetic analyzer and the software GeneMapper (Applied Biosystems). Repeats of capillary electrophoresis were carried out by reanalyzing representative subsamples from different 96-well plates together the reference samples of the most common central European haplotypes of white oaks on a single 96-well plate.

2.3. Data analysis

Chlorotypes were defined as different combinations of alleles at the 10 analyzed cpSSR loci. For chlorotype designation, loci were sorted alphabetically and alleles were sorted by increasing size.

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