

Can we use DNA to identify the geographic origin of tropical timber?

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Abstract

In many ways DNA tracking potentially offers many advantages over current methods for establishing whether a timber product has been harvested from a legitimate source. DNA methodologies can be applied at the point of consumer country importation, and can be used to overrule questionable certification documentation that may have been introduced along the supply chain. In addition, with the advancement of genomics and DNA barcoding technologies, large-scale screening of DNA variation can be done cheaply and routinely and much faster and with higher taxonomic resolution than morphological determination methods. However despite the promise, a number of problems remain; notably, DNA extraction from dried wood and old tissue sources, generation of DNA variation to distinguish between species and region of origin within species, and statistical methodologies for verifying source tests. This paper briefly outlines the latest advances in these areas. For DNA extraction methods, recent advances mean that large-scale automated methodologies could now be considered. There are several good examples of range-wide studies of phylogeography or genetic differentiation that have been undertaken, or are planned for completion in the near future, that generate the type of data needed to distinguish across the geographic range of intensively studied species (e.g. those with high timber value). Finally, statistical methodologies, particularly assignment tests, have recently advanced and can be used to phrase results within a legally interrogable context. The fruition of methodologies in each of these areas offers exciting possibilities for using DNA to verify timber source, and potentially gives a powerful new method to customers wishing to express ethical choice in a market fraught with uncertain alternatives.

Keywords: timber, tropical forests, traceability, DNA, phylogeography, assignment, certification

1 Introduction

In many ways using DNA to identify geographic origin of timber offers many advantages over the methods currently used to verify whether a timber product has been harvested from a legitimate source. DNA methodologies can be applied at the point of consumer country importation, and can be used to overrule questionable certification documentation that may have been introduced along the supply chain. In addition, with the advancement of genomics technologies and DNA barcoding initiatives, large-scale screening of DNA variation can be done cheaply and routinely, and much faster and with higher taxonomic resolution than morphological determination methods. The availability of DNA barcodes for increasing numbers of species also potentially allows rapid DNA sequence identification and is an exciting recent development.

However despite the promise, a number of problems remain with using DNA for timber certification; notably, DNA extraction from dried wood and old tissue sources, is there enough variation below the species level to identify the timber source and at what scale, and finally are the statistical methodologies for verifying source tests rigorous enough to operate with a legislative framework. This paper briefly outlines the latest advances in each of the above areas, but will not discuss species identification using DNA barcoding methodologies, as this has been the subject of numerous recent reviews (e.g. Lahaye et al 2008)

2 DNA extraction methods

DNA extraction from plant leaf and bud tissue has been a standardised and straightforward methodology for sometime (e.g. Petit et al 2002), and is now semi- or fully automatable (e.g. QIAGEN extraction kits combined with robotic workstations supporting vacuum manifold or centrifuge components). DNA extraction from freshly harvested wood, particularly incorporating cambium tissue, has also been found to yield DNA of high quality, and in some cases comparable to that from leaf material (Colpaert et al 2005).

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However, extraction of DNA from dried wood tissue has been more problematic, particularly if sampled from sapwood, and more so from heartwood.

Generally the problem with dried timber as a source is lower overall quantity of DNA and the presence of an additional range of inhibitors, e.g. carbohydrates (cellulose) and phenols (lignin), and for buried material, the level of iron can also increase. All of these components can serve to inhibit the polymerase chain reaction (PCR), and other down stream molecular methodologies, during the DNA analysis process. However a number of components can be added to the DNA extraction step to improve harvest quality, including Proteinase K, Chelex®100, PVP360. In addition, several additives can also be incorpo-

rated into the PCR step to improve amplification in the presence of some potentially inhibiting contaminants, including DMSO, and using the Stoffel Fragment (Speirs et al 2008).

In the field of DNA extraction from dried wood, much work has been done on oak timber due to the interest in timber tracking for the cooperage industry (Deguilloux et al 2002; Deguilloux et al 2003; Deguilloux et al 2004). Table 1 indicates a range of recent studies which have successfully extracted DNA from oak timber of different ages and preserved under different conditions. Using contamination-exclusion techniques, developed to work on ancient sources (Gugerli et al 2005), it has been possible to amplify DNA fragments of up to 500 bps from timber that is up to 3600 years old.

Table 1:

Listing of some recent studies to extract DNA from oak wood of various ages and conditions, with potential success indicated by size of DNA fragment (bp) that was amplifiable during the study

cpDNA Fragment Sizes Amplified From Oak Tissue of Varied Age			
Tissue	Age (years)	PCR fragment size (bp)	Reference
Leaf/Bud	Fresh	1688	Petit et al. 2002
Wood	1	1483	Deguilloux et al. 2002
Wood	2	566	Deguilloux et al. 2002
Wood	3	175	Deguilloux et al. 2002
Wood	11	187	Deguilloux et al. 2002
Wood (buried)	600	350	Dumolin-Lapègue et al. 1999
Wood (buried)	3600	500	Tani et al. 2003
Wood (marine)	3600	~200	Speirs et al. 2008

Therefore, if DNA analysis is undertaken in specifically designed laboratories, where contamination issues are minimised and traceable process is implemented (Gugerli et al 2005), there is no reason why DNA analysis from a timber source should not become standardised and automated.

3 Rangewide phylogeography and genetic differentiation within tropical trees

Several initiatives are underway to generate large-

scale DNA barcodes for tropical trees, which will potentially allow rapid, DNA-based species identification for tropical timbers. However for many of the high value timber species, once species identification has been confirmed, the main issue is usually around verifying that the timber has come from a sustainable, or at least legal, source. Or rather, that it has not been illegally sourced. To verify such a claim using DNA-based methods, genetic variation needs to exist within the species of interest and ideally to be geographically structured.

Suitable variation is established within natural populations of nearly all species due to a combination of mutational and population processes (i.e. gene flow and drift). Over time genetic structure can result at different spatial scales due to limited pollen and seed dispersal within a population, limited gene flow between populations or the historic disruption of a once continuous range of a species by climatic or geographic changes. Each of these changes will produce genetic discontinuities or clines within a species that can be identified using a range of DNA-based markers.

Several recent studies of phylogeography (generally considering genetic structuring due to

historical gene flow and discontinuity processes) and broad scale population genetic differentiation have highlighted significant genetic structure across the native range of many tropical trees (Table 2). The scale of structure depends on the species, marker type and sampling protocol used in the study. However generally, significant broad scale continental and region differences can be highlighted by phylogeographic and population structural analysis, and for many systems, variation at smaller spatial scales can be used to distinguish genetically between individual populations.

Table 2:

Some example studies of broad scale phylogeographic or genetic differentiation within tropical trees, using a range of molecular marker systems, where significant genetic structuring may be usefully used to verifying a timber source

Species	Geographic range	Molecular marker	References
<i>Swietenia macrophylla</i> (mahogany)	Central America, southern Brazil	SSRs and RAPD	Novick et al 2003, Lemes et al 2003, Gillies et al 1999
<i>Cedrela odorata</i> (Spanish cedar)	Central America (Costa Rica)	cpDNA, AFLP	Cavers et al 2003, 2005
<i>Ceiba pentandra</i>	Neotropics and Africa	cpDNA and ITS	Dick et al 2007
<i>Vouacapoua americana</i>	French Guyane and the Amazon Basin	cpDNA	Dutech et al 2000 Dutech et al 2003 Dutech et al 2004
<i>Carapa guianensis</i>	Amazon basin	cpDNA	Cloutier et al 2005
<i>Pterocarpus officinalis</i>	Caribbean Basin	AFLP	Rivera-Ocasio et al 2002
<i>Vochysia ferruginea</i>	Costa Rica	cpDNA and AFLP	Cavers et al 2005
<i>Lonchocarpus costariensis</i>	Guanacaste, Costa Rica	cpDNA and AFLP	Navarro et al 2005
<i>Irvingia wombulu</i> and <i>I. gabonensis</i>	Nigeria, Cameroon, Gabon	RAPD	Lowe et al 2000
<i>Aucoumea klaineana</i>	Gabon	cpDNA	Muloko-Ntoutoume et al 2000

There are also several range-wide analyses of high value timber species that are due for completion soon as part of ongoing collaborative project. The subject of two of these EU projects, TEAKDIV and SEEDSOURCE, include: *Tectonis grandis* (teak), *Bertholletia excelsa* (Brazil nut), *Swietenia macrophylla* (mahogany), *Cedrela odorata* (Spanish cedar), *Bombacopsis quinata*, *Carapa guianensis*, *Cordia alliodora*, *Hymenaea coubaril*, *Jacaranda copaia*, *Minquartia guianensis*, *Ochroma pyramidale*, *Schizolobium parahyba*, *Simarouba amara*, *Socratea exorrhiza*, *Symphonia globulifera*, *Virola sebifera*, *Vochysia ferruginea*.

4 Statistical tests

Statistical methodologies to match a disputed sample against a putative source have also advanced in line with the development of marker and sampling technologies. Simply matching a genotype against a map of the geographic distribution of genetic variation can be very useful for species that exhibit mainly fixed differences between populations. For example, the pattern of phylogeographic structure within European oak has been used to test whether local or translocated sources have been used in British oak plantations (Cottrell et al 2004 Lowe et al 2004).

Where genotype or allele frequency differences characterise genetic structure, the use of assignment tests to statistically differentiate between individuals of a population or region (Pritchard et al 2000), has been increasingly used. Such methods can be applied to a range of molecular marker types for distinguishing the geographic origin of timber (e.g. in oaks, Deguilloux et al 2003). There have been some very powerful demonstrations of the use of such methods in a legal framework. For example, by using a DNA assignment test against natural genetic structuring, an angling competitor was disqualified when it was proved that the winning fish could not have come from the river system where the competition was taking place (Primmer et al 2000). Despite the success of such tests, it will still be much easier to prove that a particular product does not come from a stated source, rather than to statically prove the potential source from across the range of the species. The latter case needs an extensive sampling of the variation across the range of the species, whereas the former only needs a good sampling of the contested source, and will be much easier to contest in a legislative framework. In addition for products sourced from

plantations established from a limited or even exotic source, a genetic reference profile can easily be generated using a range of markers for independent verification at the end of the market chain.

5 Outlook

The fruition of methodologies in each of the areas of DNA extraction from wood, identification of genetic variation across the natural range of tropical tree species and development of statistical assignment frameworks, offers exciting possibilities for using DNA to verify timber source. These advancements, and a process of developing a library of genetic reference profiles for large-scale sources that are sustainably and/or ethically managed potentially, gives a powerful new suit of methods to customers wishing to express ethical choice in a market currently fraught with uncertain alternatives.

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