Tracing the origin and species identity of *Quercus robur* and *Quercus petraea* in Europe: a review

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Abstract

Traceability of forest material has received recently increasing interest and European regulations already apply on forest reproductive material and timber. DNA fingerprinting methods allow identification of species and control of geographic origin, providing that genetic reference data is available. In this review, we focus on the two economically important European oak species, *Quercus robur* and *Q. petraea*. We describe the available molecular markers and data, and discuss their applicability for traceability systems of forest reproductive material at a European scale. We also provide insights on the use of DNA fingerprinting on timber material.

Keywords: *Quercus*, oak, molecular markers, traceability, Europe, forest reproductive material, timber

Introduction

During the last decades, there has been increasing interest in conservation and use of genetic resources in Europe. In particular, new European regulations imply that traded forest reproductive material (EU Council Directive 1999/105/EC; German Act on Forest Reproductive Material) and timber (EU Timber Regulation 995/2010) are of known origin. Traceability of forest reproductive material (FRM) is of significant importance, as it might avoid afforestation with maladapted material and therefore prevent the establishment of material unsuitable for the local environmental conditions. In some countries like Germany, traceability systems have been already set up to control seed lots at different steps along the chain of custody (Konnert, 2006; Konnert and Hussendörfer, 2002). In this paper, we review and discuss existing DNA analysis methods and protocols which are in use for species identification and geographic traceability in the two European white oak species *Quercus petraea* (Matt.) Liebl. (sessile oak) and *Q. robur* L. (pedunculate oak). As identification of single logs is not relevant for the trade of white oaks, we do not discuss methods for individual identification. Furthermore, our aim is to provide guidelines for white oak tracing at the European scale.

Quercus robur and *Q. petraea* are common species in European temperate forests. Their distribution range is wide, from Ireland to Ukraine and from northern Spain to Southern Scandinavia (Ducousso and Bordacs, 2003), while *Q. petraea* does not occur naturally as far in the northeast as *Q. robur* (Aas, 2000, 2002). Reproductive maturity is reached at 20 to 40 years, and trees can live at least up to 800 years (Aas, 2000, 2002). The two European white oak species have the longest target rotation period of 160 years and more (Krahl-Urban, 1959). Both species are monoecious, anemophilous and mostly outcrossing (Chybicki and Burczyk, 2013; Gerber et al., 2014; Lagache et al., 2013). Pollen dispersal kernels indicate both localized dispersal (mean 70–120 m) as well as fat tails representing long distance dispersal events, which translate in high rates of immigrant pollen within the studied stands (Buschbom et al., 2011; Chybicki and Burczyk, 2013; Gerber et al., 2014; Lagache et al., 2014; Streiff et al., 1999). Seed dispersal is more restricted but long-distance dispersal events have also been inferred (Gerber et al., 2014; Petit et al., 1997; Streiff et al., 1998). Vegetative propagation has been enhanced in the past through coppicing for regeneration purposes. However, current management regimes favour natural regeneration and plantation, which are based on generative propagation. Therefore, traceability of forest reproductive material needs to be focused on seeds. Hybridization among *Q. robur* and *Q. petraea* in mixed stands has been reported in

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many studies, but with some asymmetry due to stronger interspecific sexual barriers in *Q. petraea* (Chybicki and Burczyk, 2013; Curtu et al., 2007a; Gerber et al., 2014; Lagache et al., 2013, 2014; Lepais and Gerber, 2011), although signals of past asymmetric introgression toward *Q. petraea* could be observed at loci with high interspecific differentiation (Guichoux et al., 2013). Furthermore, both species seem to actively diverge from each other (Goicoechea et al., 2012), which probably results in successful morphological and genetic identification of pure and hybrid individuals (Chybicki et al., 2010; Curtu et al., 2007a, b; Fortini et al., 2015; Gugerli et al., 2007; Guichoux et al., 2011, 2013; Neophytou, 2014; Neophytou et al., 2015; Yücedag and Gailing, 2013).

Q. robur and *Q. petraea* are very common species in Europe, for which intensive seed transfer occurs. Oak timber is mostly used for building, furniture and barrel. Timber trade requires that the origin of the material is known, especially for certified timber from sustainably managed forests (for instance Forest Stewardship Council) and existing traceability systems are mostly based on paper documents, which could be falsified. Traceability systems based on DNA fingerprints, or at least controls along the chain of custody, could be useful to control declarations and thereby complement the existing traceability systems in order to avoid the plantation of maladapted seed stocks and the trade of illegally logged timber. Because existing studies on genetic traceability of white oaks are focusing on a small geographical scale (Degen et al., 2010; Konnert, 2006; Konnert and Hussendörfer, 2002) and do not take advantage of the large genetic data available in these species, we first review the available genetic methods and address their potential use for traceability purposes. Secondly, we discuss how a reliable, powerful, cost-effective and standardized genetic traceability system could be developed.

General overview of existing DNA extraction protocols and genetic markers for the species

Material analysed and DNA extraction

DNA has been successfully isolated from diverse material such as leaf (e.g. Dumolin et al., 1995), bud (e.g. Deguilloux et al., 2003b), cambium (e.g. Degen et al., 2010), the woody pericarp of the acorns (Ziegenhagen et al., 2003), as well as timber (Deguilloux et al., 2002, 2003b, 2004). DNA extraction protocols of wood material included a modified CTAB method (Dumolin et al., 1995) and the use of commercial DNA extraction kits like the Qiagen Plant Minikit (Deguilloux et al., 2002).

Molecular markers available

Many authors report genetic variation at plastid markers, particularly in the chloroplast genome, as it allows studies at the continent level. Four universal cpDNA fragments have been extensively studied and has shown a longitudinal genetic differentiation resulting from postglacial colonization patterns over Europe (Petit et al., 2002a, b; see also *Table 1*) as well as variation at the regional level (Ballian et al., 2010; Bordacs et al., 2002; Cottrell et al., 2002; Csaikl et al., 2002a, b; Fineschi et al., 2002; Jensen et al., 2002; König et al., 2002; Olalde et al., 2002; Petit et al., 1997, 2002c; Slade et al., 2008). Genotyping was strongly facilitated by the development of PCR-RFLP techniques, which avoids expensive Sanger sequencing for genotyping at particular SNPs (Dumolin-Lapègue et al., 1998; Petit et al., 2002b). The combination of these chloroplast markers does not allow unambiguous identification of origin, as complex patterns occur at the regional level, but allows conformity testing (Deguilloux et al., 2003b; Kelleher et al., 2004; Lowe et al., 2004; Gailing et al., 2007a, b). As cpDNA evolves clonally, there are similarities whatever the marker system used although the spatial resolution might differ. Some authors have used chloroplast microsatellites (Gailing et al., 2007a, b) developed by Deguilloux et al. (2003a) or universal markers (Sebastiani et al., 2004; Weising and Gardner, 1999) to adress the origin of a planted stand or to genetically characterize a specific region (*Table 1*). Haplotypes derived from the cpSSRs and PCR-RFLP are strongly related, and cpSSRs show many rare alleles which could be used at a local scale for traceability purposes (Chmielewski et al., 2015).

By contrast, only a few studies report variation at mitochondrial markers (Dumolin-Lapegue et al., 1998, 1999). Mitochondrial DNA is maternally inherited in oaks, like cpDNA,

and both genomes were shown to provide largely congruent results (DUMOLIN-LAPEGUE et al., 1998).

At the regional or local scale, nuclear markers are needed to study genetic structure. Up to 13 isozyme loci have been used for genetic studies in *Quercus* (DEGEN et al., 1999; HERTEL and DEGEN, 2000; KONNERT et al., 2004; MÜLLER-STARCK et al., 1993; ZANETTO et al., 1994). These markers revealed a broad genetic structure despite low levels of among-population differentiation (ZANETTO et al., 1994). However, GREGORIUS genetic distances were found to be high among seed lots in Germany (MÜLLER-STARCK et al., 1993). As expected, microsatellites (SSRs, Single Sequence Repeats) have a higher genetic diversity (e.g. DEGEN et al., 1999), which provides more resolution. Indeed, two nuclear multiplex sets of microsatellite loci [one 8-plex nSSRs set (nuclear) and a 12-plex eSSRs set (Expressed Sequence Tags SSRs (EST)] were recently developed by GUICHOUX et al. (2011), and were optimized for reproducibility and absence of null alleles. nSSRs have been substantially used for pollen flow studies, as their strong polymorphism allows reliable paternity estimation (BUSCHBOM et al., 2011; LAGACHE et al., 2013; LEPAIS and GERBER, 2011; STREIFF et al., 1999). Also many nSSRs have been until now the markers of choice to study the mating system, pollen dispersal and hybridization (BUSCHBOM et al., 2011; CHYBICKI and BURCZYK, 2013; CURTU et al., 2007a; GERBER et al., 2014; LAGACHE et al., 2013, 2014; LEPAIS and GERBER, 2010; PETIT et al., 1997; STREIFF et al., 1998, 1999). Available nSSRs loci for *Q. petraea* and *Q. robur* are reviewed in *Table 2*.

Table 1. – Organelle molecular markers available for *Q. robur* and *Q. petraea* for traceability purposes.

Fragment	Type	Genotyping method	Spatial scale	Reference
*trn*D-*trn*T	Chloroplast	PCR-RFLP	Europe/Region	BALLIAN et al., 2010; DEGUILLOUX et al 2004; GAILING et al., 2003, 2007a, b; PETIT et al., 1997, 2002a, b; SLADE et al., 2008
psaA-trnS	Chloroplast	PCR-RFLP	Europe/Region	BALLIAN *et al.,* 2010; GAILING et al., 2003, 2007b; PETIT et al., 2002a, b; SLADE et al., 2008
psbC-TrnD	Chloroplast	PCR-RFLP	Europe/Region	BALLIAN et al., 2010; PETIT et al., 2002a, b; SLADE et al., 2008
trnT-trnF	Chloroplast	PCR-RFLP	Europe/Region	BALLIAN et al., 2010; GAILING et al., 2003; PETIT et al., 1997, 2002a, b; Slade et al., 2008
trnC-trnD	Chloroplast	PCR-RFLP	Region	DEGUILLOUX et al 2004; FINESCHI et al., 2002; GAILING et al., 2003, 2007b; OLALDE et al., 2002
trnL (UAA) 3'- trnF (GAA)	Chloroplast	PCR-RFLP	Region	DEGUILLOUX et al 2004; GAILING et al., 2007a
ccmp2, ccmp6, ccmp10	Chloroplast	Fragment length	Region	CHMIELEWSKI et al . 2015; GAILING et al., 2007b; NEOPHYTOU and MICHIELS, 2013; WEISING and GARDNER 1999
µcd4, µcd5, µdt1, µdt3, µdt4, µkk3, µkk4	Chloroplast	Fragment length	Region	CHMIELEWSKI et al . 2015; DEGUILLOUX et al., 2003a, 2004; GAILING et al., 2007a, b; NEOPHYTOU and MICHIELS, 2013
cmcs5, cmcs6, cmcs7, cmcs8, cmcs9, cmcs12				CHMIELEWSKI et al . 2015; SEBASTIANI et al., 2004
nad4-1/2	Mitochondrial	PCR-RFLP	Region	DUMOLIN-LAPEGUE et al., 1998
nad4- 2/3	Mitochondrial	PCR-RFLP_SSCP	Region	DUMOLIN-LAPEGUE et al., 1998

Table 2. – Nuclear microsatellite loci available for *Q. robur* and *Q. petraea*.

Loci	Type	Developed by	References
QrZAG7, QrZAG112, QrZAG20, QrZAG96, QrZAG11, QrZAG39, QrZAG5b, QrZAG65, QrZAG87	gSSR	KAMPFER et al. (1998)	ALBERTO et al. (2010); DEGEN et al. (2010); DERORY et al. (2010) ; CURTU et al. (2007b) ; GUICHOUX et al. (2011); LEPAIS and GERBER (2011)
MsQ13	gSSR	DOW et al. (1995)	ALBERTO et al. (2010); CURTU et al. (2007b); DERORY et al. (2010) ; GUICHOUX et al. (2011)
QpZAG15, QpZAG110, QpZAG46, QpZAG9, QpZAG36, QpZAG1/5, QpZAG104	gSSR	STEINKELLNER et al. (1997)	ALBERTO et al. (2010); CURTU et al. (2007b); DEGEN et al. (1999); DEGEN et al. (2010); DERORY et al. (2010); GUICHOUX et al. (2011); LEPAIS and GERBER (2011);
PIE020, PIE223, PIE152, PIE242, PIE102, PIE243, PIE239, PIE227, PIE271, PIE267, PIE258, PIE215	eSSR	DURAND et al. (2010)	GUICHOUX et al. (2011); NEOPHYTOU et al. (2015); YUCEDAG and GAILING (2013)

In the last decade, next-generation sequencing methods allowed development of a large amount of SNP markers, for example to study adaptive variation at candidate genes (DERORY et al., 2010; GAILING et al., 2009). These markers have been recently used to differentiate the two oak species *Q. petraea* and *Q. robur* (GUICHOUX et al., 2013; VIDALIS et al., 2013) and showed higher resolution than eight microsatellite loci (GUICHOUX et al., 2013). The publication of the complete genome of one *Q. robur* reference sample will strongly facilitate the development of SNP markers in the next years (PLOMION et al., 2015) and a SNP array has been recently published (LEPOITTEVIN et al., 2015).

Allele scoring methods

The genotyping methods for fragment analysis were strongly improved over the last decades. In the past, agarose or polyacrylamide gels were used for the screening of SNP variation through PCR-RFLP (BALLIAN et al., 2010; CURTU et al., 2007b; DEGUILLOUX et al., 2003b, 2004; DUMOLIN-LAPÈGUE et al., 1998; GAILING et al., 2003, 2009; PETIT et al., 2002b; SLADE et al., 2008) or for microsatellite genotyping (BAKKER et al., 2003; DEGEN et al., 1999). Nowadays, separation on capillary sequencers allows multiplexing of up to 12 micrsatellite loci (ALBERTO et al., 2010; BUSCHBOM et al., 2011; DEGEN et al., 2010; DERORY et al., 2010; GAILING et al., 2007b; GUGERLI et al., 2007; GUICHOUX et al., 2011; HOELTKEN et al., 2012; LEPAIS and GERBER, 2011; VIDALIS et al., 2013), which reduces the genotyping costs and strongly improve the sensitivity in case of poor amplification. Besides Sanger sequencing (DERORY et al., 2010), new SNP genotyping methods have been tested, such as a GoldenGate assay (Illumina Inc.) (GUICHOUX et al., 2013), a Illumina Infinium iSelect Custom Genotyping Array (LEPOITTEVIN et al., 2015) or by a SNaPshot procedure running on an capillary sequencer (VIDALIS et al., 2013). Other methods, such as the MassARRAY technology are also available and are very promising for low-quality template DNA, such as timber (BLANC-JOLIVET, GUICHOUX, personal observation). A formal comparison of the different SNP scoring methods would be necessary to find the most appropriate technology for the screening of poor-quality DNA material, as it is often the case for material to trace.

Levels of differentiation and their use for traceability systems

Strong differentiation occurs throughout Europe at cpDNA markers, which were interpreted in the light of species history and post-

glacial recolonization routes (PETIT et al., 2002a; NEOPHYTOU and MICHIELS, 2013). Although a strong correlation has been observed between cpDNA and nuclear genetic variation (KREMER et al., 2002), pollen flow results in reduction of original differentiation pattern (FINKELDEY and MATYAS, 2003). Levels of differentiation at nuclear markers are on average low (G_{st} = 0.022 in *Q. robur*; G_{st} = 0.031 in *Q. petraea*) compared to chloroplast markers (G_{st} = 0.781 in *Q. robur*; G_{st} = 0.856 in *Q. petraea*) (PETIT et al., 2004). However, the high polymorphism at microsatellite markers probably results in the underestimation of differentiation (HEDRICK, 2005). Studies involving nuclear markers at a European scale are rare, as these markers require at least 30 samples per population and present problems of data standardization, therefore hindering collaboration among laboratories to set up large datasets. At set of 125 SNPs detected at nine candidate genes did not yield stronger among population differentiation than a set of 15 microsatellite loci (DEGEN, personal communication). As expected, variation in levels of differentiation was also observed among oak species, indicating that some species are more closely related than others (CURTU et al., 2007b).

Two different marker systems have been tested until now for traceability purposes in oak. The cpDNA marker system described by PETIT et al. (2002a) has been used to test the conformity of the declaration of origin of wood (DEGUILLOUX et al., 2003b). Given the low differentiation at the local scale and the tendency of oak species to share locally the maternal lineages, this system can typically only provide information on the region of origin, not on stand-level scale or on species. Other traceability systems are based on nuclear microsatellite markers and rely on the comparison of the genetic distance of the tested material (seed lot) with a reference sample from the declared stand of origin (KONNERT, 2006; KONNERT and HUSSENDÖRFER, 2002), or on assignment methods (DEGEN et al., 2010). The first method relies on the fact the genetic distance, based on eight microsatellite loci both for *Q. robur* and *Q. petraea*, among the test and reference samples is lower for the true stand of origin compared to other provenances. This traceability system is therefore very labour intensive, as sampling has to be repeated for each seed harvest. Further, this method has been implemented in forest tree species in Southern Germany, and little information is available on its statistical power. DEGEN et al. (2010) have shown that the origin of forest reproductive material (seedlings) can be successfully controlled with DNA fingerprints based on eight microsatellite loci. The genotypes of reproductive material were compared to data from adult individuals within the reference populations with assignment methods combined with exclusion tests (CORNUET et al., 1999; RANNALA and MOUNTAIN, 1997). The method was very powerful, as it allowed successful assignment at a very small geographic scale (DEGEN et al., 2010). Reference data based on microsatellite loci also provided good results for genetic traceability in other forest tree species, in particular to combat illegal logging. For example, theoretical assignment success was 51%, 80% and 90% for groups of one, two and three self-assigned samples at the forest concession level in the very low differentiated tropical species *Entandrophragma cylindricum* (JOLIVET and DEGEN, 2012). A blind test on timber samples also resulted in 86% correct control of the claim on the concession of origin. Similar results could be obtained with the Neotropical species *Swietenia macrophylla* (DEGEN et al., 2013), although the geographical scale was much larger and covered Central and Southern America. Genetic assignment methods are also commonly used on microsatellite data in plant (cannabis: HOWARD et al., 2009; palm: NAZARENO and DOS REIS, 2014), and animal species (cattle: MATEUS and RUSSO-ALMEIDA, 2015; salmon: GLOVER et al., 2010; red deer: FRANTZ et al., 2006) for traceability or forensic purposes. A similar method to classical genetic assignment, but including geographical information, has been successfully applied to identify the main poaching hotspots of elephant ivory (WASSER et al., 2015). The use of genetic assignment methods with microsatellite data is therefore very reliable, providing that reference data, at least from the focus area is available.

Until now, no reference data for geographic traceability purposes based on nuclear SNPs, has been published in oak, although some data has been gathered at 82 SNPs for four *Q. robur* stands across Europe (VENDRAMIN et al., unpublished). Although genetic differentiation within species was not higher than with microsatellites, the move towards the use of SNPs for traceability is underway, as shown by the use of

this marker type in several animal species (Bekkevold et al., 2015; Negrini et al., 2008; Ogden and Linacre, 2015), and even in human (O'Dushlaine et al., 2010). Screening of large amount of loci, followed by selection of loci showing high genetic differentiation and/or outliers (Bekkevold et al., 2015; Glover et al., 2010; Ogden and Linacre, 2015) might be a good strategy to develop cost-effective marker sets for genetic traceability. First results on *Larix* spp. are encouraging (Blanc-Jolivet et al., in preparation).

Discussion

Species identification

Although hybridization among white oak species occurs, it is generally rare in mixed stands (Curtu et al., 2007a; Gugerli et al., 2007). Phenotype variation such as leaf morphology and pubescence, and genetic analysis of adults and progenies among species occurring in sympatry showed that low inter-specific pollen-flow (Curtu et al., 2007a), backcross events (Lepais and Gerber, 2011), genetically controlled pollen discrimination (Lepais and Gerber, 2011), selection against hybrids at the juvenile stage (Gugerli et al., 2007) and post-mating prezygotic reproductive barriers (Abadie et al., 2012; Lagache et al., 2013) have been identified as potential mechanisms maintaining species integrity. The two species have contrasting environmental optima (Chybicki et al., 2010; Guichoux et al., 2013; Neophytou and Michiels, 2013), different reproductive strategies (Lagache et al., 2014) and within-stand environmental heterogeneity sometimes affects the spatial arrangement of the two species (Chybicki and Burczyk, 2013; Gugerli et al., 2007; Lagache et al., 2013, 2014). Further, signatures of divergent selection have been identified (Goicoechea et al., 2012).

Co-dominant markers (isoenzymes and microsatellites) have been successfully applied to distinguish oak species (Curtu et al., 2007a, b; Gomory, 2000; Gugerli et al., 2007; Guichoux et al., 2011, 2013; Hertel and Degen, 2000; Hoeltken et al., 2012; Lepais and Gerber, 2011). In contrast, cpDNA data provides virtually no information on species status for sympatric species (Curtu et al., 2007a; Neophytou and Michiels, 2013), suggesting that codominant nuclear markers are needed to indentify the species. Further, species identification in oak requires a substantial amount of data at microsatellite or at SNP loci over the distribution range, as geographic differentiation within species needs to be distinguished from between-species differentiation. Applying the set of loci as in Guichoux et al. (2013) over many well-dispersed European populations might be useful to build a reliable set of molecular markers for oak species identification of samples stemming from all Europe.

Another technical issue includes standardization of microsatellite data among laboratories. High polymorphism at most microsatellite loci (Alberto et al., 2010; Degen et al., 2010) will not facilitate the establishment of large-scale data. Well-validated markers with reference samples and procedures are needed for this purpose (Guichoux et al., 2011).

Geographical origin

As discussed above, cpDNA markers could be useful to control the region of origin at a broad scale, and to check the conformity of a given material to its population of origin (Deguilloux et al., 2003b; Petit et al., 2002b). However, at a local scale, only nuclear microsatellite markers allow a reliable control of the origin, as most cpDNA *Q. robur* and *Q. petraea* haplotypes are widely distributed across Europe, although some haplotypes are restricted to a particular region (e.g. Slavonia, Petit et al., 2002b; Gailing et al., 2007b). Microsatellite data for reference populations across the whole European range would therefore be needed to verify the geographical origin, at least for autochthonous material. This task would not be easy, as many populations are planted, and genetic exchanges between native forests and planted material probably occur (Gailing et al., 2007b; König et al., 2002; Neophytou and Michiels, 2013). In the frame of traceability of forest reproductive material where source stands are clearly identified, assignment methods applied on nSSRs data have however already provided very strong results (Degen et al., 2010). Estimation of exclusion probabilities for each putative stand of origin can reliably address whether the material possibly originates from one of several reference stands or from another stand (Cornuet et al., 1999). The second step is to find the most likely population among the stands exhibiting non-significant exclusion probability (Jolivet and Degen, 2012). Although many studies pro-

vided results on genetic assignment, only a subset used this two-step procedure (DEGEN et al., 2013; FRANTZ et al., 2006; HONJO et al., 2008; HOWARD et al., 2009; JOLIVET and DEGEN, 2012; MAUDET et al., 2002; NEGRINI et al., 2008), which provides high security in forensic applications, as the true origin might have not been sampled (MANEL et al., 2002). Other methods for exclusion testing include calculation of likelihood ratios (BEKKEVOLD et al., 2015; OGDEN and LINACRE, 2015). In other words, assignment methods associated with exclusion probabilities estimation can address the question – can the material originate from a given stand? – but the true origin will not be necessarily identified. However, in order to avoid a non-exclusion of too many reference populations or similar likelihood values, presence of genetic differentiation is required. Some studies addressed the power of the genetic assignment on their reference data to address its suitability for the questions raised. The authors concluded that several factors including strong genetic differentiation, polymorphism, number of reference samples per population and number of loci are affecting the outcome of assignment tests (BEKKEVOLD et al., 2015; GLOVER et al., 2009; HOWARD et al., 2009; MANEL et al., 2005; MAUDET et al., 2002). Also, the use of Bayesian clustering analysis to identity gene pools can substantially improve the accuracy of genetic assignment (BEKKEVOLD et al., 2015; GLOVER et al., 2009; MANEL et al., 2005; MATEUS and RUSSO-ALMEIDA, 2015) but identification at the stand level, as required for seed harvests, might be in this case not possible. Clustering analysis would therefore only be the method of choice for traceability at a regional level. A way to overcome this problem could be the use of group assignment, as shown in *Quercus robur* (DEGEN et al., 2010), *Entandrophragma cylindricum* timber (JOLIVET and DEGEN, 2012) and in *Prunus avium*, where seed stand of origin could be assigned with 99% accuracy when assignment testing was conducted on groups of five individuals (BLANC-JOLIVET, unpublished). Such very reliable methods should thus provide cost-effective ways to verify the origin of FRM.

Recently developed molecular marker development (RAD sequencing) and genotyping methods (MassARRAY) will facilitate the use of nuclear SNPs in genetic traceability systems. As shown by GUICHOUX et al. (2013), discrimination among *Q. robur* and *Q. petraea* was higher at 262 SNPs selected for high interspecific differentiation than at 12 SSRs. Efficient marker detection and screening methods will allow the setup of SNPs sets containing a strong geographical signal (OGDEN and LINACRE, 2015). Another advantage of SNP over SSR data is the straightforward standardization among laboratories and genotyping methods, which should make possible the development of large-scale datasets for reference stands.

During the last decades, tracing back timber to its region of origin has raised much interest as control tool against illegal logging. DNA from wood samples is highly fragmented and degraded, causing technical problems for DNA extraction and amplification. Template contamination is also frequent. Amplification of nuclear microsatellites in wood is complicated by allele drop-in and drop-out, contamination and non-amplification (BLANC-JOLIVET, personal observation, but see GILL et al., 2012). Also, cpSSRs which are based on fragment size variation of 1 bp should be avoided due to frequent shifts and/or genotyping errors during fragment analysis, which are sometimes related to the Taq Polymerase used for the PCR reaction (BLANC-JOLIVET, personal observation). Therefore, when chloroplast DNA is used, SNP markers or indels, which could be scored by PCR-RFLP and/or fragment analysis should be favoured. Further, allele identification is sometimes difficult due to low signal quality resulting in slight shifts. The use of well-designed SNP sets might therefore be an alternative to the use of SSR markers, as genotyping errors due to stuttering and shifts are impossible. Most researchers in the field thus concentrate on cpDNA, which is present in more copies per cell, resulting in higher and more reliable amplification. The traceability system based on cpDNA was successfully tested on wood material (DEGUILLOUX et al., 2004). Statistical tools only include conformity tests, which relies on available data on the population of origin and can only answer the question: is the genotype of the material conform to the genetic data from its declared origin? By contrast, assignment methods provide information on the most likely population of origin, providing that reference data exists, and can be used to control logs without any declaration. Unfortunately, most assignment methods have not been adapted yet to chloroplast data, which often violate the assumption of non-independency of loci. A new

method combining markers into haplotypes seem to be promising, because the assumptions of independency and linkage equilibrium among loci are not required (DUFORET-FREBOURG et al., 2015). However, high fragmentation of data due to amplification failure from timber samples might not allow haplotype construction.

Conclusion

This review shows that a lot of genetic data is available for *Quercus*, and that two markers systems can be used for traceability studies. One system includes cpDNA markers characterized over the whole distribution range in Europe, while the other uses nuclear co-dominant markers with only limited information across the European distribution range. Although statistical tools have been well implemented and successfully tested at low spatial scale for microsatellites, this type of data requires strong standardization among laboratories. In fact, fragment size varies among machines and binning of alleles might therefore be different among laboratories. Furthermore, the high polymorphism in *Q. robur* and *Q. petraea* at this type of markers might exacerbate these difficulties. Intensive work is thus needed on the standardization of microsatellite data. Alternatively, SNP data might become the marker of choice to trace back fresh FRM material (seeds, seedlings, adults) and perhaps eventually wood material to its stand of origin, providing that the number of loci to screen is not too large. Efficient marker selection methods might support the development of markers sets for each specific traceability question.

The marker system based on cpDNA is currently the most suitable to trace low-quality DNA material such as timber. However, this marker system should be further developed to increase spatial resolution based on SNP markers or indels over Europe, to shorten DNA fragment size in order to increase amplification success, to find allele redundancies which would help overcoming the problems with poor DNA amplification on timber and to allow multiplexing of loci. Next-generation sequencing technologies could be useful to detect spatially structured polymorphism in the chloroplast, mitochondrial or nuclear genomes. However, more work is required on statistical tools to allow the development of powerful tests on haplotype data. Robust SNP genotyping methods such as the MassARRAY technology might make also the use of nuclear markers possible for timber tracking, which would overcome the statistical and lack of polymorphism problems of plastid markers.

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