Novel perspectives in wood certification and forensics: dry wood as a source of DNA

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The importance of wood for human societies can hardly be understated. If dry wood were amenable to molecular genetic investigations, this could lead to major applications in wood forensics, certification, archaeology and palaeobotany. To evaluate the potential of wood for molecular genetic investigations, we have attempted to isolate and amplify, by PCR, DNA fragments of increasing size corresponding to all three plant genomes from different regions of 10 oak logs. Stringent procedures to avoid contamination with external DNA were used in order to demonstrate the authenticity of the fragments amplified. This authenticity was further confirmed by demonstrating genetic uniformity within each log using both nuclear and chloroplast microsatellites. For most wood samples DNA was degraded, and the sequences that gave the best results were those of small size and present in high copy number (chloroplast, mitochondrial, or repeated nuclear sequences). Both storage conditions and storage duration play a role in DNA conservation. Overall, this work demonstrates that molecular markers from all three plant genomes can be used for genetic analysis on dry oak wood, but outlines some limitations and the need for further evaluation of the potential of wood for DNA analysis.

Keywords: woody tissues; PCR; DNA retrieval; contaminations; genotyping

1. INTRODUCTION

Despite continuous deforestation during the past millennia, an estimated 27% of the terrestrial surface of the Earth is still covered by forests, representing some 380 billion m³ of wood (FAO World Resources 2000–2001). Wood remains are by far the most abundant biomass available for human activities, even if the claim that the highest percentage of the Earth's biomass must lie in the wood of plants has been challenged (Gold 1992).

In humans and animals (Hardy *et al.* 1995; Bailey *et al.* 1996; Hagelberg 1997; Krings *et al.* 1997), remains such as bones have been shown to provide an appropriate source of DNA for forensics and for a range of evolutionary studies, but the retrieval of DNA from the much more abundant wood of forest trees has never been studied extensively. The presence in wood of substances that strongly inhibit PCR (Lee & Cooper 1995) and the general problems of contamination encountered when attempting to amplify degraded DNA from dead tissues (Kwok & Higuchi 1989) have been barriers to these applications. Studies involving the isolation and amplification of DNA from fresh wood are still rare (De Filippis & Magel 1998; White *et al.* 2000). In the case of dry wood, the only report, to our knowledge, is the joint demonstration by two groups that chloroplast DNA can be successfully recovered from dry oak wood using PCR (Dumolin-Lapègue *et al.* 1999). This prompted us to test more systematically the potential of dry oak wood as a source of DNA.

The feasibility of genetic analysis on dry wood could have major applications in wood ecocertification, foren-

sics, archaeology and palaeobotany. Timber identification (of tree species or of geographical origin) is a technical prerequisite for monitoring and controlling wood trade or to control the chain of custody in the context of certification.

Species identification based on wood anatomy is difficult and sometimes impossible in the case of taxonomically related species that have similar wood structure. Chemical composition of trees, which depends on the tree's environment but also part of its genotype, can sometimes allow us to check the conformity with its inferred taxonomic identification or place of origin (Marco *et al.* 1994; Medina & Vivas 2000), but the high level of individual variability makes assignment difficult. If techniques were available, molecular markers could be used, alone or more probably in a complementary manner with already existing methods. Taxonomic identification will necessitate databases of nuclear or cytoplasmic markers discriminating the species of interest (Chen & De Filippis 1996). Controlling the geographical origin of wood will require DNA markers showing enough geographical structure as well as careful genetic description of the stands, forests or regions where the wood may have been harvested, in the form of detailed reference maps. Maternally inherited DNA markers may be particularly suited for testing conformity with geographical origin, because they are known to be highly structured in comparison with biparentally inherited nuclear markers (Petit *et al.* 1997). Other highly variable markers such as nuclear microsatellites could permit individual tree genotyping and may have applications in forest crime prosecutions or protection of valuable works of art or antiques. Other applications of a more academic nature could be imagined in archaeology, palaeobotany or phylogeography, as molecular markers could be developed to assist in the taxonomic identification of fossil

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Table 1. Conservation, origin and age of studied logs.

log	origin	felling date	analysis date	conservation	rings outer sap- wood	rings inner sap- wood	rings transition wood	rings outer heart- wood	rings central heart- wood	rings inner heart- wood
1	Petite-Charnie, Sarthe	06/12/2000	15/12/2000	indoors	1–9	10–17	18–20	21–46	47–65	66–97
2	Petite-Charnie, Sarthe	27/12/2000	27/12/2000	indoors	1–7	8–15	16–18	19–41	42–64	65–94
3	Petite-Charnie, Sarthe	06/12/2000	09/01/2001	indoors	1–8	9–18	19–21	22–37	38–59	60–85
4	Darney, Vosges	11/1999	10/2000	outdoors	1–8	8–19	20–22	23–47	48–85	86–118
5	Handelotte, Haute-Marne	11/1999	10/2000	outdoors	1–12	13–28	29–31	32–49	50–84	85–116
6	Lure, Haute-Saone	11/1999	10/2000	outdoors	1–15	16–32	33–35	36–88	89–127	128–157
7	Chatenois, Vosges	11/1999	10/2000	outdoors	1–7	8–18	19–23	24–50	51–74	75–150
8	Pierroton, Aquitaine	12/1998	12/2000	indoors	1–7	8–13	14–17	18–22	23–26	27–35
9	Céreste, Alpes de Htes Provence	1981	12/2001	indoors	1–14	15–22	23–27	28–51	52–73	74–85
10	Vitrolles, Vaucluse	1981	12/2001	indoors	1–14	15–24	25–29	30–36	37–47	48–68

remains, or to compare the patterns of past diversity with present-day patterns. This may help us understand the origin of our modern forests and evaluate past modifications in their genetic structure. Clearly, the possibility of applying modern DNA techniques to dry wood would be of considerable interest in many fields.

However, a simple evaluation of basic information on wood anatomy, structure and biochemistry makes it clear that the adaptation of DNA extraction procedures to dry wood by the usual laboratory methods used for fresh leaves, buds or other living organs will not be straightforward. In living trees, cambium and to a lesser extent sapwood should yield suitable DNA. Difficulties may then be expected, as it is suspected that once a tree is cut, the quantity and quality of DNA that can be retrieved will quickly diminish and DNA is likely to be degraded into smaller fragments (Bär *et al.* 1988; Lindhal 1993; Cano 1996).

To evaluate the potential of wood for molecular genetic investigations and the accessibility to all three genomes for the different purposes mentioned above, several logs of French oaks were analysed and DNA was isolated from various parts of the trunk. Amplification of fragments of increasing sizes from the three genomes (nuclear, mitochondrial and plastid) was carried out to compare the effect of copy number and of amplicon size on the efficiency of the PCR. Stringent precautions were taken throughout to guarantee the authenticity of the amplified fragments.

2. MATERIAL AND METHODS

(a) Samples

Ten logs of sessile oak (*Quercus petraea* (Matt.) Liebl.) were obtained from different regions in France (see table 1). Three logs (numbers 1 to 3) coming from Sarthe (centre of France) were analysed respectively one, three and five weeks after felling. Four logs (numbers 4 to 7) coming from northeast France were analysed six months after felling. Log number 8 originated from southwest France and had been stored for 3 years after felling. Finally, logs 9 and 10 came from southeast France and were analysed 11 years after felling. Logs 1, 2, 3, 8, 9 and 10 were conserved indoors, in a dry room, while log numbers 4 to 7 came

from trees that had remained six months outdoors after felling. All analyses were performed on one-quarter of each log using internal tissues that had not been directly in contact with the air.

(b) DNA isolation

Initial tests demonstrated the frequency of contamination with external DNA. As a consequence, all DNA isolation and PCR experiments were subsequently carried out under sterile conditions in separate dedicated rooms. High-pressure systems, filtering of incoming air, UV light irradiation, laminar flow hood, bleach cleaning of every surface, and specific precautions during manipulations were used to avoid contamination with other plant DNA. Surface tissues of wood fragments were washed with diluted bleach and removed. About 200 mg of wood shavings was obtained with a scalpel from each log region, ground into a fine powder using a Retsch-mill apparatus (Fischer-Bioblock) and then used for genomic DNA isolation with the DNeasy Plant minikit (Qiagen). During each isolation manipulation, four samples coming from different logs were analysed simultaneously in order to detect possible cross-contamination. Two negative controls per isolation were used. They were treated in the same way as the normal samples except that no sample sawdust was added.

(c) DNA amplification by PCR

Twelve primer pairs specific to the plastid, mitochondrial and nuclear genomes were tested on all DNA samples isolated from wood (see table 2). Each amplification was performed on four samples as well as on two negative DNA isolation controls and on four negative PCR controls (PCR mixture without addition of target DNA). For chloroplast DNA, the seven pairs of primers were all located in the intergenic spacer between *trnD* and *trnT* (DT) (Demesure *et al.* 1995). One of these primer pairs had already been tested on wood DNA in a previous study (Dumolin-Lapègue *et al.* 1999) and six new primer pairs were designed to amplify fragments of increasing size, ranging from 87 to 1483 bp. The shortest fragment spans a polymorphic mononucleotide (A/T) repeat (chloroplast microsatellite). Three pairs of primers were used to amplify nuclear fragments: *ssrQrZAG20* (80 bp) and *ssrQrZAG119* (178 bp) correspond to two microsatellites (Kampfer *et al.* 1998; Steinkellner *et al.* 1997) and 5S to the 5S ribosomal DNA (230 bp) (Szymanski *et al.* 1999). Two pairs of primers, *cox2-1/2* and *nad4-3/4*, were

Table 2. Sequences of the primer pairs used for the amplification tests.

primer pair	sequence 5'–3'	primer	genome	fragment length (bp)	reference
ssrQrZAG119	GATCAGTGATAGTGCCTCTC	ssrQrZAG119F	nuclear	80	Steinkellner <i>et al.</i> (1997)
μdt1	GATCAACAAGCCCAAGGCAC ATCTTACACTAAGCTCGGAA TTCAATAACTTGTTGATCCC	ssrQrZAG119R μdt1u μdt1l	microsatellite chloroplast microsatellite	87	this study
ssrQrZAG20	CCATTAAAAGAAGCAGTATTTTGT	ssrQrZAG20F	nuclear	178	Steinkellner <i>et al.</i> (1997)
D7T7	GCAACACTCAGCCTATATCTAGAA GATCAATTCAATTAGGGTCG ATATCTTATGCACATGGTGG	ssrQrZAG20R D7 T7	microsatellite chloroplast	187	this study
5S	TGGGAAGTCCTCGTGTTGCA	5Sd	nuclear	230	Szymanski <i>et al.</i> (1999)
D1T1	ATTAGTGCTGGTATGATCGC GAGACCAGAAAGGGTAATGA	5Sr D1	ribosomal chloroplast	360	Dumolin-Lapègue <i>et al.</i> (1999)
cox2/1-2	CTAAATAGCAACGCAAGAAA TTTTCTTCCTCATTCTKATTT	T1 cox2/1	mitochondrial	378	Demesure <i>et al.</i> (1995)
D6T6	CCACTCTATTGTCCACTTCTA TTGATATTCATTATAAATCG ATATCTTATGCACATGGTGG	cox2/2 D6 T6	chloroplast	566	this study
DT800	TATTGTAACATAAATCCGCA AATTAGAAATAGAATTGGAA	DT800u Dt800l	chloroplast	822	this study
DT1000	TATTGTAACATAAATCCGCA AATGGAGTTTCATTACCCTT	DT1000u Dt1000l	chloroplast	1039	this study
DT1500	TATTGTAACATAAATCCGCA TCTTATGCACATGGTGGACC	DT1500u DT1500l	chloroplast	1483	this study
nad4/3-4	GGAGCTTTCCAAAGAAATAG GCCATGTTGCACTAAGTTAC	nad4/3 nad4/4	mitochondrial	2430	Demesure <i>et al.</i> (1995)

used to amplify mitochondrial fragments of 378 and 2430 bp (Demesure *et al.* 1995). All chloroplast DNA primer pairs used in this study were designed using oak sequences as a reference; however, one of them (D7T7) turned out to be relatively conserved, allowing amplification of many dicotyledonous tree species (M.-F. Deguilloux, unpublished data). Conversely, the primers used to amplify the 5S nuclear ribosomal gene and the two mitochondrial introns *cox2*-1/2 and *nad4*-3/4 are highly conserved (Demesure *et al.* 1995).

The 5S fragment was amplified according to Szymanski *et al.* (1999) on 2.5 μl of pure DNA extract. All other amplifications were performed according to Demesure *et al.* (1995). Amplifications were performed with 50 cycles except for chloroplast and nuclear microsatellites, for which only 40 cycles were used (increasing the number of cycles often resulted in fuzzy PCR products with those primers). PCR products were checked on 8% polyacrylamide gel followed by ethidium bromide staining and visualized under UV light, whereas the microsatellites were viewed on a Li-Cor model 4000L automatic DNA sequencer.

3. RESULTS

(a) Checking for contamination

No amplification product was noted both for isolation and for PCR negative controls throughout the whole study (out of a total of 480 DNA isolation and 960 PCR controls). Despite the large number of PCR cycles used

(up to 50), it appears that contamination by external DNA had been efficiently avoided.

(b) Amplification rates

The results for the 10 logs are shown in table 3. Out of a total of 80 separate DNA extracts, 43 (54%) led to DNA amplification with at least some primer pairs (312 successful amplifications out of 960 performed, i.e. 32.5% successful PCRs). A main cause of failure was isolation data problems in cambium, sapwood and transition zone for the logs analysed three weeks to six months after felling. DNA extracts obtained from these logs' regions were dark coloured and showed inhibition of amplification. This involved 22 of the 80 DNA extracts (27.5%). With the exception of two trees, no DNA amplification was obtained from bark extracts. In these two cases (logs 1 and 3), however, different genotypes were found for DNA isolated from bark compared with DNA isolated from other parts of the same logs, using one or both nuclear microsatellites (table 4), indicating contamination in the DNA isolated from the bark. In contrast, 33 of the 43 extracts (77%) that gave some amplification could be genotyped with at least one of the two nuclear microsatellites, and a single genotype was found in the different parts of each log, demonstrating the expected genetic uniformity within a log. Interestingly, for the nuclear microsatellites, only one of the two alleles observed in the cambium

Table 3. Amplification results on oak logs (numbers 1–10).
(Each case gives fragment amplification results for one log region, each number corresponds to one log ('1', good amplification for log '1' region; '—', no amplification; numbers in bold concern contaminations.)

	ssrQrZAG119	μdt1	ssrQrZAG20	D7T7	5S	D1T1	cox2/1–2	D6T6	DT800	DT1000	DT1500	nad4/3–4
bark	1-3-----	1-3-----	1-3-----	1-3-----	1-3-----	1-3-----	1-3-----	1-3-----	1-3-----	1-3-----	1-3-----	1-3-----
cambium	8910	12-----8910	1-----8-10	12-----8910	12-----8910	1-----8910	1-----8--	1-----8--	1-----8--	1-----8--	1-----	1-----
outer sapwood	1-----8910	1-----8910	-----89-	1-----8910	1-----8910	1-----8910	1-----8--	1-----8--	1-----	-----	-----	-----
inner sapwood	1-----8910	12-----8-10	12-----8-10	12-----8910	12-----8910	12-----8910	12-----8--	12-----8--	12-----8--	12-----8--	12-----8--	12-----
transition wood	-----8--	12-----8910	12-----8--	12-----8910	12-----8910	12-----8910	12-----8--	12-----8--	12-----	12-----	12-----	12-----
outer heartwood	123-5678--	123-5678--	12--5-----	12345678--	123-5678--	123-5678--	123-5678--	123-567--	123-----	123-----	-2-----	-2-----
central heartwood	--3-56-----	123456-8--	-23-----	123456-8--	123456-8--	123456-8--	123456-8--	123-----	-23-----	-23-----	-23-----	-23-----
inner heartwood	12--6-8--	123456-8--	-2-----	123456-8--	123456-8--	12345-8--	123--8--	1234-----	-23-----	123-----	-23-----	-2-----

Table 4. Nuclear ('Q119' for ssrQrZAG119, 'Q20' for ssrQrZAG20) and chloroplast (μdt1) microsatellite genotypes obtained for each log region.
(Each allele is coded by one number, and the numbers in bold concern contaminations.)

	1	2	3	4	5	6	7	8	9	10
	Q119	μdt1	Q20	Q119	Q20	Q119	μdt1	Q20	Q119	μdt1
bark	25	1	33	—	—	—	—	—	—	—
cambium	—	1	33	—	—	—	—	26	2	23
outer	—	—	—	—	—	—	—	—	—	—
sapwood	26	1	—	—	—	—	—	26	2	23
inner	—	—	—	—	—	—	—	—	—	—
sapwood	26	1	33	—	—	—	—	26	2	23
transition	—	1	33	—	—	—	—	26	2	23
wood	—	1	33	—	—	—	—	26	2	23
outer	—	1	33	—	—	—	—	26	2	23
heartwood	26	1	33	46	1	33	22	46	2	26
central	—	—	—	—	—	—	—	—	—	—
heartwood	—	1	33	33	2	33	2	33	2	33
inner	—	—	—	—	—	—	—	—	—	—
heartwood	26	1	—	46	1	33	2	—	46	2

Figure 1. Mean amplified fragment length and amplification success rate on different regions of logs 1 and 8–10 (grey bars, mean fragment length; diamonds, success rate).

Figure 2. Amplification success rate related to amplified fragment length (black circles, chloroplast, open squares, nuclear; open triangles, mitochondrial).

could be amplified in the inner and outer sapwood of log number 10 (the smaller). The chloroplast microsatellite locus had a higher success rate than the nuclear loci (0.50 versus 0.26) but was less variable.

The amplification rates were higher for the outer part of the logs, from cambium to transition zone (excluding those logs where oxidation problems were apparent; see figure 1). Moreover, the mean size of fragments amplified from these regions is also greater. Furthermore, higher amplification success rates were obtained for short and/or repeated target sequences. In particular, there is a good relationship between amplification success rate and amplicon size ($r^2 = 0.85$, excluding the two single-copy nuclear genes; see figure 2).

Finally, a great variability of DNA availability is noted among logs that had been conserved in different ways (figure 3). Log numbers 1, 2 and 3 analysed one, three and five weeks after felling could provide information about DNA conservation during the first steps of wood drying. In the heartwood, little DNA degradation was detectable after several weeks but there is significant variability between individual trees. In log numbers 4, 5, 6 and 7, only short fragments could be amplified from heartwood. Log number 8 (kept for 3 years indoors, immediately after felling) yielded much better results with

Figure 3. Mean amplified fragment length and amplification success rate on different heartwood logs (grey bars, mean fragment length; diamonds, success rate).

sapwood; longer fragments (1500 bp) could be amplified and oxidation was no longer a problem. Nevertheless, heartwood amplification rates were lower than those obtained with younger logs. In particular, no amplification was obtained on heartwood from logs 9 and 10 (kept 11 years indoors after felling).

4. DISCUSSION

Working with small amounts of low-quality DNA has forced us to optimize PCR conditions, probably up to the point that a single molecule of target DNA could be amplified. The absence of contamination by external DNA (pollen, foreign DNA molecules) during all laboratory experiments is therefore particularly noteworthy. The set-up of fully dedicated rooms and equipment with all precautions typically found in laboratories dealing with ancient DNA proved very effective. The use of three microsatellites showed that internal contamination (i.e. contamination into the sample structure) had occurred in the bark of two of the 10 trees studied. They also indicated that contamination across samples or contamination by foreign DNA was probably absent from the remaining samples. A limit to the use of the genotyping approach to

control for contamination is that most polymorphic markers are single-copy nuclear microsatellites. These sequences cannot be amplified as readily as DNA sequences present in multiple copies, especially with extremely degraded DNA. As very conserved primer pairs were used, this means that contamination by DNA from other plant species had also been avoided. Furthermore, the consistent results obtained in terms of amplification frequency as a function of amplicon size and age of the logs support the claim that the DNA that has been amplified is authentic.

Only surface sample cleaning seems to be problematic, especially if the sample is very porous or presents a complex structure, as in the case of bark (presence of cracks), where pollen grains or sawdust from other oak trees appeared to have remained in some cases.

The DNA obtained from oak wood is clearly degraded. This degradation appears to be gradual during drying, following the felling of the tree, as expected from the limited chemical stability of DNA (Lindhal 1993). Indeed, while relatively long chloroplast fragments were recovered from fresh sapwood, shorter fragments were obtained from sapwood of logs that had dried outside. In the transition wood, degradation of DNA molecules is more significant and results in shorter fragments for all three genomes. Finally, in heartwood, fragments longer than 380 bp could not be amplified. Differential DNA access in sapwood versus heartwood may be simply explained by the presence of living cells in sapwood that are absent from heartwood. This can be related to the gradual transformation of wood cells during ageing (Fengel 1970). The trunk of typical dicotyledonous trees such as the oak is composed of differentiated tissues: the bark, a thin cambial layer, the sapwood and the heartwood. Only the cambium is composed exclusively of living cells that actively divide to allow tree growth. The sapwood is composed of both living (ray parenchyma cells) and dead cells (vessels and wood fibres) (Mia 1972; Nobuchi *et al.* 1987). All cells in the heartwood are dead, as are those found in the bark. The ultrastructure of ray parenchyma cells is gradually modified at increasing distances from cambium (Frey-Wyssling & Bosshard 1959; Saranpää 1988) and no organelles or nuclei are detectable in the heartwood (Bamber 1976). Thus, in heartwood, all cells are dead and empty, but short fragments of DNA may nevertheless have remained adsorbed on cell walls (Cano 1996), as indicated by the encouraging results obtained with DNA isolated from this part of the trunk. However, no DNA could be obtained from the bark, another tissue made up of only dead cells but having a very different structure.

The mode of conservation of wood seems to have a strong effect on subsequent DNA availability, as shown by the results obtained with log 8 (which was stored for 3 years indoors) compared with the logs conserved for only six months outdoors. DNA conservation among logs exposed to outside conditions was also quite heterogeneous, perhaps as a consequence of different microsite conservation conditions. Exposure to water is probably the most destructive force acting on the DNA molecules (Cano 1996). Logs conserved outdoors are exposed to environmental climatic conditions and as a consequence maintain a relatively high humidity for several months (Vivas *et al.* 2000), a situation that will result in increased

DNA damage. Moreover, under these conditions, UV and micro-organisms well known for their effect on DNA molecules may also explain the accelerated rate of decay (Murmanis *et al.* 1987; Vivas *et al.* 1997). Relatively rapid dehydration of tissues (as in the case of wood stored indoors) seems to be the best way to preserve DNA; this factor has been identified as crucial for DNA conservation in fossil materials (Cano 1996).

Regardless of the conservation process, a major problem encountered during wood DNA studies is the presence of PCR inhibitors. For cambium, sapwood and transition wood, problems appeared during DNA isolation in samples stored a few weeks indoors or several months outdoors (but not for the oldest logs). DNA isolation from these samples produced very dark DNA solutions that gave no amplification. As water-soluble ellagitannins are known to be easily oxidized in solution (Vivas *et al.* 2000), this finding could be related to ellagitannin modifications in wood following tissue death. A gradual oxidation of these ellagitannins takes place during the ageing of wood cells, from sapwood to heartwood (Vivas *et al.* 2000). It is during heartwood formation, in the transition wood, that most of the insolubilization of those compounds takes place by polymerization and oxidation (Hillis 1968; Masson *et al.* 1996). The same phenomenon of ellagitannin insolubilization occurs after felling, during the wood drying process (Vivas *et al.* 2000; De Simon *et al.* 1999). In this way, in recently felled logs, increased amounts of water-soluble ellagitannins are present in cambium, sapwood and transition wood, and could lead to coloration in solution (i.e. in the DNA isolation buffer) and inhibition of PCR. The testing of numerous isolation or purification protocols did not improve these results (Geneclean kit for Ancient DNA, BIO 101; Nucleospin Plant Kit, Macherey-Nagel; Qiaamp DNA Stool, Qiagen; Chelex 100, Bio-Rad; QIAquick Spin, Qiagen; Microcon, Amicon).

Independently of the isolation problems, the chance of successful amplification clearly increases as the size of amplicon decreases and the copy number of the target sequence increases. Heterogeneous priming efficiency could in principle alter these relationships. Moreover, copy number effects could be obscured by differential degradation of some genomes or sequences. For instance, it has been reported that mitochondrial DNA is more prone to oxidative lesions than are nuclear sequences (Bohr & Dianov 1999). Nevertheless, a combination of amplicon size and copy number effects does account for much of the amplification variability in our experiments. With two samples (logs 9 and 10), amplicon size effects were apparent within the same PCR experiment: using nuclear microsatellite *ssrQrZAG119*, only the shorter allele was amplified in two of three PCRs, a phenomenon well known when working with degraded DNA and called allelic dropout (Taberlet *et al.* 1996). Previous studies (e.g. Cano 1996) have also reported that if DNA is degraded, the chance of successful amplification increases if the target gene sequence is present in multiple copies in each cell. Here, better results were obtained with nuclear ribosomal gene 5S, which is highly repeated (350–400 times in *Arabidopsis thaliana*; see The Arabidopsis Genome Initiative 2000) compared with the two single-copy nuclear microsatellite fragments, despite its greater length.

Chloroplast and mitochondrial genomes are also present in many copies per cell. In wood tissues, the plastids are differentiated in amyloplasts (in living parenchyma cells (Pizzolato 1978)), which play a major role for starch and lipids storage, and participate in phenol and terpene synthesis (Bamber 1976). Even if the number of plastids in living parenchyma cells is not as high as the number of chloroplasts in photosynthetic tissues (Mia 1972), they will still greatly outnumber single-copy nuclear genes, and will as a consequence be more easily isolated and amplified.

The present results demonstrate the potentialities of genetic analyses on dry wood, as chloroplast, mitochondrial and nuclear DNA sequences could all be recovered through PCR amplification. This is an important finding that could lead to major applications, as it means that the geographical origin or taxonomic status of wood samples may now be checked, provided some surveys and databases are available. In the case of the European white oaks, a very detailed European reference map of chloroplast variants exist, with as many as 2600 forests typed by a consortium of 16 laboratories (Petit *et al.* 1997, 2002; Dumolin-Lapègue *et al.* 1997). The determination of the same variants on wood samples could help confirm the claimed geographical origin of the wood used to age wine by the barrel industry. Taxonomic identification of wood samples, sometimes difficult or costly on the basis of wood anatomy studies alone (Feuillat *et al.* 1997), could be enhanced by the analysis of appropriate genetic markers selected from one of the three genomes, for instance ribosomal DNA from the nuclear genome, repeated in many copies and often considered as an excellent tool for taxonomic purposes (Szymanski *et al.* 1999). Also, nuclear microsatellites could be used for tracing individual trees, at least when working with relatively fresh wood samples. Finally, as in ancient DNA studies relying on bones, DNA analysis could also be attempted in the case of wood samples uncovered during archaeological or palaeobotanical investigations, if exceptionally well-conserved specimens are found (Golenberg 1991). Although only results on oak have been presented here, we also obtained encouraging results with beech and pine wood (M.-F. Deguilloux, unpublished data), and we expect that the methods might be adapted to many woody species.

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REFERENCES

- Bailey, J. F., Richards, M. B., Macaulay, V. A., Colson, I. B., James, I. T., Bradley, D. G., Hedges, R. E. M. & Sykes, B. C. 1996 Ancient DNA suggests a recent expansion of European cattle from a diverse wild progenitor species. *Proc. R. Soc. Lond. B* **263**, 1467–1473.
- Bamber, R. K. 1976 Heartwood, its function and formation. *Wood Sci. Technol.* **10**, 1–8.
- Bär, W., Kratzer, A., Mächler, M. & Schmid, W. 1988 Post-mortem instability of DNA. *Forensic Sci. Int.* **39**, 59–70.
- Bohr, V. A. & Dianov, G. L. 1999 Oxidative DNA damage processing in nuclear and mitochondrial DNA. *Biochimie* **81**, 155–160.
- Cano, R. J. 1996 Analysing ancient DNA. *Endeavour* **20**, 162–167.
- Chen, D. M. & De Filippis, L. F. 1996 Application of genomic DNA and RAPD-PCR in genetic analysis and fingerprinting of various species of woody trees. *Aust. Forestry* **59**, 46–55.
- De Filippis, L. & Magel, E. 1998 Differences in genomic DNA extracted from bark and from wood of different zones in *Robinia* trees using RAPD-PCR. *Trees* **12**, 377–384.
- Demesure, B., Sodzi, N. & Petit, R. J. 1995 A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Ecol.* **4**, 129–131.
- De Simon, B. F., Cadahia, E., Conde, E. & Garcia-Vallejo, M. C. 1999 Evolution of phenolic compounds of Spanish oak wood during natural seasoning. First results. *J. Agric. Food Chem.* **47**, 1687–1694.
- Dumolin-Lapègue, S., Demesure, B., Fineshi, S., Le Corre, V. & Petit, R. J. 1997 Phylogeographic structure of white oaks throughout the European continent. *Genetics* **146**, 1475–1487.
- Dumolin-Lapègue, S., Petit, R. J., Gielly, L. & Taberlet, P. 1999 Amplification of DNA from ancient and modern oak wood. *Mol. Ecol.* **8**, 2137–2140.
- FAO World Resources 2000–2001 Food and agricultural organization of the United Nations and Forest Stewardship Council. <http://www.fao.org>.
- Fengel, D. 1970 Ultrastructural changes during aging of wood cells. *Wood Sci. Technol.* **4**, 176–188.
- Feuillat, F., Dupouey, J. L., Sciamia, D. & Keller, R. 1997 A new attempt at discrimination between *Quercus petraea* and *Quercus robur* based on wood anatomy. *Can. J. Forest Res.* **27**, 343–351.
- Frey-Wyssling, A. & Bosshard, H. H. 1959 Cytology of the ray cells in sapwood and heartwood. *Holzforschung* **13**, 129–137.
- Gold, T. 1992 The deep, hot biosphere. *Proc. Natl Acad. Sci. USA* **89**, 6045–6049.
- Golenberg, E. M. 1991 Amplification and analysis of Miocene plant fossil DNA. *Phil. Trans. R. Soc. Lond. B* **333**, 419–427.
- Hagelberg, E. 1997 Ancient and modern mitochondrial DNA sequences and the colonization of the Pacific. *Electrophoresis* **18**, 1529–1533.
- Hardy, C., Callou, C., Vigne, J. D., Casane, D., Dennebouy, N., Monolou, J. C. & Monnerot, M. 1995 Rabbit mitochondrial DNA diversity from prehistoric to modern times. *J. Mol. Evol.* **40**, 227–237.
- Hillis, W. E. 1968 Heartwood formation and its influence on utilization. *Wood Sci. Technol.* **2**, 260–267.
- Kampfer, S., Lexer, C., Glössl, J. & Steinkellner, H. 1998 Characterization of (GA)_n microsatellite loci from *Quercus robur*. *Hereditas* **129**, 183–186.
- Krings, M., Stone, A., Schmitz, R. W., Krainitzki, H., Stoneking, M. & Pääbo, S. 1997 Neandertal DNA sequences and the origin of modern humans. *Cell* **90**, 19–30.
- Kwok, S. & Higuchi, R. 1989 Avoiding false positive with PCR. *Nature* **339**, 237–238.
- Lee, A. B. & Cooper, T. A. 1995 Improved direct PCR screen for bacterial colonies: wooden toothpicks inhibit PCR amplification. *Biotechniques* **18**, 225–226.
- Lindhal, T. 1993 Instability and decay of the primary structure of DNA. *Nature* **362**, 709–715.

- Masson, G., Puech, J. L. & Moutounet, M. 1996 Composition chimique du bois de chêne de tonnellerie. *Bull. de l'OIV* **69**, 635–657.
- Marco, J., Artajona, J., Larrechi, M. S. & Rius, F. X. 1994 Relationship between geographical origin and chemical composition of wood for oak barrels. *Am. J. Enol. Viticulture* **45**, 192–200.
- Medina, B. & Vivas, N. 2000 Vers une garantie de l'origine géographique. II. La méthode ICP/MAS. V Colloque des Sciences et Techniques de la Tonnellerie, connaissances actuelles et Avenir de l'Elevage en barriques. *J. Sci. Tech. Tonnellerie* 11–13.
- Mia, A. J. 1972 Fine structure of the ray parenchyma cells in *Populus tremuloides* in relation to senescence and seasonal changes. *Texas J. Sci.* **24**, 245–260.
- Murmanis, L., Highley, T. L., Madison, W. I. & Palmer, J. G. 1987 Cytochemical localization of cellulases in decayed and non-decayed wood. *Wood Sci. Technol.* **21**, 101–109.
- Nobuchi, T., Tokuchi, N. & Harada, H. 1987 Variability of heartwood formation and cytological features in broad-leaved trees. *Mokuzai Gakkaishi* **33**, 596–604.
- Petit, R. J., Pineau, E., Demesure, B., Bacilieri, R., Ducouso, A. & Kremer, A. 1997 Chloroplast DNA footprints of post-glacial recolonization by oaks. *Proc. Natl Acad. Sci. USA* **94**, 9996–10 001.
- Petit, R. J. (and 26 others) 2002 Identification of refugia and postglacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. *Forest Ecol. Mgmt* **156**, 49–74.
- Pizzolato, T. D. 1978 A tannic acid–ferric chloride–toluidine blue stain for wood amyloplasts embedded in epoxy resin. *Forest Sci.* **24**, 49–51.
- Saranpää, P. 1988 Plastids and glycolipids in the stem wood of *Pinus sylvestris* L. *Trees* **2**, 180–187.
- Steinkellner, H., Fluch, S., Turetschek, E., Lexer, C., Streiff, R., Kremer, A., Burg, K. & Glössl, J. 1997 Identification and characterisation of (GA/CT)_n microsatellite loci from *Quercus petraea*. *Plant Mol. Biol.* **33**, 1093–1096.
- Szymanski, M., Barciszewska, M. Z., Barciszewski, J. & Erdmann, V. A. 1999 5S ribosomal RNA databank. *Nucleic Acid Res.* **27**, 158–160.
- Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L. P. & Bouvet, J. 1996 Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acid Res.* **24**, 3189–3194.
- The Arabidopsis Genome Initiative 2000 Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**, 796–813.
- Vivas, N., Amrani-Joutei, K., Glories, Y., Doneche, B. & Brechenmacher, C. 1997 Développement de microorganismes dans le bois de cœur de chêne (*Quercus petraea* Liebl) au cours du séchage naturel à l'air libre. *Annals Sci. Forest.* **54**, 563–571.
- Vivas, N., Saint-Cricq De Gaulejac, N. & Absalon, C. 2000 Les méthodes de séchage du bois. II. Affinage et maturation du bois: pratiques et intérêts respectifs. V Colloque des Sciences et Techniques de la Tonnellerie, connaissances actuelles et Avenir de l'Elevage en barriques. *J. Sci. Tech. Tonnellerie* 21–25.
- White, E., Hunter, J., Dubetz, C., Brost, R., Bratton, A., Edes, S. & Sahota, R. 2000 Microsatellites markers for individual tree genotyping: application in forest crime prosecutions. *J. Chem. Technol. Biotechnol.* **75**, 923–926.

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