Johann Heinrich
von Thünen-Institut

**Landbauforschung**
*vTI Agriculture and Forestry Research*

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## October 8-9 2007, Bonn/Germany

Bernd Degen (Editor)

Bundesministerium für
Ernährung, Landwirtschaft
und Verbraucherschutz

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Johann Heinrich von Thünen-Institut
Bundesforschungsinstitut für
Ländliche Räume, Wald und Fischerei (vTI)
Bundesallee 50, 38116 Braunschweig,
Germany

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[1] Institute of Forest Genetics, Johann Heinrich von Thünen Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries, Sieker Landstraße 2, D-22927 Großhansdorf, Germany

**Proceedings of the international workshop**

# *"Fingerprinting methods for the identification of timber origins"*

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**Editor**

Photo from B. Degen 2003

**The international workshop was organised by**

Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz

WWF

**World Wide Fund for Nature**

**Landbauforschung**
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**Introduction by the Editor**

Illegal logging and trade with illegal timber and wood products are the cause for many economic and ecological problems both in the producer and in the consumer countries

Although instruments have been established against illegal logging and the trade, some on a national level (purchasing rules for public administrations) and some on the EU level (EU FLEGT Action Plan), we lack practicable control mechanisms to identify the origin of timber and wood products. Such methods of identifying types of wood and timber origins are the fundamental prerequisites for efficient import controls or corresponding origin testing by industry and the trade. The tests presently used, for example in the scope of the CITES international species protection convention, meet their limits in many tropical tree species.

The solution to precisely this problem was the aim of the international workshop, which was organised by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) and the World Wide Fund for Nature (WWF) in Königswinter on 8 and 9 October 2007. A group of 40 leading scientists from nine countries discussed new methods of identifying timber species and timber origins.

The goals of the workshop were:

- to offer scientists working with genetic (DNA markers) and chemical (isotopes) testing methods to identify timber origins a forum for interdisciplinary exchange of information,
- to reveal the current standard of knowledge for both methods and analyze their strengths and weaknesses,
- to pinpoint gaps in research and set up alliances and networks,
- to identify technical and other requirements for setting up databases,
- and to devise political requirements and discuss steps and make recommendations for an effective control system.

In two sessions, the scientists discussed new methods of identifying the origins of timber. Session A explored genetic and chemical testing methods for identifying timber origin and tree species, while Session B focused on setting up and managing relevant databases.

This special issue contains the proceedings of most presentations given during the workshop. During the manuscript preparation all authors used the possibility to update information according to new results and developments in 2008.

I hope the information of these proceedings will stimulate the further development and application of methods to control and reduce illegal logging.

Bernd Degen

Johann Heinrich von Thünen Institute (vTI), Institute of Forest Genetics, Sieker Landstrasse 2, 22927 Grosshansdorf, Germany
bernd.degen@vti.bund.de

**Foreword from the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV)**

Illegal logging continues to be one of the major causes of deforestation and forest degradation. At the same time, illegally sourced timber competes with sustainably produced timber and causes market distortions. As early as 2001, Germany convened a first international scoping meeting on timber fingerprinting, i.e. methods to help identifying or verifying places of origin of traded timber. At that time scientists of different disciplines explored comparative advantages and disadvantages of genetic, chemical and biological options basically available. A multidisciplinary approach was recommended to further explore the complementary use of the various methods. Further political support for the subject and continued research in a number of countries, including Germany, was encouraged.

This year, Germany felt that holding the G8 presidency and hosting the G8 forest expert meeting offered a good opportunity for stock taking and feeding the results directly into the ongoing policy debate on further strengthening the fight against illegal logging. Several potential users of timber fingerprinting are envisaged once it would be practical. They reach from customs and timber importing industry to independant organisations and institutes. The lucky coincidence that, in the meantime, WWF had started investigating the usefulness of the stable isotope method, commonly applied in the case of food products, also for timber fingerprinting, led to a cooperation between the responsible Ministry of Agricuture, Food and Consumer Protection (BMELV) and WWF Germany on the latter subject and in organising this Workshop.

It is highly appreciated that so many and diverse institutes and experts accepted the invitation and, thus, contributed to the impressive overview of already ongoing activities worldwide, including related ones, like barcoding. Our expectations that the Workshop would inform about the relevance of the various methods for the potential development of concrete control systems – governmental and non-governmental - were more than fulfilled. We believe that sufficient momentum has been created for enhanced international cooperation to this end. Networking, creation of synergies and focusing activities on most important traded and/or endangered timber species using standardised methods are wishful features of that and were recommended. Publicly available data banks and practical applicability within a period of three to four years are further important objectives resulting from the workshop. The German government will continue supporting related follow up activities. For an international approach further supporting partners are needed and most welcome.

Matthias Schwoerer

International Forest Policy Division, Federal Ministry of Food, Agriculture and Consumer Protection
Rochusstrasse 1, D-53123 Bonn, Germany
Matthias.Schwoerer@bmelv.bund.de

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Matthias Schwoerer

International Forest Policy Division, Federal Ministry of Food, Agriculture and Consumer Protection
Rochusstrasse 1, D-53123 Bonn, Germany
Matthias.Schwoerer@bmelv.bund.de

**Foreword from WWF**

Some years ago WWF Germany learned, through a magazine article, of a method with which it is possible to scrutinise the declaration of the origin of foodstuffs. From WWF Germany's viewpoint, this was (and still is) exactly what was badly needed for the wood sector.

For years WWF has been working intensively on the international level to decrease deforestation and extinction of species, today's principal environmental debacles. There is no doubt that, apart from advancements in establishing protected areas and establishing sustainable certified forestry, forest loss proceeds at great speed. Between 14 and 16 million hectares of forest are lost each year. Most of these forests disappear in tropical regions. At the same time tropical forests accommodate the highest density of species.

Beyond forest loss and extinction of species, global warming has come into political and public awareness with just cause: between 20 and 25 % of global $CO_2$ emissions result from deforestation. Indonesia, for example, is one of the world's largest $CO_2$ emitters following the USA and China. More than 80 % of the $CO_2$ in Indonesia results from deforestation.

WWF identifies several causes of deforestation, which are often combined or dependent on each other:

- use of wood, both legal und illegal
- land use conversion, e.g. expansion of agriculture, livestock farming, bio-fuel and paper industry
- settlements / infrastructure
- exploitation of mineral resources

Fingerprinting methods help considerably in distinguishing legal from illegal wood and thus create the basis with which to fight illegal logging – one of the main causes of deforestation. Degradation resulting from legal deforestation can also be minimized via credible voluntary certification such as the FSC (Forest Stewardship Council).

With these threats in mind, strong action is needed to stop deforestation. WWF appraises the current legal situation as insufficient in most countries to fight the production of illegal wood. WWF believes there is a strong need to establish a legal basis to make the import and trade of illegal wood a legal offence. As a first step, WWF calls for the establishment of an obligatory declaration of the origin of wood. With a legal basis established, there will be a need for control methodologies.

Parallel to WWF's support to further develop the stable-isotope method, scientists all over the world have started to work on the same topic – mostly via genetic markers. The international scientific workshop organized by the German Federal Ministry of Food, Agriculture and Consumer Protection with WWF Germany offered a unique occasion to bring together top scientists working on the scrutinization of the declaration of wood origin to learn about state-of-the-art advancements. WWF Germany is pleased and proud to have helped this idea become reality in October 2007. With the workshop WWF hopes that both scientists and politicians are even more motivated to jointly develop tools to help to save our forests.

In principle it is safe to say: knowledge about the origin of timber is not a sufficient, but always a minimum pre-condition in order to make a statement regarding its legality! All scientific methods that contribute to more transparency are highly welcome!

Johannes Zahnen

WWF Deutschland, Rebstöcker Strasse 55 , 60326 Frankfurt am Main, Germany
Zahnen@wwf.de

# Use of DNA-markers for tracing illegal logging

Bernd Degen[1], Matthias Fladung[1]

## Abstract

Illegal logging is one of the main reasons of deforestation in natural forests and is the cause of high ecological and economic damage. Genetic methods are useful to infer species identity and are promising tools to control the geographic origin of logged timber. DNA barcoding and multilocus approaches using nuclear and chloroplast microsatellites as well as Single Nucleotide Polymorphism (SNPs) are the main methods in use to determine species identity. Due to recolonisation after the last glacial periods and limited pollen and seed dispersal we observe a spatial genetic structure for most species in natural forests. Genetic inventories with extensive and systematic samples over the whole species distribution area are the basis to identify the country of timber origin. In order to control for the origin of timber on the level of a logging concessions genetic inventories with a higher spatial resolution are needed. DNA extracted from wood generally is degraded in comparison to that from fresh leaf material. Thus for the practical application only gene markers that can be easily amplified in DNA extracted from unprocessed and processed timber are usable for geographic timber tracking. This has been sucessfully tested for microsatellites (cpSSRs, nSSRs) with amplified DNA fragments usually not larger than 500 bp. The power and possible spatial resolution of gene markers to identify the geographic origin of timber depends on the spatial genetic structure in the species distribution area and the quality of the genetic reference data base (the number and distribution of sampled populations, sample size within each sampled population) and the geographic distance between the geographic origin of the unknown timber probe and the next sampled population in the reference data base. Here computer simulations are very useful to design suitable sample strategies for timber tracking.

[1] Johann Heinrich von Thünen Institute (vTI), Institute of Forest Genetics,
Sieker Landstrasse 2, 22927 Grosshansdorf, Germany.
bernd.degen@vti.bund.de

*Keywords: Chloroplast, DNA barcoding, gene marker, identification, illegal logging, micro-satellite, recolonisation, refugia, spatial genetic structure, simulation studies, tropics*

## 1 Introduction

Illegal logging and trade with illegal timber and wood products are the cause for many economic and ecological problems both in the producer and in the consumer countries. Illegal logging is believed to be one of the chief causes of worldwide deforestation and trade with illegal timber and wood products creates market disadvantages for products from sustainable forestry. Moreover, fallow land produced by illegal logging contributes to climate change by releasing greenhouse-relevant gases, The OECD assesses global damages through illegal timber at approx. € 150 billion per year. According to estimates, approx. 50 % of timber exports from the Amazon Basin, Central Africa, South-East Asia and the Russian Federation originate from illegal logging. Since illegal logging poses also a major threat to forest biodiversity, the 9th Conference of the Parties of the Convention on Biological Diversity (CBD) most recently in May 2008 has urged countries to strengthen forest law enforcement and governance and to prevent illegal logging and related trade.

So far, wood anatomical methods have been made available to identify species identity for many of the traded tree species (see Koch et al. this issue). We lack, however, practicable control mechanisms to identify the species identity of a remaining set of important timber tree species and we need methods to trace the geographic origin of timber and wood products. Genetic methods have the potential to address the topic of tracing illegal logging on three scales:

### 1.1. Species identification

It is relatively common for tropical producer countries to prohibit the logging or export of certain species in certain forms. The Convention on International Trade in Endangered Species (CITES) has also the objective to protect a set of endangered tree species. CITES controls seldom

ban trade entirely, but do require that special legal documents be presented at export and import. A common way in which criminal traders seek to circumvent CITES controls is by providing a false declaration of the species involved. To overcome this problem genetic methods to control species identity would be very helpful.

1.2. Control of the geographic origin of timber

The control of the geographic origin in timber is an important issue as well. The falsification of the country of origin is another well-documented area of illegality in the trade in tropical timber. This occurs at the point of import for timber that is in international trade, and usually involves the production of false paperwork such as phyto-sanitary certificates, invoices and certificates of origin. An actual example is the ban of the EU and the USA on teak from Birma.

Another common problem of illegal logging on smaller spatial scales is the false declaration of timber that has been logged outside a registered concession, or within a protected area. On this scale certified forest companies might have an economic interest to apply genetic fingerprints to proof their efforts of sustainable forest management.

## 2 Genetic approaches

2.1. DNA extraction

A number of different DNA extraction protocols from dried and processed wood from different tree species have been developed in the last 10 years (De Filippis and Magel 1998, Deguilloux et al. 2002, Rachmayanti et al. 2006, Asif and Cannon 2007). These protocols have in common the challenge to avoid contamination with external DNA and to minimise DNA degradation (Deguilloux et al. 2002). In an early study, De Filippis and Magel (1998) demonstrated for *Robinia* that the procedures and protocols developed for leaves are applicable to wood and that RAPD-PCR technology is a versatile and sensitive method of detecting genomic changes in trees. However, all proceeding paper claim that markers of choice to circumvent both problems are those that (a) show species specificity and (b) are of small size following PCR amplification and are present in high copy number (chloroplast, mitochondrial, or repeated nuclear sequences; Deguilloux et al. 2002, Rachmayanti et al. 2006).

DNA markers of choice fulfilling the mentioned requirements and that were already successfully applied in paternity analyses and species identification are microsatellite (SSR) markers (Ziegenhagen et al. 2003, Ziegenhagen and Fladung 2004). The fact that dry wood is accessible for molecular genetic investigations opens a wide horizon of potential downstream applications. In a study to control the geographic origin of oak wood destined for the French barrel industry Deguilloux et al. (2004) detected the existence of unlabeled oak woods originating from Eastern Europe and the incorrect use of the names of famous French forests. We extracted DNA from wood in another study to detect foreign genes in wood sampled from genetically modified trees (Fladung et al. 2004). In comparison to stem wood taken from herbarium specimens, Asif and Cannon (2007) investigated the possibility of identification of an endangered tropical timber species using sequencing technology for wood DNA.

2.2. Species identification

DNA-barcoding is a genetic approach to distinguish between different species. Here differences of the nucleotide sequence at specific target DNA-regions are used for identification. These target DNA regions show genetic differences among different species but not or only to a small degree within different individuals of a species (Taberlet et al., 2007, Kress et al., 2005, Hebert et al., 2003, 2005). Other characteristics required from these target DNA-regions are that they are present in most taxa and that they are easy to sequence. For species that perform photosynthesis the so called ITS-region (Internal Trancribed Spacer) of the nucleus has been successfully used for taxonomic purposes during the last ten years (Syring et al., 2007, Barker et al., 2007, Mort et al., 2007, Kenicer et al., 2005, Erikson et al., 2003). The genome of the chloroplast of plants is highly conserved in terms of size (120-170kb), structure and linear order of genes. With a few exceptions the chloroplast genome includes two so called “Inverted repeat Regionen (IR a/b)” These two regions are interrupted by a SSC- (small single copy) and a LSC (large singly copy)-region (Shaw et al., 2007). Taxonomists have used sequence differences in standardised target regions within the chloroplast genome to distinguish among species. The LSC-region has been in the focus of these studies (Taberlet et al. 1991, Timme et al., 2007; Butcher et al., 2007; Feldberg et al., 2007; Tsai et al., 2006).

In many cases the highly variable nuclear micosatellites can be applied for different species within a genus or even within a family (see for example White and Powell 1997). Microsatellites, or Simple Sequence Repeats (SSRs), are polymorphic loci present in nuclear and organelle DNA that consist of repeating units of 1-6 base pairs in length. In most cases there are strong differences of the allele frequencies between species of the same taxonomic genus. Thus multilocus approaches have the potential to assign individuals to a given species (Duminil el al. 2006, Hertel u. Degen 2000). The advantage of this approach is that the classification is not restricted to the pure species approach. Thus even different levels of hybridisation between species can be detected.

In a recent study SNP-markers have been made available to differentiate between different *Populus* species and to detect hybrids between different *Populus* species (Fladung 2006). The "problem" with *Populus* species is the high introgressive hybridisation capacity. The genus *Populus* is divided into five sections. From about 35 poplar species of the northern hemisphere only three species are native to Central Europe: *Populus nigra* (black poplar), *Populus alba* (white poplar) and *Populus tremula* (aspen). Four of the five sections of *Populus* are represented in North America which are important for gene introgression studies when species are transferred to Central Europe because members of these sections are freely interbreeding with European poplar species. Introgressive hybridisation and gene flow from domesticated poplar species into their wild relatives can have a profound effect on the persistence and evolution of wild populations. Today, particularly the European black poplar (*P. nigra)* is a threatened species mainly caused by gene introgression from the North-American cottonwoods *P. deltoides* and *P. trichocarpa* (Vanden Broeck et al. 2004; EUFORGEN, www.ipgri.cgiar.org/networks/euforgen/euf_home.asp).

However, the risk of loosing species purity through hybridisation concerns not only black poplar, but also the two other Central Europe poplar species with wide distribution, aspen and white poplar.

Single nucleotide polymorphisms (SNPs) are DNA sequence variations at the DNA level occurring when a single nucleotide differs at a homologous position between members of a species, genus or family. SNPs are more easily detected since fast and high-throughput sequencing and genotyping techniques are available. In the last decade SNPs have been used for widespread applications, for example, in the construction of high-density genomic maps (Cho et al. 1999), in gene detection and identification, and the analysis of the genetic structure of populations (Garcia-Gil et al. 2003). Further, genetic diversity within a species which is believed to be fundamental for the ability of individuals to adapt to different/changing environments is most commonly realized on the basis of nucleotide differences. For the human genome, for instance, it has been estimated that SNPs occur in at least 10 million nucleotide positions (Wang et al. 1998), in mean every 200 to 300 bp a SNP.

In a pilot study the gene for polyphenoloxidase (PPO) was used to distinguish between *P. tremula* and *P. tremuloides*. In the EMBL-data bank two sequences from *Populus* species are available. Both sequences are 1689 bp long but differ in 66 SNPs. A fragment of 834 bp of the PPO gene was sequenced of six to 13 different genotypes per *Populus* species and "consensus" sequences specific for each species were designed. Based on these results the two aspen species could clearly be differentiated: at position 381 and 411, *P. tremula* carries a "T" and a "G" instead of a "C" and an "A" in *P. tremuloides*, respectively (red letters in Table 1, Figure 1). The interspecific hybrid of the two species is heterozygous in the two loci (Figure 1).

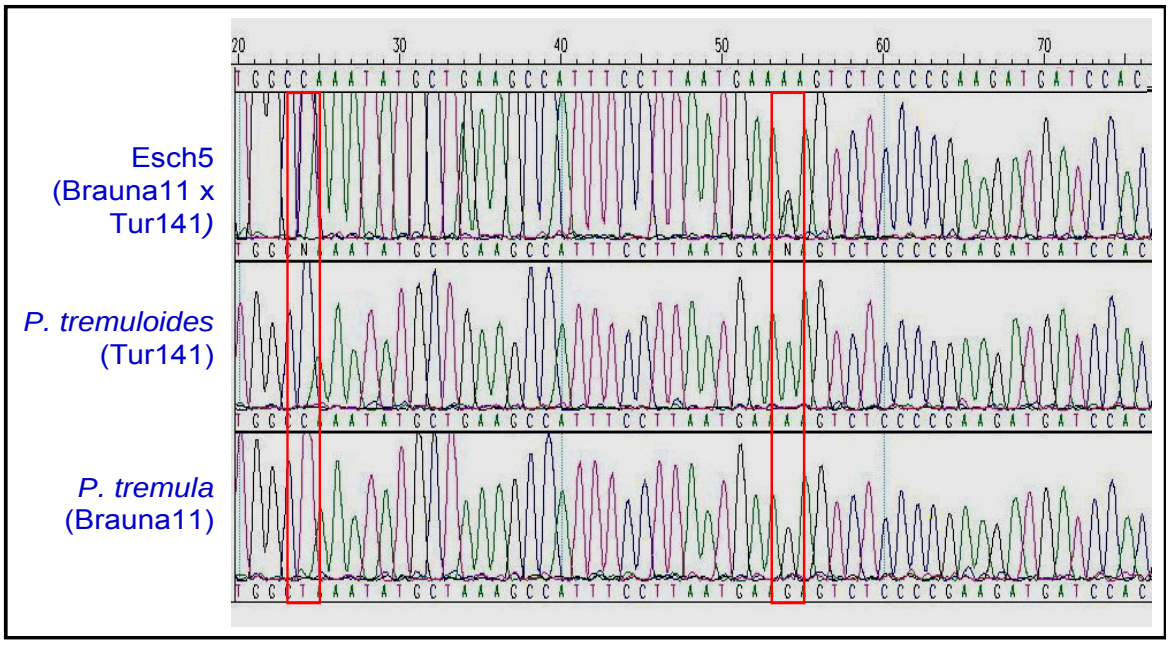



Figure 1:
Differentiation of the two aspen species by SNPs in partial sequences of the polyphenoloxidase (PPO) gene in *P. tremula* (line Brauna11) and *P. tremuloides* (line Tur141) and an interspecific hybrid (line Esch5)

Analyzing the same PPO fragment *P. alba* (green letters in Table 1), *P. nigra* (blue letters in Table 1), *P. deltoides* (brown letters in Table 1) can be distinguished from *P. trichocarpa* as well as from the other *Populus* species. A similar approach has already been performed with five more genes (Fladung and Buschbom, unpublished).

Table 1:
Partial "consensus" nucleotides of the PPO gene of six different *Populus* species. For each species six to 13 different trees have been sequenced. The sequence of *P. trichocarpa* serves as a control

| | 381 | 411 | 405 | 673 | 444 | 781 | 342 | 511 | 378 | 424 |
|---|---|---|---|---|---|---|---|---|---|---|
| *P. trichocarpa* | C | A | A | G | C | C | T | G | C | A |
| *P. tremula* | **T** | **G** | A | G | C | C | T | G | G | G |
| *P. tremuloides* | **C** | **A** | A | G | C | C | T | G | G | G |
| *P. alba* | C | A | **G** | **A** | C | C | T | G | G | G |
| *P. nigra* | C | A | A | G | **T** | **A** | T | G | G | G |
| *P. deltoides* | C | A | A | G | C | C | **A** | **A** | G | G |
| | 381 | 411 | 405 | 673 | 444 | 781 | 342 | 511 | 378 | 424 |

2.3. Controlling the geographic origin within a species

In natural forests usually a genetic structure at local and regional spatial scales can be observed. In temperate forests as well as in tropical forests, the glacial periods changed the vegetation drastically. In the temperate zone large areas were covered by ice and were free of any vegetation and in the tropics former rain forests were transformed to dry savannas. After each glacial period trees recolonised their distribution area starting from different refugia. As a result of this recolonisation in many cases a clear genetic differentiation can be identified between tree populations from different regions. The extent of genetic differences depends on the recolonisation routes and the genetic differences in these refugia. Chloroplast gene markers and nuclear microsatellites have been successfully used to elaborate reference data about this genetic differentiation (Petit et al. 1997, Caron et al. 2000, Dutech et al. 2000, 2003, Koenig et al. 2003). Figure 2 shows one example for the tropical tree species *Carapa guianensis* in the Amazon (Cloutier et al. 2005). Limited pollen and seed dispersal are the main factors casing spatial genetic structure on smaller scales in natural tree populations (Degen et al. 2001, 2004; Hardy et al. 2006). The spatial resolution of a possible control of timber origin depends on local and regional genetic differences and on the number of sampled populations used to generate a genetic reference data base (Degen et al. 2001, Cavers et al. 2005). There are already a few good data sets for the spatial genetic structure of tree populations on a large scale with a spatial resolution of 50 to 200 km (Caron et al. 2000, Dutech et al. 2000, 2003). And even more studies have found spatial genetic structure on very small scales with a resolution of a few meters to a few hundred meters (Degen et al. 2001, figure 3, 4).

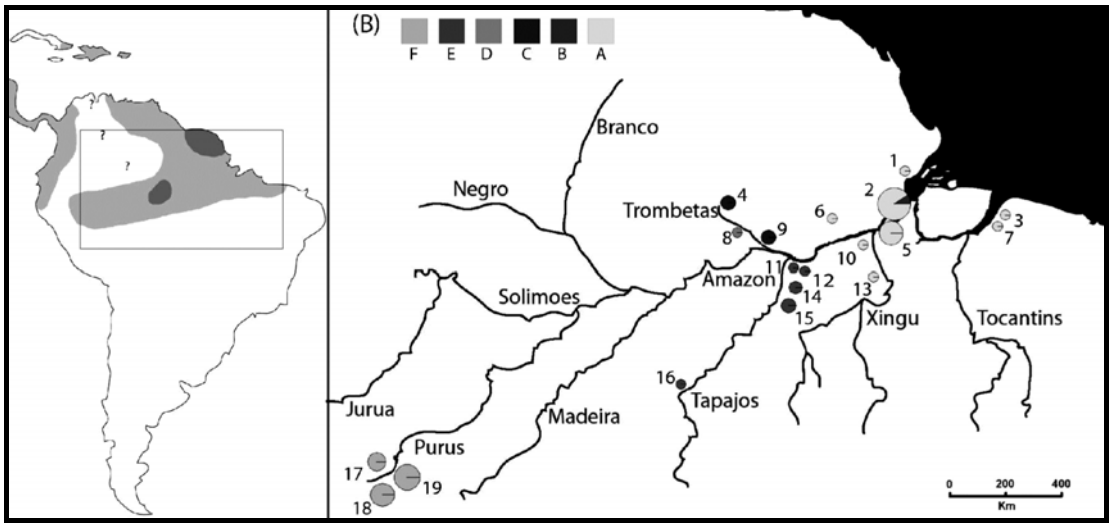



Figure 2:
(a) Geographic range of *Carapa guianensis* (light grey) and areas where both *C. guianensis* and *C. procera* are found (dark grey). (b) Map of the 19 locations sampled for chloroplast DNA variation in *Carapa guianensis* in the Amazon basin. Each location is represented by a numbered circle of size proportional to the number of individuals sampled, and each haplotype is represented by a different colour (Cloutier et al. 2005)

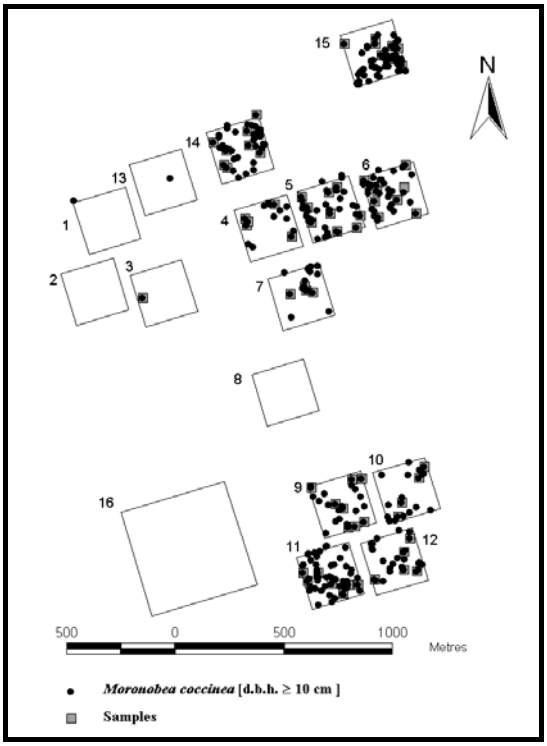



Figure 3:
Position of all trees of the tropical tree species *Moronobea coccinea* [d.b.h. > 10 cm] and position of trees that have been sampled for genetic studies in the experimental trial Paracou in French Guiana (Degen et al. 2001)

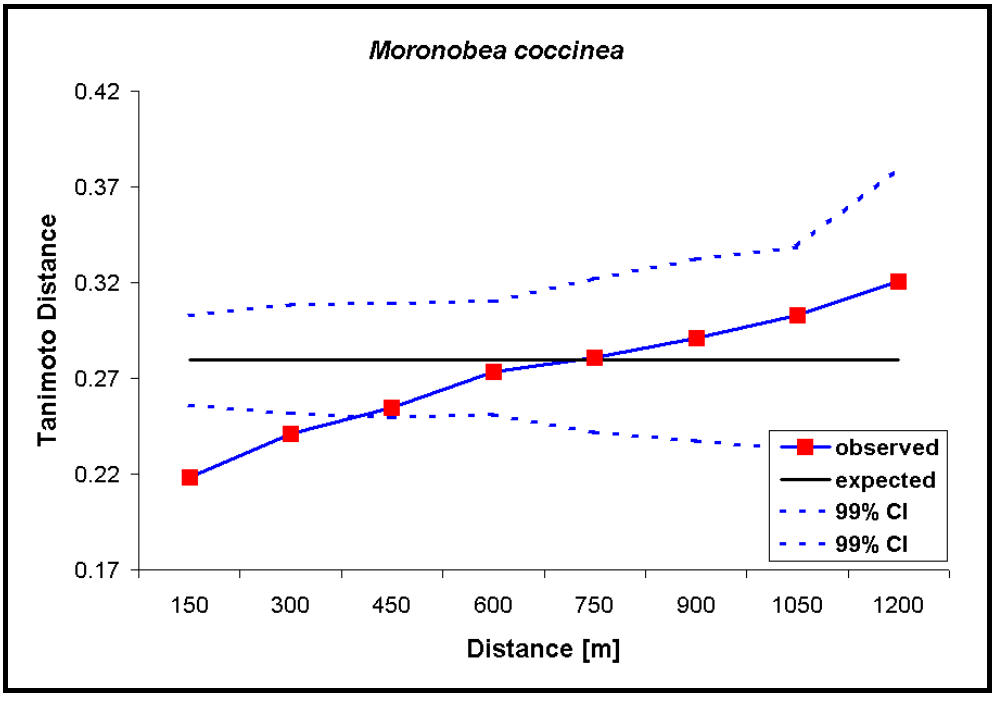



Figure 4:
On a scale of up to 1200m increasing genetic distance (Tanimoto) is detected with increasing spatial distance among Monorobea trees in Paracou in French Guiana (Degen et al. 2001)

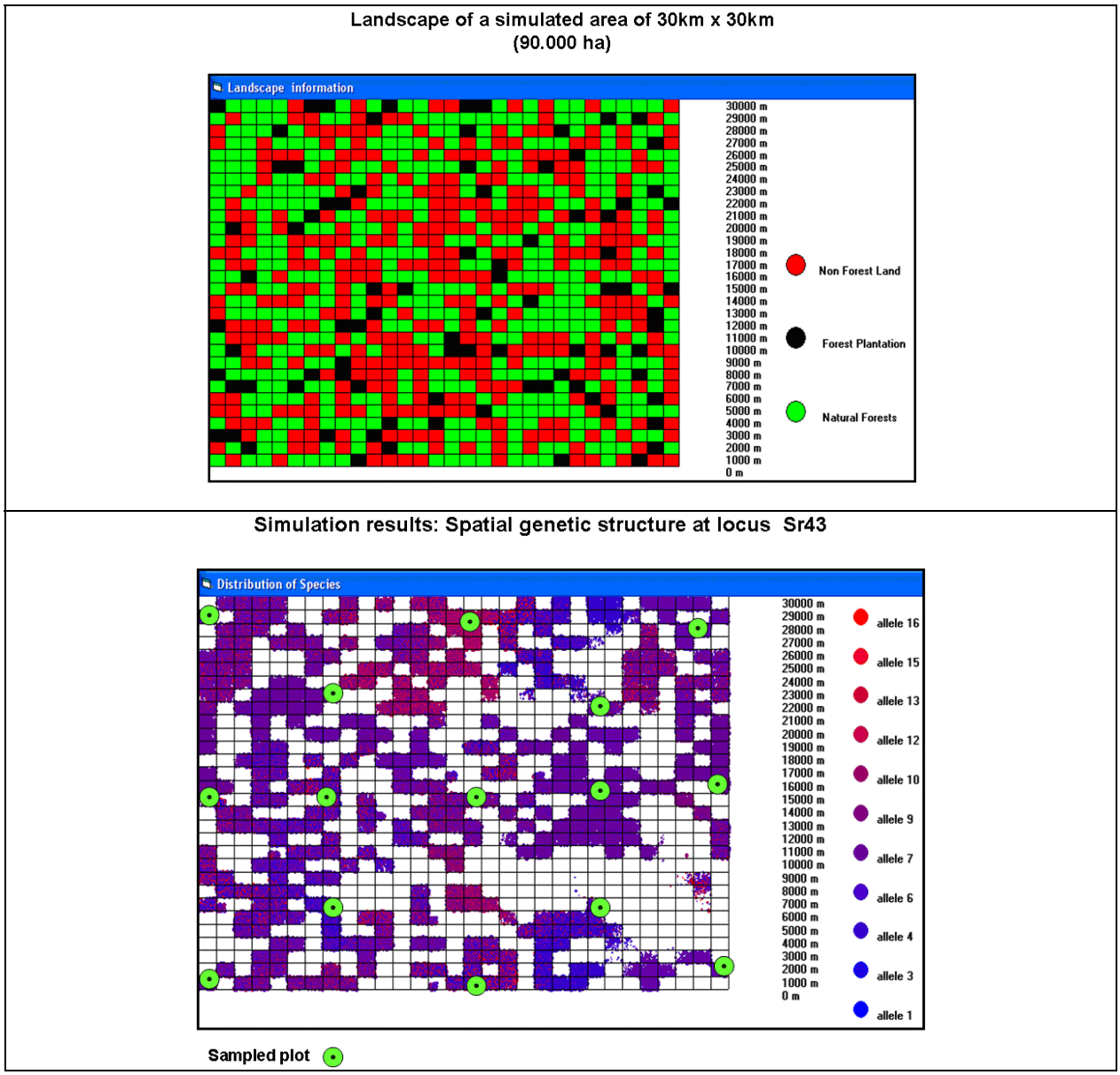



Figure 5:
a): Distribution of natural forest, forest plantation and non forest land in a large scale simulation with the computer model Eco-Gene. The simulated area covers 90.000 ha. Each grid cell represents 100 ha. (b) After 3000 simulated years clear genetic differences (illustrated for one locus as different colours) were observed. Based on this spatial genetic structure at several gene loci a distribution of 15 sample plots along transects (green circles) is expected to be a good starting point for successful assignment of randomly selected individuals to the next sample plot.

So far missing are results on genetic patterns at spatial scales that can be used to control the origin of timber from a logging concession with a usual size of several thousand hectares. We used the simulation model Eco-Gene (Degen et al. 2006) to run first simulations on the expected spatial genetic pattern for a tropical tree species at a scale of 30 km by 30 km (figure 5). The simulations included typical allele frequencies for nuclear microsatellites, densities of a commercial tropical tree species, diameter distribution and important population genetic processes like re-colonisation, pollen and seed dispersal. The simulation results suggested that there is a correlation between genetic and geographic distance up to a few kilometres. In these cases genetic assignment of unknown individuals has a chance of success if there is a sampled population in the reference data base every 5 km (figure 5b).

Here we need more simulation studies to consider the special situation of each timber species and timber concession.

The power of genetic fingerprinting to trace the geographic origin of timber depends on the spatial genetic pattern in the natural tree populations, the sample design for the genetic reference data base and the possibility to amplify the gene markers from DNA extracted from wood.

### 3 References


Asif MJ, Cannon CH (2007) DNA extraction from processed wood: a case study for the Identification of an endangered timber species (Gonystylus bancanus). Plant Mol Biol Report 23:185-192

Barker NP, Galley C, Verboom GA, Mafa P, Gilbert M, Linder HP (2007) The phylogeny of the austral grass subfamily Danthonioideae: Evidence from multiple data sets . Plant Sys Evol 264:135-156

Caro H, Dumas S, Marque G, Messier C, Bandou E, Petit RJ, Kremer A (2000) Spatial and temporal distribution of chloroplast DNA polymorphism in a tropical tree species. Mol Ecol 9:1089-1098

Cavers S, Degen B, Caron H, Lemes MR, Margis R, Salgueiro F, Lowe AJ (2005) Optimal sampling strategy for estimation of spatial genetic structure in tree populations. Heredity 95:281-289

Cho RJ, Mindrinos M, Richards DR, Sapolsky RJ, et al (1999) Genome-wide mapping with biallelic markers in Arabidopsis thaliana. Nature Genetics 23:203-207

Cloutier D, Povoa JSR, Procopio LC, Leao NVM, Wadt LHO, Ciampi AY, Schoen DJ (2005) Chloroplast DNA variation of Carapa guianensis in the Amazon basin. Silvae Genet 54:270-274

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

Degen B, Bandou E, Caron H (2004) Limited pollen dispersal and biparental inbreeding in Symphonia globulifera in French Guiana. Heredity 93:585-591

Degen B, Caron H, Bandou E, Maggia L, Chevallier MH, Leveau A, Kremer A (2001) Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. Heredity 87:497-507

Degen B, Petit R, Kremer A (2001) SGS - Spatial genetic software: A computer program for analysis of spatial genetic structures of individuals and populations. J Hered 92:447-448

Deguilloux MF, Pemonge MH, Petit RJ (2002) Novel perspectives in wood certification and forensics: dry wood as a source of DNA. Proc R Soc Lond, Ser B-Biol Sci 269:1039-1046

Deguilloux MF, Pemonge MH, Petit RJ (2004) DNA-based control of oak wood geographic origin in the context of the cooperage industry. Ann For Sci 61:97-104

Duminil J, Caron H, Scotti I, Cazal SO, Petit RJ (2006): Blind population genetics survey of tropical rainforest trees. Mol Ecol 15:3505-3513

Dutech C, Maggia L, Joly HI (2000) Chloroplast diversity in Vouacapoua americana (Caesalpinniaceae), a neotropical forest tree. Mol Ecol 9:1427-1432

Dutech C, Maggia L, Tardy C, Joly HI, Jarne P (2003) Tracking a genetic signal of extinction-recolonization events in a neotropical tree species: Vouacapoua americana aublet in french guiana. Evolution 57:2753-2764

Erikson T, Hibbs MS, Yodrer AD, Delwiche CF, Donoghues MJ (2003) The phylogeny of rosideae (Rosaceae) based on sequences of the internal transcribed spcers (ITS) of nuclear ribosomal DNA and the TRNL/F region of chloroplast DNA. Int. J. Plant Sci. 164:197-211

Fladung M (2006) Entwicklung von SNP-Markern in putativ anpassungsrelevanten Genen. Vortr Pflanzenzüchtg Göttingen 70:139-146

Fladung M, Buschbom J (submitted) Identification of Single Nucleotide Polymorphisms (SNPs) in different Populus species.

Fladung M, Nowitzki O, Ziegenhagen B, Markussen T (2004) Identification of transgenes from wood of genetically transformed poplar trees. Wood Sci Technol 38:207-215

Garcia-Gil MR, Mikkonen M, Savolainen O (2003) Nucleotide diversity at two phytochrome loci along a latitudinal cline in Pinus sylvestris. Mol Ecol 12:1195–1206

Hardy OJ, Maggia L, Bandou E, Breyne P, Caron H, Chevallier MH, Doligez A, Dutech C, Kremer A, Latouche-Halle C, Troispoux V, Veron V, Degen B (2006) Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. Mol Ecol 15:559-571

Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. Proc R Soc Lond, Ser B-Biol Sci 270:313-321

Hebert PDN, Gregory, TR (2005) The promise of DNA barcoding for taxonomy. Syst Biol 54:852-859

Hertel, H, Degen B (2000) Unterscheidung von Stiel- und Traubeneichen (Quercus robur L. und Q. petraea [Mattuschka] Liebl.) mit Hilfe von genetischen und morphologischen Merkmalen. For Snow Landsc Res 75:169-183

Indrioko S, Gailing O, Finkeldey R (2006) Molecular phylogeny of Dipterocarpaceae in Indonesia based on chloroplast DNA. Plant Syst Evol 261:99-115

Kencier GJ, Kajita T, Pennington RT, Murata J (2005) Systematics and biogeography of Lathyrus (Leguminosae) based on internal transcribed spacer and cpDNA sequence data. Am. J. of Bot. 92:1199-1209

Konig AO, Ziegenhagen B, Dam BCV, Csaikl UM, Coart E, Degen B, Burg K, Vries SMGD, Petit RJ (2002) Chloroplast DNA variation of oaks in western Central Europe and genetic consequences of human influences. For Ecol Manage 156:147-166

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of barcodes to identify flowering plants. Proc. Natl. Acad. Sci. USA 102:8369-8374

Lemes MR, Gribel R, Proctor J, Grattapaglia D (2003) Population genetic structure of mahogany (Swietenia macrophylla King, Meliaceae) across the Brazilian Amazon, based on variation at microsatellite loci: implications for conservation. Mol Ecol 12:2875-2883

Mort ME, Archibald JK, Randle CP, Levsen ND, O'Leary TR, Topalov K, Wiegand CM, Crawford DJ (2007) Inferring phylogeny at low taxonomic levels: Utility of rapidly evolving cpDNA and nuclear ITS loci. Am. J. of Bot. 94:173-183

Novick RR, Dick CW, Lemes MR, Navarro C, Caccone A, Bermingham E (2003) Genetic structure of Mesoamerican populations of Big-leaf mahogany (Swietenia macrophylla) inferred from microsatellite analysis. Mol Ecol 12:2885-2893

Petit RJ, Pineau E, Demesure B, Bacilieri R, Ducousso A, Kremer A 1997: Chloroplast DNA footprints of postglacial recolonization by oaks. Proc. Natl. Acad. Sci. USA 94:9996-10001

Rachmayanti Y, Leinemann L, Gailing O, Finkeldey R (2006) Extraction, amplification and characterization of wood DNA from Dipterocarpaceae. Plant Mol Biol Report 24:45-55

Shaw J, Lickey E, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for Phylogenetic studies in angiosperms: The Tortoise and the Hare III. Am J Bot 94:275-288

Syring J, Farrell K, Businsky R, Cronn R, Liston A (2007) Widespread genealogical nonmonophyly in species of Pinus subgenus Strobus. Syst Bio 56:163-181

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105-1109

Vanden Broeck A, Storme V, Cottrell JE, Boerjan W, Van Bockstaele E, Quataert P, Van Slycken J (2004) Gene flow between cultivated poplars and native black poplar (Populus nigra L.): a case study along the river Meuse on the Dutch–Belgian border. For Ecol Manage 197:307–310

Wang DG, Fan JB, Siao CJ, Berno A, et al (1998) Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science 280:1077-1082

White G, Powell W (1997) Cross-species amplification of SSR loci in the Meliaceae family. Mol Ecol 6:1195-1197

Ziegenhagen B, Fladung M (2004) DNA markers for identification and evaluation of genetic resources in forest trees – Case studies in Abies, Picea and Populus. In: Lörz H, Wenzel G (eds) Molecular Marker Systems in Plant Breeding and Crop Improvement. Springer Verlag. pp 413-429

Ziegenhagen B, Liepelt S, Kuhlenkamp V, Fladung M (2003) Molecular identification of individual oak and fir trees from maternal tissues of their fruits or seeds. Trees 17:345-350

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

Andrew Lowe[1]


## 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 interogable 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.


[1] Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Science, University of Adelaide, North Terrace, SA5000, Australia
andrew.lowe@adelaide.edu.au

*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).

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 additional, several additives can also be incorporated 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 |
|---|---|---|---|
| *Swietenia macrophylla* (mahogany) | Central America, southern Brazil | SSRs and RAPD | Novick et al 2003, Lemes et al 2003, Gillies et al 1999 |
| *Cedrela odorata* (Spanish cedar) | Central America (Costa Rica) | cpDNA, AFLP | Cavers et al 2003, 2005 |
| *Ceiba pentandra* | Neotropics and Africa | cpDNA and ITS | Dick et al 2007 |
| *Vouacapoua americana* | French Guyane and the Amazon Basin | cpDNA | Dutech et al 2000<br>Dutech et al 2003<br>Dutech et al 2004 |
| *Carapa guianensis* | Amazon basin | cpDNA | Cloutier et al 2005 |
| *Pterocarpus officinalis* | Caribbean Basin | AFLP | Rivera-Ocasio et al 2002 |
| *Vochysia ferruginea* | Costa Rica | cpDNA and AFLP | Cavers et al 2005 |
| *Lonchocarpus costariensis* | Guanacaste, Costa Rica | cpDNA and AFLP | Navarro et al 2005 |
| *Irvingia wombulu* and *I. gabonensis* | Nigeria, Cameroon, Gabon | RAPD | Lowe et al 2000 |
| *Aucoumea klaineana* | 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.

## 6 References


Cavers S, Navarro C, Hopkins P, Ennos RA, Lowe AJ (2005) Regional and population-scale influences on genetic diversity partitioning within Costa Rican populations of the pioneer tree Vochysia ferruginea Mart. Silvae Genet 54:258-264

Cavers S, Navarro C, Lowe AJ (2003) Chloroplast DNA phylogeography reveals colonisation history of a Neotropical tree, Cedrela odorata L., in Mesoamerica. Mol Ecol 12:1451-1460

Colpaert N, Cavers S, Bandou E, Caron H, Gheysen G, Lowe AJ (2005) Sampling tissue for DNA analysis of trees: trunk cambium as an alternative to canopy leaves. Silvae Genet 54:265-269

Cloutier D, Povoa JSR, Procopio LC, et al (2005) Chloroplast DNA variation of Carapa guianensis in the Amazon basin. Silvae Genet 54:270-274

Cottrell JE, Samuel CJ, Sykes R (2004) The species and chloroplast DNA haplotype composition of oakwoods in the Forest of Dean planted between 1720 and 1993. Forestry 77:100-106

Deguilloux MF, Pemonge MH, Bertel L, Kremer A, Petit RJ (2003) Checking the geographical origin of oak wood: molecular and statistical tools. Mol Ecol 12:1629-1636

Deguilloux MF, Pemonge MH, Petit RJ (2002) Novel perspectives in wood certification and forensics: dry wood as a source of DNA. Proc R Soc Lond B: Biological Sciences 269:1039-1046

Deguilloux MF, Dumolin-Lapègue S, Gielly L, Grivet D, Petit RJ (2003) A set of primers for the amplification of chloroplast microsatellites in Quercus. Mol Ecol Notes 3:24-27

Dick CW, Bermingham E, Lemes MR, et al (2007) Extreme long-distance dispersal of the lowland tropical rainforest tree Ceiba pentandra L. (Malvaceae) in Africa and the Neotropics. Mol Ecol 16:3039-3049

Dumolin-Lapègue S, Petit RJ, Gielly L, Taberlet P (1999) Amplification of DNA from ancient and modern oak wood. Mol Ecol 8:2137-2140

Dutech C, Joly HI, Jarne P (2004) Gene flow, historical population dynamics and genetic diversity within French Guianan populations of a rainforest tree species, Vouacapoua americana. Heredity 92:69-77

Dutech C, Maggia L, Tardy C, et al. (2003) Tracking a genetic signal of extinction-recolonization events in a neotropical tree species: Vouacapoua americana Aublet in French Guiana. Evolution 57:2753-2764

Dutech C, Maggia L, Joly HI (2000) Chloroplast diversity in Vouacapoua americana (Caesalpiniaceae), a neotropical forest tree. Mol Ecol 9:1427-1432

Gillies ACM, Navarro C, Lowe AJ, Newton AC, Hernandez M, Wilson J, Cornelius JP (1999) Genetic diversity in Mesoamerican populations of mahogany (Swietenia macrophylla), assessed using RAPDs. Heredity 83:722-732

Gugerli F, Parducci L, Petit RJ (2005) Ancient plant DNA: review and prospects. New Phytol 166:409-418

Lahaye R, van der Bank M, Bogarin D, Warner J, Pupulin F, Gigot G, Maurin O, Duthoit S, Barraclough TG, Savolainen V (2008) DNA barcoding the floras of biodiversity hotspots. Proc Natl Acad Sci USA 105:2761-2762

Lemes MR, Gribel R, Proctor J, et al (2003) Population genetic structure of mahogany (Swietenia macrophylla King, Meliaceae) across the Brazilian Amazon, based on variation at microsatellite loci: implications for conservation. Mol Ecol 12:2875-2883

Lowe AJ, Munro RC, Samuel S, Cottrell J (2004) The utility and drawbacks of chloroplast DNA for identifying native British oak stands. Forestry 77:335-347

Lowe AJ, Wilson J, Gillies ACM, Dawson I (2000) Conservation genetics of bush mango from central/west Africa, implications from RAPD analysis. Mol Ecol 9:831-841

Muloko-Ntoutoume N, Petit RJ, White L, et al (2000) Chloroplast DNA variation in a rainforest tree (Aucoumea klaineana, Burseraceae) in Gabon. Mol Ecol 9:359-363

Navarro C, Cavers S, Colpaert N, Hernandez G, Breyne P, Lowe AJ (2005) Chloroplast and total genomic diversity in the endemic Costa Rican tree Lonchocarpus costaricensis (J.D.Smith) Pittier (Papilionaceae). Silvae Genet 54:293-300

Novick RR, Dick CW, Lemes MR, et al (2003) Genetic structure of Mesoamerican populations of Big-leaf mahogany (Swietenia macrophylla) inferred from microsatellite analysis. Mol Ecol 12:2885-2893

Petit RJ, Csaikl U, Bordacs S, Burg K, Coart E, Cottrell J, van Dam B, Deans JD, Glaz I, Dumolin-Lapègue S, Fineschi S, Finkeldey R, Gillies ACM, Goicoechea PG, Jensen JS, König A, Lowe AJ, Madsen SF, Matyas G, Munro RC, Olalde M, Pemonge MH, Popescu F, Slade D, Tabbener H, Taurchini D, de Vries SGM, Ziegenhagen B, Kremer A (2002) Chloroplast DNA variation in European white oaks: Phylogeography and patterns of diversity based on data from over 2,600 populations. For Ecol Manage 156:5-26

Primmer CR, Koskinen MT, Piironen J (2000) The one that did not get away: individual assignment using microsatellite data detects a case of fishing competition fraud. Proc R Soc Lond B: Biological Sciences 267:1699–1704

Pritchard JK, Stephens M, Donnelly PJ (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959

Rivera-Ocasio E, Aide TM, McMillan WO (2002) Patterns of genetic diversity and biogeographical history of the tropical wetland tree, Pterocarpus officinalis (Jacq.), in the Caribbean basin. Mol Ecol 11:675-683

Speirs A, Loy T, McConnachie G, Lowe AJ (2008) Genotyping the Mary Rose: the hunt for DNA in a 16th century flagship. In: Haslam, Robertson, Crowther, Kirkwood, Nugent (eds) Archaeological Science Under a Microscope: Studies in Residue and DNA Analysis in Honour of Tom Loy. In press

Tani N, TsumuraY, et al (2003) Nuclear gene sequences and DNA variation of Cryptomeria japonica samples from the postglacial period. Mol Ecol 12:859-868

# Identification of the timber origin of tropical species by molecular genetic markers – the case of dipterocarps

Reiner Finkeldey[1], Yanti Rachmayanti, Hani Nuroniah, Nga Phi Nguyen, C. Cao and Oliver Gailing

## Abstract

Illegal logging continues to be a main cause for the destruction of tropical forest ecosystems. The development of non-manipulable tools to control the origin of timber and timber products from tropical tree species will greatly contribute to distinguish legally from illegally harvested wood. This will promote the marketing of tropical timber from sustainable managed forests and will eventually support the ban of illegally harvested material.

We tested the application of molecular genetic markers to identify the origin of tropical Dipterocarpaceae. Dipterocarps are a very species-rich family dominating tropical forests in South- and Southeast-Asia. They are the main source of tropical timber (trade name, for example, meranti) from this region. Since most species have a restricted distribution, species identification is an important and in many cases sufficient indication of the origin of timber.

In total, more than 3000 dipterocarps representing over 110 different species have been sampled. Sampling has been most intensive on the Indonesian islands of Borneo and Sumatra. Locations from Vietnam, Thailand, and the Philippines are represented as well. We developed a simple and reliable method to extract DNA from dipterocarp wood based on a frequently used extraction kit. The success and efficiency of the method to extract DNA of good quality for PCR amplification from freshly cut timber and processed wood products was tested. The success rate for amplification was influenced by the age of wood, the degree of processing, and inhibitory substances. It was possible to increase the success rate in many cases to 100% of all investigated samples by a careful selection of the amplified DNA fragment (fragment length; genomic origin, repeat number), appropriate dilution of template DNA, repeated eluation of DNA, and choice of the most suitable position for investigation (inner or outer wood). The method proved to be applicable for the majority of investigated dipterocarp wood samples and for most other investigated material as well.

In a parallel attempt, we developed markers to distinguish between closely related species from the same timber group and between geographic regions from widely distributed, common species. Species distinction is often possible by the investigation of cpDNA fragments of different length. The identification of the region of origin is hampered by a moderate degree of genetic differentiation for the two common dipterocarps *Shorea leprosula* and *S. parvifolia*. However, we observed strong geographic differentiation at several AFLP markers, which were converted to SCAR (*Sequence Characterized Amplified Region*) markers.

In summary, dipterocarps are suggested as a suitable group of species to implement a system for the identification of the origin of tropical timber.

*Keywords: timber origin, DNA extraction, DNA marker, genetic variation, tropical tree, Dipterocarpaceae*

## 1 Introduction

Forest destruction and degradation continue to be main threats to global biodiversity and cause severe environmental damage. Considerable losses of forest cover diminish the forested area in all main regions of the tropics (FAO 2001). Alarming rates of deforestation are reported for several tropical countries in Southeast-Asia. Several attempts to promote forest conservation and to combat deforestation aim at promoting the trade, marketing and use of timber and wood

*Forest Genetics and Forest Tree Breeding, Büsgen Institute, Faculty of Forest Sciences and Forest Ecology, Göttingen University, Büsgenweg 2, 37077 Göttingen, German*
rfinkel@gwdg.de

products from sustainably managed forests and to exclude illegally harvested timber from na- to apply tools allowing to proof the origin of wood and wood products or to reliably test the declaration of the origin of timber. Customs offices in producer and consumer countries, forest certification schemes such as the Programme for the Endorsement of Forest Certification Schemes, (PEFC, www.pefc.org/internet/html/ index.htm) and the Forestry Stewardship Council (FSC, http://www.fsc.org/en/), forest enterprises producing timber according to the principles of sustainable forest management, as well as the timber industry and end consumers potentially benefit from improved methods to infer the origin of wood which might be potentially illegally harvested. Currently available methods only rarely allow to prove false declarations of the origin of wood in court. Thus, the development of innovative methods to test the proclaimed origin of timber based on non-manipulable characters is important in this context.

DNA is a stable and highly variable molecule. Hence, variation of DNA is potentially useful to conclude on the origin of any biological material. Two basic requirements need to be fulfilled in order to use DNA variation for the identification of the origin of wood: (i) It is possible to isolate DNA from wood of different age and processing status, and (ii) markers need to be developed which are informative with regard to the identification of the origin of wood.

We developed molecular tools to infer the origin of dipterocarps (Dipterocarpaceae). The family is pantropical with comparatively few species occurring in the neotropics and in Africa. The subfamily Dipterocarpoideae is very species-rich and common in Asian evergreen and Monsoon forests. The centre of species diversity is reached in Borneo (Kalimantan) with more than 260 described species; few species are native to forests east of the Wallace-Line.

More than 50% of all trees including the majority of emergents and trees of the canopy are dipterocarps in many natural forests in tropical Asia, which are regarded as a centre of global biodiversity. Dipterocarps are not only a keystone resource in particular in tropical Southeast-Asia (Whitmore 1975), but they are also among the most important tropical timbers (trade names: meranti, balau for Shorea spp., keruing for Dipterocarpus spp., kapur for Dryobalanops, etc.). In many regions dipterocarps are critically endangered due to forest destruction and non-sustainable forest management leaving only secondary tional and international markets. In this context, it is of prime importance to develop and forests of little commercial value after logging. Dipterocarps are rarely grown in plantations although attempts are made to establish man-made dipterocarp forests.

Sustainable management of dipterocarp forests is feasible, if harvesting is carefully controlled and natural regeneration promoted (Lamprecht 1986). Thus, the development of tools to identify dipterocarp wood from sustainably managed forests will contribute to the application of sustainable management practices and the conservation of dipterocarps and their associated species. The large number of commercial species needs to be taken into consideration for the development of tools for wood identification. For example, the wood from more than 100 species belonging to the species-rich genus *Shorea* is differentiated into only a few trade names (white meranti, yellow meranti, dark red and light red meranti).

We developed a simple protocol for the extraction of DNA from the wood of dipterocarps (Rachmayanti et al. 2006), which was tested for a large number of wood samples. We also investigated DNA variation among and within dipterocarp species in order to develop informative markers with regard to the origin of material.

## 2 Materials and methods

### 2.1.Material

Out of the 332 wood samples belonging to the family Dipterocarpaceae, 181 were collected from natural forests or plantations in South-East Asia, and 151 were from wood enterprises or wood processing facilities in Germany. At least 40 samples were taken in each of the following four countries: Thailand, Vietnam, Philippines, Indonesia. In each country, samples were collected at at least four different sites. The following taxa were sampled: *Anisoptera, Dipterocarpus, Hopea, Parashorea, Shorea,* and *Vatica*. Leaves collected from 25 dipterocarps trees and corresponding wood samples from Indonesia and the Philippines were used for the verification of the DNA extraction method. Wood from 74 tree species other than dipterocarps was included for comparisons.

Leaf material from over 3,000 dipterocarp trees was sampled throughout the Asian range of the family and is available for investigation. Most material is from natural forests in Indonesia; the

species-rich genus *Shorea* is particularly well-represented in these samples. A total of 116 different species were sampled in natural forests, botanical gardens, and plantations and arboreta.

**3 DNA Isolation**

DNA from wood was extracted with the DNeasy Plant Mini Kit (Qiagen) applying the same modifications and optimizations as reported in Rachmayanti et al. (2006). Prior to extraction the surface tissues (including cambium and bark) of wood samples were removed using a sawing machine to avoid contamination with other DNA. 50 – 100 mg of shavings produced by drilling of the clean inner part of wood samples were used for DNA extraction. Polyvinylpyrolidone was added into the lysis buffer (step 8.a, Rachmayanti et al. 2006) up to 2.6% (w/v). The effect of PVP addition on PCR inhibition was tested for different concentrations. The last step of the extraction protocol (elution) was performed twice; the second eluate was collected separately from the first. Genomic DNA of leaf samples was extracted with the DNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen).

DNA isolated from leaf and wood from the same tree were analyzed by PCR amplification, genotyping and sequencing. Chloroplast microsatellite primers (Weising and Gardner, 1999) were applied for genotyping. The intergenic spacer between *trnL* and *trnF* was amplified and sequenced as well. PCR amplification, genotyping and sequencing was carried out as reported by Rachmayanti *et al.* (2006) except that direct sequencing of purified PCR products (without cloning) was performed.

PCR amplification was performed to examine the success of DNA extraction from wood. Three primer pairs were chosen to amplify three fragments of different length (short fragment of about 100-200 bp, middle length fragment of about 500-600 bp and long fragment of about 1100bp). PCR–reaction mixtures were prepared and amplification protocols were used as reported previously (Rachmayanti *et al.* 2006).

In order to study the effectiveness of PVP addition on the reduction of PCR inhibitory substances, three DNA isolation methods differing in the concentration of PVP in the lysis buffer (without, with 2.6% and with 5.0 %[w/v] PVP were compared (step 8a of DNA isolation method, Rachmayanti *et al.* 2006). PCR inhibitory tests were performed using the same parameters as in normal PCR except that mixed DNA (DNA extract from wood plus another known high-quality DNA) was applied as PCR template. A total of 2.5 µl volume of PCR template (2 µl of wood-DNA extract + 0.5 µl of high quality leaf DNA) was applied for each PCR reaction. In this PCR inhibitory test, a series of 2.5 µl volume of PCR templates was prepared. Each template contained 0.5 µl of leaf DNA mixed with 2 µl of undiluted, 10 times, 20 times, 40 times, 80 times and 160 times diluted wood DNA extract, respectively. This test was performed for the wood DNA extract from each isolation method.

The quantity and quality of the DNA extract from three different zones of wood were analyzed, i.e. zone a: outer sapwood (without cambium or bark), m: transition zone and zone i: inner heartwood. PCR amplification was performed on the first and the second eluate of each zone. Three markers with different fragment lengths (*ccmp2*, 0.15 kb; *trnL*, 0.6 kb; *trnLF*, 1.1 kb) were used for PCR. The PCR reaction mixture and the amplification protocol were carried out according to Rachmayanti *et al.* (2006). 2 µL of undiluted eluate was used as template in each PCR.

**4 Genetic variation and marker development**

Variation among species was assessed by means of PCR-RFLPs, cpSSRs, AFLPs, and sequencing. Methods are described in detail by Indrioko et al. (2006) for PCR-RFLPs and cpSSRs, and Cao et al. (2006a) for AFLPs. DNA sequence variation of selected cpDNA fragments and the ITS region is under investigation by Nuroniah et al. (2008; in prep.) and Nguyen et al. (2008; in prep.)

Intraspecific variation was assessed in detail for two common *Shorea* species in Indonesia: *S. leprosula* and *S. parvifolia* by means of AFLPs (Cao et al. 2006b). The conversion of AFLP markers to SCAR markers is described by Nuroniah et al. (2008; in prep.).

**5 Results and discussion**

5.1. DNA Isolation

In order to test the DNA isolation method, wood and leaf DNA extracts from the same tree were amplified with three chloroplast microsatellite primers and genotyped. The result shows that the microsatellite fragments of wood and leaf from the same tree have the same length. The chloroplast region *trnF* (approximately 400 bps)

was sequenced for five trees sampled in the Philippines in order to verify the method. The results show that the *trnF* sequences obtained from wood and leaf from the same tree were identical (100% homolog), but that the sequences differ among species. A Blast search against the EMBL data base confirmed that the sequences belong to the expected genomic regions.

A concentration of 2.6% (w/v) of PVP was routinely added to the lysis buffer in most extractions reported here. Results after the addition of 2.6% PVP were generally satisfactory. However, the extraction of high quality DNA from a few wood samples was difficult due to strong PCR inhibition. These samples were used to evaluate the effect of different PVP additions to the lysis buffer on amplification success.

Three DNA isolation methods differentiated by the different PVP quantity added into the lysis buffer (0%, 2.6% and 5.0% [w/v]) were compared. The effect of different PVP quantity on PCR inhibition was tested. DNA extraction without PVP treatment left a high content of PCR inhibitory substances so that even 160 times diluted wood extract inhibited the PCR reaction. The addition of 5% (w/v) of PVP shows that a 40 times diluted extract had no inhibitory effect showing that PVP addition to the lysis buffer (step 8.a, Rachmayanti 2006) can effectively reduce PCR inhibition in the DNA extract.

Eleven wood samples from Indonesia were used for this analysis of DNA quantity and quality in different parts of the wood. Undiluted DNA extracts (both of 1st and 2nd eluate) from the three different zones of wood (outer sapwood (a), transition zone between sapwood and heartwood (m) and inner heartwood (i)) were used as template. PCR success rate was tested with the three chloroplast markers. In each PCR test a PCR positive control of dipterocarp leaf DNA and PCR negative controls of water were included (Fig. 1). An analysis of PCR success rates of the second eluate suggests that DNA quantity is decreasing along the wood regions from outer sapwood to inner heartwood. This is illustrated by the decreasing PCR success rate for short (0.15 kb), middle (0.6 kb) and long fragments (1.1 kb) along regions a (1.0; 1.0 and 0.91 for short, middle and long fragment, respectively), m (0.73; 0.64 and 0.55) and i. (0.45; 0.27 and 0.10). These results suggested that DNA quantity in the outer ring of sapwood (a) is higher than that in the middle rings (m), and lowest in the inner ring of heartwood (i).

Generally, the amplification success rate for the longer fragment (1.1 kb) is lower than for shorter fragments (0.6 kb and 0.15 kb). This tendency is observed on all DNA extracts of a, m and i suggesting that genomic DNA in wood samples is generally degraded into small fragments.

Further analysis of PCR inhibition on extracts from different parts of wood was performed on seven Indonesian wood samples which showed PCR inhibition after a first test. PCR inhibition was tested on the DNA extracts from three different parts (a, m and i) of each wood sample. PCR inhibition rate of concentrated DNA extracts from outer sapwood (a) is 100%. However, in the case of undiluted DNA extracts from the transition zone (m) and from the inner ring of heartwood (i) the inhibitory rate drops to about 70% (5 out of 7 samples). PCR inhibition in wood DNA extract was removed in zone i after 20 times dilution. In zone m after 80 times, and in zone a after 320 times dilution. These results suggest that the content of PCR inhibitory substances is decreasing from the outer sapwood to the inner heartwood.

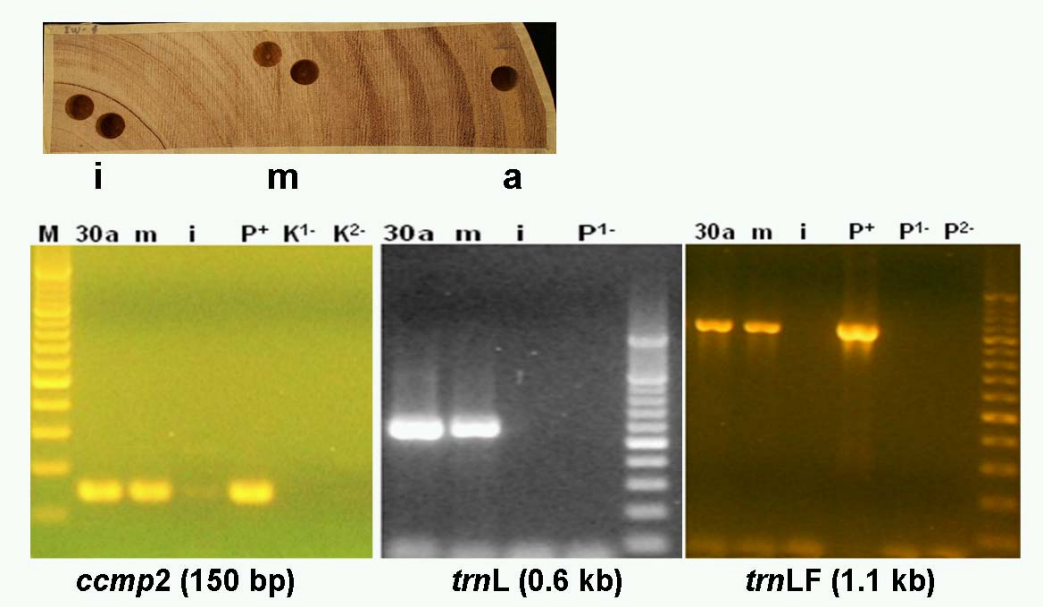
The standard extraction protocol as described in Rachmayanti et al. (2006) with the addition of 2.6% (w/v) PVP was used to extract DNA from a total of 332 dipterocarp wood samples. Shavings from outer sapwood were used for most unprocessed samples.

The success of DNA isolation was evaluated by applying PCR amplification to the DNA extracts instead of measuring DNA concentration spectrophotometrically since in many cases the ratio of λ260/280 and λ260/230 were very low indicating high impurity of the DNA extracts due to proteins, aromatic groups, phenols, carbohydrates or other substances.

Three chloroplast DNA markers which amplify DNA fragments of different length (0.15 kb for *ccmp2*, 0.6 kb for *trnL*, 1.1 kb for *trnLF*) were applied for PCR amplification in order to evaluate DNA quality (degradation level of wood DNA).

Successful amplification was achieved in 369 out of 408 PCR reactions (90.9 %) for the fragment *ccmp2* (approximately 150 bps), in 319 out of 408 reactions (70.8 %) for the *trnL* fragment (approximately 600 bps), and in 234 out of 408 reactions (57.6 %) for the *trnLF* fragment (approximately 1,100 bps). Thus, the average success rate for the three fragments was 75.7 %. All samples which were directly collected from natu

Figure 1:
Electrophoresis of PCR products obtained from the amplification of 3 chloroplast markers on wood extracts from different zones (a: outer of sapwood, without cambium or bark, m: transition of sapwood and heartwood and i: inner of heartwood). 30a, m, i = wood sample Iw30 zone a, m, and i, respectively; M = DNA size standard; $P^{+}$ = PCR positive control (leaf DNA as PCR template); $K^{1-}$, $K^{2-}$, $P^{1-}$, $P^{2-}$ PCR negative controls (water as PCR template)




ral forests or plantations (unprocessed wood and storage duration until DNA extraction from 1 to 4 years) have a good result after PCR amplification, i.e. the PCR success rate is 100% for ccmp2 (short amplicon) and *trnL* (middle length amplicon) and 50% – 100% for *trnLF* depending on the geographic region or species. PCR amplification was highly successful (success rate 100% for short, middle and long amplicons) in all dipterocarps samples from Vietnam.

In case of dipterocarp samples from Indonesia and the Philippines PCR inhibition was found in DNA extracts of some samples in the PCR inhibitory test. The inhibitory effect could be reduced significantly by 10 or 20 times dilution of wood DNA extracts before performing PCR amplification. After dilution up to 1:20 a successful amplification of the long fragment (*trnLF*, 1.1 kb) could still be achieved in all samples. However, in some samples dilution until 80, 160 or even 320 times was needed to remove the PCR inhibition.

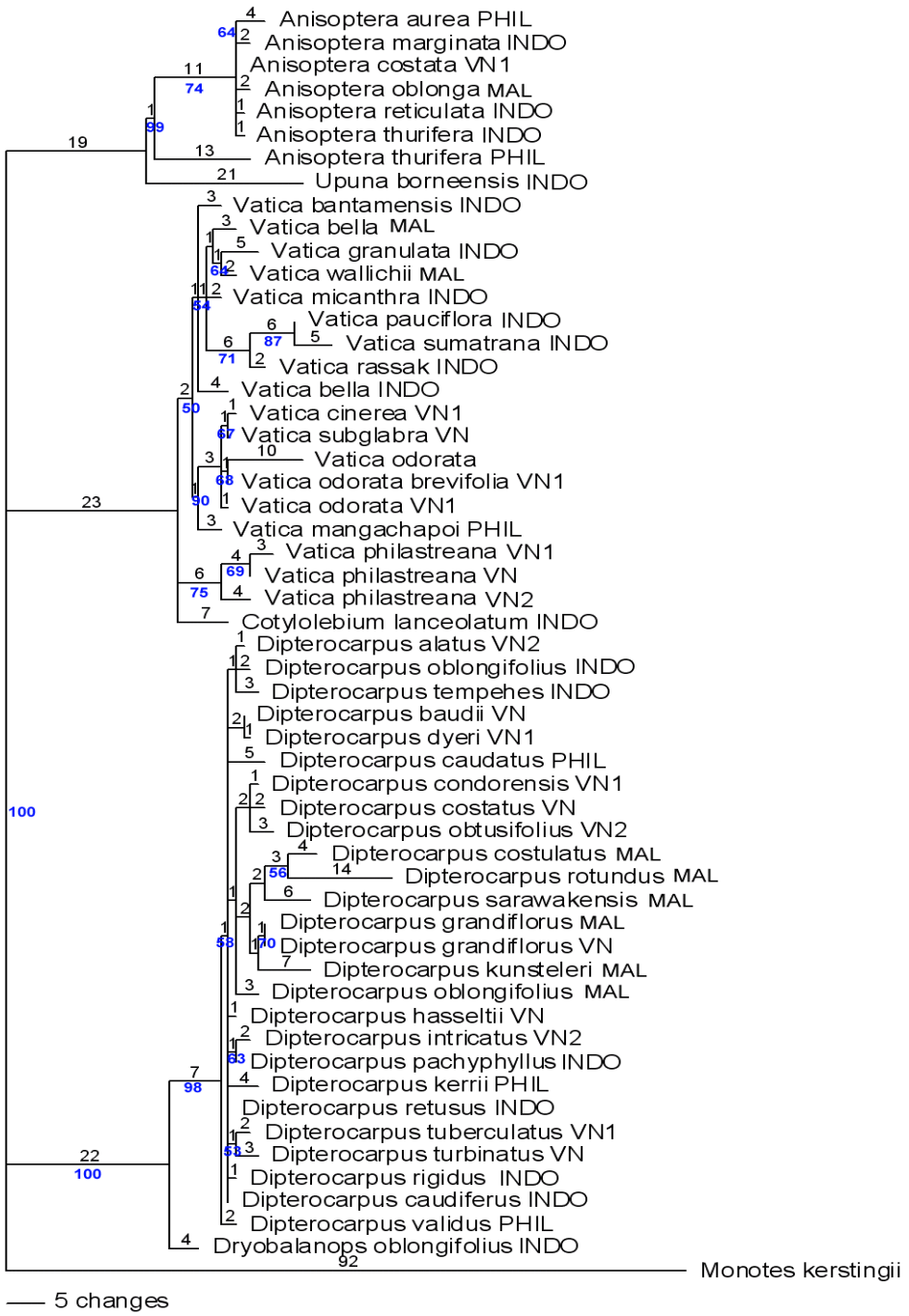
Low amplification success rate was obtained in the samples of Meranti (Shorea *spp.*) from wood enterprises (75 – 85% for short fragment of *ccmp2*, 42 – 49% for middle length fragment of *trnL* and about 15% for long fragment of *trnLF*), although the inhibition test revealed no PCR inhibitory substance in the DNA extract. This result might be caused by the high degradation level of the genomic DNA due to very long storage duration and processing of wood after shipment from producer to consumer countries (gluing, pressure, oven heating, etc.). However, the use of short DNA fragments as molecular markers for the identification of processed wood seems to be feasible in most cases even for processed wood.

5.2. Genetic variation and marker development

A phylogenetic tree based on the variation of cpDNA using PCR-RFLPs and cpSSRs (Indrioko et al. 2006) is consistent with the conventional taxonomy of the subfamily Dipterocarpoideae as described, for example, by Ashton (1982). Most species can be unambiguously recognized based on the observed cpDNA variation. Numerous diagnostic characters allow the assignment of samples to a particular taxon (species, genus, or tribe). The observation of diagnostic characters is particularly noteworthy for endemic species since their observation in a particular sample allow not only to identify the species, but gives at the same time information on the possible origin of the material, which is for obvious reasons restricted to the distribution area of the endemic species.

Tribe Dipterocarpae was further investigated by sequencing of the *trnL* intron and the *trnL-trnF* intergenic spacer region (Nguyen *et al.* 2008; Nguyen *et al.* in prep.). An unambiguous assignment of material up to the species level proved to be feasible based on the investigated sequences in almost all cases (Fig. 2).

Figure 2:
One of the most parsimonous trees of tribe Dipterocarpae based on sequence variation of the cpDNA regions *trnL* and *trnLF*. Branch length above, bootstrap value below branches (Nguyen et al. 2008)



Intraspecific variation was assessed for selected taxa of the genus *Shorea* (Cao *et al.* submitted). All species revealed considerable levels of genetic diversity within populations (Table 1). The common Indonesian species *S. leprosula* and *S. parvifolia* were studied in particular detail. Preliminary results suggest low variation levels of cpDNA within species, and the absence of strong phylogeographic signals at cpDNA markers (Indrioko 2005). This observation severely impedes the identification of the origin of wood by this type of marker. However, ample genetic variation was observed both within and among populations at AFLPs for both species (Cao et al. 2006b).

Table 1. Genetic variation of *Shorea* species from two locations (NM: Nanjak Makmur, Sumatra; S:Sumalindo, Borneo) in Indonesia at AFLPs

| Pop. ID | Location | N | *PPL* | $n_a$ | $n_e$ | $H_e$ | *I* |
|---|---|---|---|---|---|---|---|
| *S. parvifolia* | NM | 26 | 44.71% | 1.447 | 1.176 | 0.110 | 0.174 |
| *S. acuminata* | NM | 32 | 49.41% | 1.494 | 1.159 | 0.100 | 0.162 |
| *S. dasyphylla* | NM | 20 | 55.29% | 1.553 | 1.273 | 0.164 | 0.251 |
| *S. blumutensis* | NM | 21 | 62.35% | 1.624 | 1.266 | 0.165 | 0.257 |
| *S. leprosula* | NM | 16 | 42.35% | 1.424 | 1.224 | 0.134 | 0.204 |
| *S. macroptera* | NM | 26 | 52.94% | 1.529 | 1.259 | 0.155 | 0.238 |
| Mean | NM | 24 | 51.18% | 1.512 | 1.226 | 0.138 | 0.214 |
| *S. parvifolia* | S | 31 | 51.76% | 1.518 | 1.199 | 0.122 | 0.193 |
| *S. leprosula* | S | 26 | 45.88% | 1.459 | 1.192 | 0.115 | 0.178 |
| *S. palembanica* | S | 25 | 61.18% | 1.612 | 1.245 | 0.149 | 0.232 |
| *S. platyclados* | S | 27 | 65.88% | 1.659 | 1.235 | 0.144 | 0.230 |
| *S. johorensis* | S | 24 | 55.29% | 1.553 | 1.183 | 0.115 | 0.186 |
| Mean | S | 27 | 56.00% | 1.560 | 1.211 | 0.129 | 0.204 |

*PPL*, percentage of phenotypically polymorphic loci; $n_a$, observed number of alleles per locus; $n_e$, effective number of alleles per locus; $H_e$, Nei's gene diversity; *I*, Shannon's information index.

AFLP loci showing strong differentiation among the islands of Sumatra and Borneo were successfully converted to simple SCAR markers (Nuroniah et al. 2008; Nuroniah et al. in prep.). This marker allows to unambiguously assign material of this common dipterocarp to one of the two main Indonesian islands.

## 5.3. Conclusions

Dipterocarps are the most important group of timber trees from tropical Asia. The family Dipterocarpaceae contains both important common timber species and endangered taxa. Thus, the development of tools to identify the origin of dipterocarp wood deserves high priority within the context of both the international timber trade and biodiversity conservation.

We sampled material from more than 3000 dipterocarp trees belonging to 116 species with the main objective to develop tools for the identification of the origin of tropical timber. This material is a suitable basis for the development of reference data banks containing relevant genetic data on species and regions.

Simple and reliable methods to isolate DNA from wood of dipterocarps were developed which proved to be efficient for the isolation of DNA from wood of other tropical and temperate tree species as well. Untreated wood is a suitable source for DNA extraction even several months to years after felling. Success rates from treated wood are lower, but it is often possible to isolate short fragments of a few hundred bps even from processed wood. More systematic research is needed to investigate the effect of wood treatment on DNA isolation success.

Species identification is an important requirement in particular for endangered taxa of the very diverse dipterocarp family, and allows inferences on the possible origin of material due to the large number of endemic species. In most cases, variation of chloroplast DNA allows a reliable identification up to the species level.

Intraspecific variation is abundant, but mainly within populations. However, it is demanding, but possible to develop markers showing strong phylogeographic variation patterns within species. For example, a SCAR marker developed

from an AFLP locus allows to distinguish *S. leprosula* trees from Sumatra island from trees of the same species growing on Borneo.

In summary, currently available tools allow to test the declaration of the origin of dipterocarp timber by means of molecular markers. The available techniques and the current knowledge will allow to identify false declarations in many, but not all cases. More research is required to develop informative markers showing strong intraspecific differentiation at the regional scale. The dipterocarps are a suitable group to prove the usefulness of molecular genetic markers to trace the origin of tropical timber.

## 6 References


Ashton PS (1982) Flora malesiana, Series I – Spermatophyta, flowering plants. Vol. 9, Part 2. Dipterocarpaceae. Martinus Nijhoff. The Hague, Boston, New York

Cao CP, Gailing O, Siregar I, Indrioko S and Finkeldey R (2006a) Genetic variation at AFLPs for the Dipterocarpaceae and its relation to molecular phylogenies and taxonomic subdivisions. J Plant Res 119:553-558

Cao CP, Finkeldey R, Siregar IZ, Siregar UJ, Gailing O (2006b) Genetic diversity within and among populations of *Shorea leprosula* Miq. and *Shorea parvifolia* Dyer (Dipterocarpaceae) in Indonesia detected by AFLPs. Tree Genetics and Genomes 2:225-239

FAO (2001) State of the world's forests. FAO, Rome

Indrioko S (2005) Chloroplast DNA variation in Indonesian Dipterocarpaceae - Phylogenetic, taxonomic, and population genetic aspects. Forstwissenschaftliche Dissertation Universität Göttingen : Cuvillier Verlag, 127 pp, ISBN 3-86537-401-8

Indrioko S, Gailing O, Finkeldey R (2006) Molecular phylogeny of Dipterocarpaceae in Indonesia based on chloroplast DNA. Pl. Syst. Evol. 261:99-115

Lamprecht H (1986) Waldbau in den Tropen. Hamburg and Berlin : Parey

Nguyen PN, Gailing O, Finkeldey R (2008) Molecular phylogeny of tribe Dipterocarpeae (family Dipterocarpaceae) based on sequence data of chloroplast DNA. International Conference "Systematics 2008", Göttingen

Nuroniah H, Gailing O, Finkeldey R (2008) Diagnostic Marker to identify the geographic origin of Shorea leprosula (Dipterocarpaceae). International Conference "Systematics 2008", Göttingen

Rachmayanti Y, Leinemann L, Gailin, O, Finkeldey R (2006) Extraction, amplification and characterization of wood DNA from Dipterocarpaceae. Plant Mol Biol Report 24:45-55

Weising K, Gardner RC (1999) A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. Genome 42:9-19

Whitmore TC (1975) Tropical Rain Forest of the Far East. Oxford : Clarendon Press

**Application of the natural variation of stable isotope composition in wood – a brief view of the history and outlook to the future.**

Hilmar Förstel[1]

**Abstract**

The application of the natural variation of the stable isotopic composition of wood and its compounds has a long tradition. Most of the research has focussed on wood as a record of the climate in the past. Tree rings have been used as a calendar. Hydrogen and oxygen isotopes of the local precipitation follow a well-known global pattern, which is closely correlated with the local mean annual temperature. The local isotopic composition is reflected by the biomass formed at that certain site, e.g. in the wood and especially in the cellulose. The measurements of the isotopic ratios of hydrogen and oxygen of organic materials require pyrolytic techniques which have been improved subsequently. Especially the reaction tubes have to be made from inert materials gastight at high temperatures, as recently Agroisolab has introduced silicon carbide. With the open-split technique a larger amount of samples can be measured. Additionally to the isotopic composition of hydrogen and oxygen, resulting from the local water regime, other isotopic pairs of carbon, nitrogen and sulphur are useful as well. In contrast to the global orientation of the "water isotopes" their variations report the local conditions of water supply, of the geological situation and of the history of land use. In such a manner the stable isotopes will stamp its "isotopic fingerprint" on the wood. The stable isotope method differs from other applications: The trunk itself can be used to trace back the origin without other certification systems and the results depend on the physical and soil chemical conditions of the site of growth. A long experience, an improved technique and an independent result are the advantages of the stable isotope method. The isotopic composition can be followed even down to products as paper and furniture. The paper follows the increasing application of the method, especially on the field on tree ring research.

Out of a large amount of papers only some examples were chosen to demonstrate the development in the past and to get the basis for the application in future.

*Keywords: stable isotopes, isotope ratios, tree rings, hydrogen, oxygen*

## 1 Introduction

To prevent illegal trade with timber and its products it is mandatory to trace the product back to its geographical origin. Documentation on paper is not sufficient, as experiences from the food market demonstrate this gap again and again. Therefore it is necessary to use properties of the material itself to confirm or to reject the declared origin of timber. As a final goal the complete chain should be traceable from forest stand to the timber cut there down to the end products as furniture and even paper.

The stable isotopic composition of any material in nature is a property which hardly can be influenced or falsified. This holds especially for timber as a solid material of complicated structure which is built up under the specific conditions at the site of its growth. Biomass can only be formed from the material available directly in the surrounding environment. This material consists not only of chemical elements, but most of these elements themselves are composed of more than one stable isotope. Especially the elements which built up the major portion of the biomass, as carbon, oxygen, hydrogen, nitrogen and sulphur, consist of at least two stable isotopes. This property has been used for many years by a lot of projects and has been documented by an extensive literature since more than 60 years, successfully covering nearly all fields of natural products. Timber was in focus as a record of past climatic conditions. In temperate climates tree rings are a memory of the annual climatic conditions, for tree rings are formed freshly each year mainly built up by cellulose, which has been used as a favourite substance in the past. Some other substances, as lignins, have not included usually. Possibly well-experienced techniques from the paper and pulp industry were transferred to the sample preparation and to obtain samples of pure material. Nevertheless other

[1] Agroisolab GmbH, Technology Centre Jülich, Karl-Heinz-Beckurts-Strasse 13, 52428 Jülich, Germany
h.foerstel@agroisolab.de

components as lignin should be taken into consideration in future applications.

Companies which trade in wood usually demand answers to the following questions: At a first step they look for the confirmation of the identity of the material delivered from a particular site and, if there are doubtful results, as a second step they ask for real geographic origin of the timber. Both questions differ distinctly in their expense and effort, for the second inquiry needs a global database and more experience. In most of the cases it will be sufficient to confirm or reject the identity of a specimen with the commodity. Both questions are summarized by the term authenticity of a material, but should no be mixed.

Databases cannot include each specific situation of growth everywhere on the earth, even if knowledge of the global patterns of the isotopic composition of some elements can be applied. Therefore it is mandatory to call for a declaration of the origin of timber and of its products. But even if this requirement will be accepted, within some states as Russia or Brazil the large differences of the environmental conditions prevent from an efficient control of their timber exports by that measure alone. Therefore contracts between the suppliers of timber from defined areas and the trade will be necessary. Specimens have to be deposited to ensure the traceability of the product and will not need too much space and effort. But finally independent methods have to be available to test the end product. Experiences with the stable isotope method in the food sector may serve as promising measure, according to the European Community regulation 178/2002, which has introduced the traceability of food and fodder.

## 2 Material and Methods

### 2.1. Stable isotopes as experimental tool

Chemical methods usually analyse the elemental composition of materials only, neglecting that most of the elements consist of more than one stable isotope. Therefore all materials around us, including ourselves, consist of stable isotopes. Exact measurements also have demonstrated that the isotopic compositions of various materials in nature differ significantly. The extent of the effects depends on the atomic weight of the element. They are higher for elements with low atomic weight, and decrease with increasing atomic weight. Therefore the isotopic compositions of the biological important elements hydrogen, oxygen, nitrogen, carbon and sulphur differ significantly from one material and from one location to the other. Organisms have to use that isotopically different material at that specific site to build up their biomass. Thus results in a site-specific isotopic fingerprint during the formation of biomass. The isotopic fingerprint is stable and can be changed only, if the material is transferred incompletely. This fact, observed for all plants growing in a certain environment, is also valid for timber.

Tracing back timber to its geographical origin necessitates a sufficient knowledge of the global pattern of the isotopic composition of an element. Only if this is confirmed, the stable isotopic composition of the timber preserves a memory of its geographical origin. Such a well-known pattern is observed in the global water cycle resulting from the fractionation of the isotopes of hydrogen and oxygen and is an important base of the application of the natural variation of stable isotopes. Consequently each trunk reflects the isotopic composition at the site of its growth, for nearly all of the biochemical substances will be synthesized in a aqueous phase. Additionally during the different metabolic pathways isotopic fractionations have been observed. Biochemical fractions as e.g. lipids differ significantly from the isotopic composition of carbohydrates. Therefore any variation of the chemical composition between different samples may alter the isotopic data of a biomass. Therefore the preparation and use of purified and isolated materials as cellulose are preferred. Wood has the advantage that material of single years can be separated by the tree rings and consists mainly of cellulose and lignin. Thus, tree rings have been a favoured tool especially in paleaeoclimatological research, and stable isotopes, corresponding to the application of the width of tree rings, have been examined since the 1970´s, or even earlier. Present studies have shown that not only temperature alone but water supply also may be a governing factor of the formation and consequently isotopic storage in tree rings (Weidner et al. 2006). Annual variations between tree rings are observed and therefore used as a tool of palaeoclimatology, but for the control of authenticity a certain number of tree rings will combined in a mean representative enough for a particular site. Tests of Agroisolab GmbH have demonstrated that seven tree rings are sufficient to get a representative mean, but in daily routine a minimum of 10 was set as requirement.

Beside hydrogen and oxygen other isotopic climate, for an increased water loss in warm and arid climates initiates an increasing opening of the stomata. Different opening width of the stomata have an influence on the conditions of the diffusion of carbon dioxide to the sites of photosynthesis and consequently change the isotopic composition of carbon slightly.

The reservoirs of nitrogen and sulphur in the soil at a particular site are reflected in the biomass. Under human cultivation nitrogen depends mainly on the fertilizer, but the nitrogen regime in forests differs from that influence. Atmospheric deposition has a contribution to the nitrogen budget of forests. Additionally, nitrogen has an intense turnover both in the ecosystem and in biochemical cycles and therefore is fractionated in different ways. Sulphur seems to be less sensitive because of its simpler biochemical pathways.

According to this experience the results are much more statistically significant as more or better all of the biological relevant five elements are measured. Agroisolab GmbH has improved and developed methods to perform this specific demand. The stable isotopes of strontium are of interest if the geological situation will be taken into account and its use for this purpose shall be tested in the future.

2.2. Experimental conditions

Specifically adapted instruments were developed to get reproducible and internationally accepted data of the stable isotopic variations within natural materials: the stable isotope ratio mass spectrometry IRMS. For the first studies of isotopic variations a specific isotope ratio mass spectrometer was constructed by NIER 1942 as part of the Manhattan project during World War II and afterwards improved in 1947, serving to more civil applications as cosmo-chemistry, geochemistry, hydrology and ecology. In contrast to other applications in mass spectrometry the IRMS devices record the small differences between a standard and a sample. Disturbing influences of the instrument have to be minimized, for the differences in the isotopic compositions are small. For the same reason only simple gases as hydrogen, carbon dioxide, carbon monoxide, nitrogen and sulphur dioxide can be used. All of the materials have to be converted completely to these sample gases, otherwise isotopic fractionations will falsify the result. The data obtained are related to the standard gas of pairs can be used too. Carbon is an indicator of the measurement. This reference gas has to be calibrated to international reference materials.

These international reference materials are distributed by the International Atomic Agency IAEA in Vienna/Austria and the National Institute of Standards and Technology NIST in Gaitherburg Md./USA. They are accepted by all professional laboratories as a basis of their statements. For each element a certain reference material is set as the zero point of the scale. For hydrogen and oxygen a specific water sample, the Standard Mean Ocean Water SMOW, was chosen, for carbon the Pee Dee Bee PDB carbonate (from the sceleton of a fossil squid), for nitrogen the air and for sulphur the Cañon Diabolo Triolith CDT (an iron meteorite). The variations are reported as deviations from internationally accepted zero point of a primary reference material (IAEA 1995) in permil. An enrichment against the standard will result in positive and vice versa a lower content of the heavier stable isotope in negative values. For the atom-% of the minor stable isotope differ between the elements significantly (e.g. 1.1 atom-% for $^{13}C$ and 0.015 atom-% for D) δ-values must be read carefully.

The definition is:

$$\delta_{sample} = (R_{sample} - R_{standard}/R_{standard}) - 1 \bullet 1000 \quad [‰]$$

while R is the ratio between the content of the minor and the major isotope, and sample means the gas measured corrected internally against the laboratory standard and finally against the international reference material. In regular intervals the laboratory standards are calibrated against the international reference materials. Since 2002 the laboratories compare their results in the Proficiency Tests FIT-PTS, organized regularly by the European Joint Research Centre JRC Ispra/Italy. The test materials are shipped by Eurofins Nantes/France, usually wine, juice, honey and vanillin.

The use of the international standards and the intercomparison by the FIT-PTS ensure that data of all professional laboratories are comparable within a narrow range. Presently it is more and more recommended to express the isotopic data in accordance with the SI standardisation, but two problems must be taken into account: The absolute isotopic content in atom-% of the basic reference materials is not known exactly enough, and additionally more than fifty years of literature may become hard to read and to understand

without conversion a huge amount of data. As a result of this break a treasure of experience may be lost.

The technique of IRMS in early times was limited to people very experienced in vacuum technology. After preparing each sample separately under the enclosed conditions in a vacuum line specifically built for each pair of isotopes the sample was brought to the inlet system of the mass spectrometer. Each gas sample was enclosed in a glass volume closed by a cock. The inlet system was a set of valves to pump of the atmospheric air between sample volume and inlet of the IRMS. The sample was then flushed into the analyser of the IRMS through a dual inlet system. This dual inlet system used two bellows, one for the sample and the other for the reference gas, ensuring that both gases are introduced into the mass spectrometer under the same pressure.

This procedure was precise, but the sample preparation and transfer to the mass spectrometer were time consuming. Under the past conditions this was not of too much concern measuring samples of pure scientific interest, but an increasing demand for applications and an advanced information technology lead to the introduction of new on-line techniques of the combination between sample preparation and IRMS in the 1980´s. An elemental analyser was coupled to the IRMS via an open split (more details see Förstel 2007)

In the elemental analyser the samples are burnt to carbon dioxide and nitrogen oxides under oxidizing conditions. Afterwards the nitrogen oxides are reduced to nitrogen by a subsequent column. Both gases are then separated by a gas chromatographic column. The outlet of the capillary is sucked into the IRMS through an adapted capillary driven by the pressure difference between atmosphere and vacuum of the mass spectrometer, the capillary serving as a resistance. Helium as a transport gas does not disturb the measurement.

Step by step single compounds as well as the whole system were adapted to quicker und easier handling, the pumping system, the collectors, the registration etc. The contacts between user and manufacturer have been always very close. This can be seen from the discussions of the Annual Meetings of the German Working Group on Stable Isotopes, GASIR. Fields in biology, forensic science, medicine and environmental observation were opened more and more. Thus, the traceability of food and other materials according to the EU regulation 178/2002 necessitates a very rapid sample turnover, for the dealer needs quick information about the goods offered to the customer. Today this experience can be transferred to timber and its products.

In the case of timber liquid water cannot be used. But since many years various papers have demonstrated that cellulose may serve as that memory. Organically-bound hydrogen and oxygen are prepared by pyrolysis under high temperatures. This procedure needs special materials of the reaction tubes and high temperatures.

### 2.3. Current techniques

An overview of the methods presently applied by Agroisolab GmbH is summarized in Table 1. The instruments have been supplied by the company which originally has started as Vacuum Generators Ltd. and later on has been named as Micromass and GV and recently managed by Elementar GmbH. Beside the changing labels up to now the instruments have been distributed under a constant strategy of design.

Conversion under high temperatures under exclusion of air is necessary to obtain valuable data for the isotopic composition of the organic fraction of organically bound hydrogen and especially of oxygen. Former materials of the reactions tubes, as a combination of an outer tube of oxygen containing ceramics and an inner tube of glassy carbon, did not prevent from an increasing background at temperatures rising up above 1300°. But higher temperatures are necessary to obtain valuable results. The silicon carbide, introduced by Agroisolab GmbH, serves as a very suitable material to ensure both a high temperature and gas tight conditions. A single tube is sufficient to isolate the hot reaction zone (Figure 1). The background of both system are compared in figure 2.

Figure 1:
Application of the silicon carbide reaction tube. Both ends of the tube can easily be cooled by a simple laboratory system and therefore tightly sealed to the connections. The SiC tube is inside the furnace

The measurement of the carbon and nitrogen isotope ratio is carried out by an elemental analyser to prepare the gas suitable for the measurement. But both the low concentrations of nitrogen as well as sulphur require specifically adapted methods. The diluter which reduces the high carbon dioxide content of the gas outflow down to the level of the nitrogen peak is not sufficient to obtain the best results. Because of the low sulphur content a new method had to be developed. First the sulphur will be bound in a chemical reaction and then can be leached after changing the chemical composition. In the forthcoming projects both methods will be improved.

Table 1:
Summary of the methods applied by AgroisolabGmbH to measure all isotopic composition of the "bioelements"

| **Element** | **Device**** | **Conditions** |
|---|---|---|
| Carbon/nitrogen | Elementary analyser, GC and Optima | Oxidation at 1021°C copper oxide, added with tungsten oxide and silver/cobalt oxide, subsequent reduction at 650°C with copper Diluter operation for normal nitrogen content in biomass, for wood increased amount of material* |
| Hydrogen and oxygen organically bound | High temperature furnace under air free conditions (pyrolysis) coupled with an Isoprime | 1500° C reaction tube silicon carbide filled with pieces of glassy carbon and a small layer of carbon covered with nickel. |
| Sulphur | Optima | The usual method, if enough sulphur is present , uses a column at 1000°C filled with copper and tungsten oxide* |
| *methods have to be improved and adapted for wood<br>ᵗcombination may change according to the incoming materials | | |

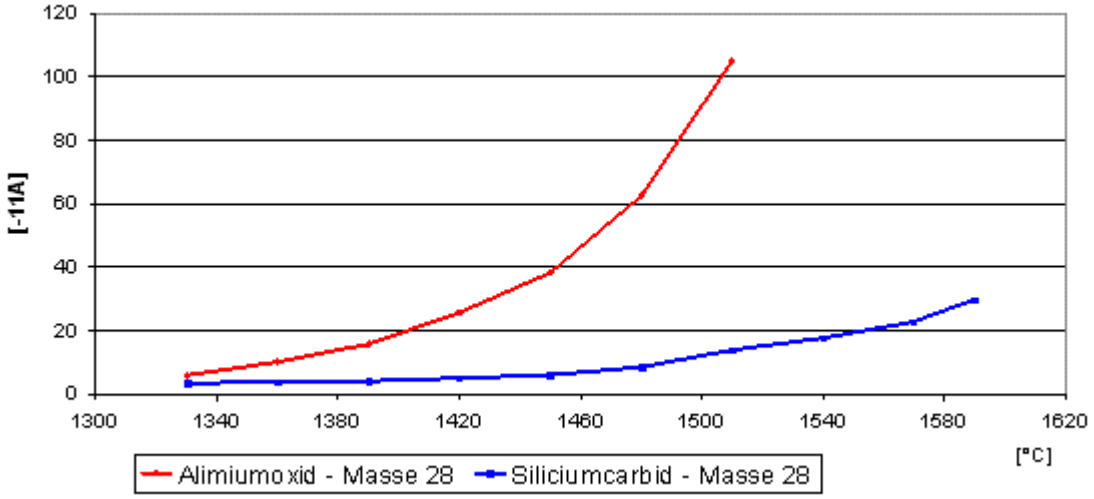



Figure 2:
Comparison of the background at mass 28 between the double tube system and the silicon carbide tube.

## 3 Assumptions of the application

### 3.1. Global pattern of the water isotopes

A global pattern of the isotopic concentration of precipitation is used as a basic tool. The observation of the isotopic composition of the stable isotopes in water has been initiated by Friedmann 1953 after improving the NIER type mass spectrometer. He has reported distinct differences of the isotopic composition between waters of various natural origin. He was also the first one to draw attention to the close correlation between the isotopic composition of hydrogen and oxygen in precipitations. This was finally confirmed and summarized by Craig 1961 (Figure 3).

Dansgaard 1964 could include observations from the polar regions to extend the meteoric water line. He proposed that both temperature and humidity are the governing factors for the isotopic composition of the local precipitation. Since these first publications the correlation is known as “meteoric water line” (whereas “meteoric” means natural precipitation) and has been confirmed afterwards repeatedly. Only data from evaporating water bodies out of isolated ponds deviate from this correlation, assembling around a so-called evaporation line with another correlation between both isotopic pairs. This evaporation line includes also data from overground parts of plants as leaves and fruits, and is of importance for food analysis. Of concern for this study is the general observation that the position at the meteoric water line depends on the mean annual temperature at a certain location. More depleted values of the isotopic composition of hydrogen and oxygen are observed in cold climate, higher ones in a warm environment.

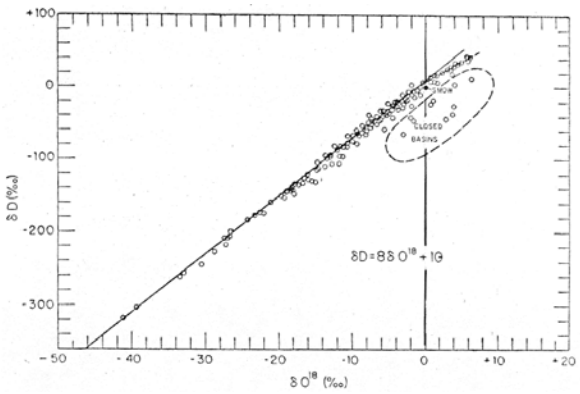



Figure 3:
Historical figure from the publication of the “meteoric water line” by Craig 1961 demonstrating the close correlation of the stable isotopic composition of hydrogen and oxygen isotopes in precipitations (meteoric waters). Only water from closed and evaporating water bodies deviate from this close correlation

The globally valid correlation between the isotopic data of hydrogen and oxygen cannot explain until today by simply working chemical or physical mechanisms. But soon after the first measurements, using the data from the IAEA global precipitation network, a distinct global pattern became visible. The IAEA started its

sample collection to follow the radioactive fallout of nuclear weapon tests and continues this sampling and measuring procedure and data collection until now (IAEA 1953-1991). The data are available via the internet from the IAEA under the GNIP data files. An early map using the IAEA data was published by Förstel et al. 1975 (Figure 4) and improved later on by Bowen 2002.

The map shows the isotopic pattern of precipitation as a result of the movement of air masses from the equator towards the poles, transporting water vapour and resulting in subsequent precipitations. Two third of the evaporation of water into the atmosphere originates from the region around the equator. After the evaporation the water vapour condenses to precipitation. Evaporation and condensation are typical fractionation steps: During evaporation the lighter mass will leave the liquid phase preferentially and forms the water vapour of the atmosphere. The following precipitation reversely is a condensation process and consequently a step of enrichment of the heavy isotope in the rain droplets. But the condensation of water vapour is not complete and the water vapour will be refilled repeatedly both by the evapotranspiration of soil and of the vegetation with the light isotopes. Therefore during the transport of air masses across land a subsequent decrease of the heavy isotopes in water vapour and in precipitation will be observed, the so-called continental effect.

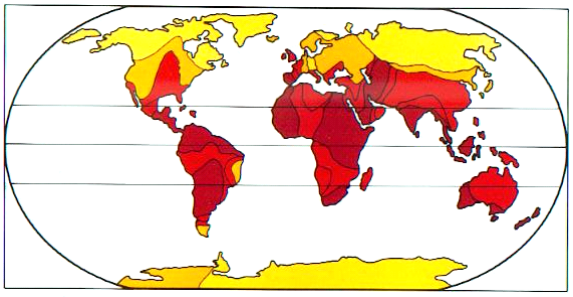


Figure 4:
Global pattern of oxygen isotopes in meteoric precipitation using the data collected by the IAEA network (Förstel et al. 1975)

Correlation studies in Germany, including 900 samples equally distributed across the whole area, were used to calculate the different influences on the isotopic pattern of the precipitation. The result of the isotopic fractionation reads as follows: 45 % of the decrease result from the distance to the sea, 45 % are attributed to the altitude and only 10% to the amount of precipitation (Förstel and Hützen 1982, 1983). The first two parameters depend on the mean annual temperature, whereas in Germany distance from the sea and altitude are also correlated together. This dependence may explain the close correlation of the isotopic content of precipitation and the mean annual temperature at a particular site.

Remarkable is the influence of the mountains on the northern hemisphere. In North America the mountains are orientated in a north-south direction which is followed by the corresponding isotopic pattern (and the movement of the air masses). In Europe the Alps turn away the air masses in a northwest-southwest direction. Therefore in Europe two processes overlay each other: the movement of water vapour from the equator towards the cold poles in a south-north direction and the general movement of the air masses from the Atlantic Ocean across the land mass from the west to the east. As a general consequence each location has a separate and (in our days) constant isotopic composition in the precipitation and consequently in the water pool of the soil and in the groundwater (Förstel and Hützen 1982, 1983)

Dongmann et al. (1974) and Förstel 1978 have reported an enrichment of the heavy isotopes of water in the leaves due to the transpiration. A distinct diurnal cycle will be observed, following the diurnal rhythm of temperature and air humidity depending on this course. This means that the fractionation of water in the leaves and needles depends on the physical conditions of their environment. The relative humidity seems to be the governing parameter. Usually the absolute humidity remains more or less constant during the day, but the relative humidity in dependence on the temperature curve has a minimum during noon, the isotopic enrichment of water its maximum. Craig and Gordon 1965 proposed a model to describe the effect of evaporation which is applied until today. The model includes terms of an enrichment by transpiration and an exchange back from the adjacent air humidity. The application is demonstrated by Dongmann et al. (1972). Farquhar and Lloyd (1993) use a resistance model which is common in plant physiology. Both models consider the isotopic enrichment in the leaves and needles as a pure physically driven process. Because of the fact, that this effect depends on the physical conditions only, the systematic position of plant species plays only a secondary role. The governing parameter is the local water regime. Plants of different water physiology show significant variations, as observed between C3- and C4-plants or succulent species regulating their water ecology by the CAM metabolism (CAM = Crassulacean Acid Metabolism). Another difference is observed comparing deciduous trees and conifers.

## 4 Isotopic archive in tree rings

### 4.1. Climatic archive

This report of the development of the research cannot claim to be a complete review, for palaeoclimatology is a broad field and discusses many global as well as local details (see for more recent results Haneca et al. 2006). Only the general tendencies are demonstrated by some selected publications to explain the suitability of the stable isotope method. Even if the aim has been to include most of the important groups involved in this research, only a first glance on the literature can be given.

For the D/H and $^{18}O/^{16}O$ ratios in the biological materials represent the isotopic variations of the meteoric waters and depend on the actual temperature and humidity (Epstein et al. 1976), sampling tap water at a site usually will be representative enough to predict the isotopic composition of water entering the local plants throughout the growing season. The abundance of deuterium between tree rings as a calendar has been applied to trace back climatic tendencies in *Picea* (Schiegl 1974). But following authors (Libby and Pandolfi 1974) have focussed climatic tendencies to temperature alone and entitled one of their papers "Isotopic thermometers" (Libby et al. 1976). They collected wood samples from English oaks, cutting tree rings from 1712 till 1954 and were able to correlate the annual air temperature during winter with the D/H, $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratio of the cellulose (Figure 5).

As the time scale has been expanded (Epstein and Yapp 1976) taking samples from a Scots pine ranging from 970 till 1970, the isotopic exchange of the hydroxyl group with the hydrogen of water came into focus. Even presently the influence of the actual laboratory conditions of the atmosphere in the laboratory are discussed. Some authors have proposed to eliminate this influence and have removed this exchangeable hydrogen by the addition of nitric acid to form nitrocellulose (Timmel 1963). They also have shown a close correspondence between the resulting isotope data and tree ring width.

Cellulose has been chosen to diminish the biochemical heterogeneity within a plant and between different species, and is common to all photosynthetic plants. The observation that the isotopic composition of cellulose from aquatic plants follows the meteoric water line was seen as a confirmation that cellulose is a memory of the isotopic conditions of water present during its formation (Epstein et al. 1976). Cellulose from terrestrial plants at the other hand was enriched, later on understood as a result of the isoto-

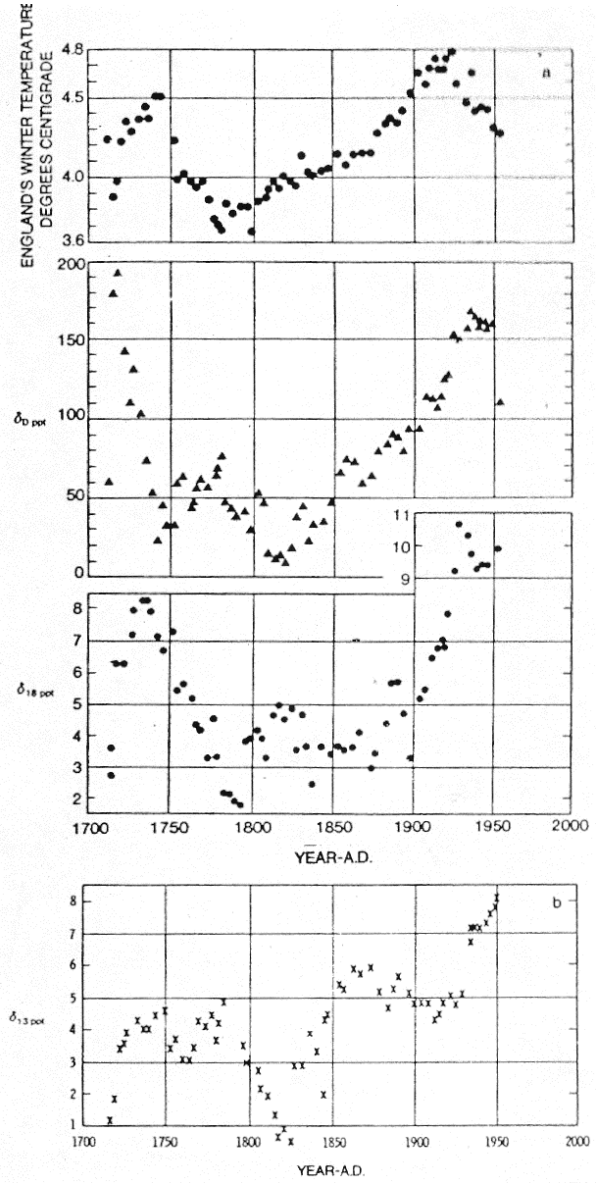



Figure 5:
Comparison of the winter temperature with (from top to bottom) the $^{18}O/^{16}O$-, $^{13}C/^{12}C$- and D/H-ratios of cellulose. Tree rings were collected ranging from 1712-1954 (Libby and Pandolfi 1974) The arrow points to the duration of the so-called Little Ice Age

pic enrichment during transpiration. Transpiration and starch formation are correlated for the plant has to open its stomata to get the carbon dioxide from the air. Simultaneously the plant losses water via the widely opened stomata, resulting in an enrichment of the stable isotopes of water. Cellulose from peat of lakes has been measured to reconstruct the history of water bodies of lakes (Brenningmeyer et al 1982).

Following publications often have reduced the correlation between isotopic content, cellulose and climate to the precipitation only. Burk and Stuiver (1981). proposed that the organically bound oxygen correlates distinctly with the local mean annual temperature. The limited number of data suggest that 1° degree of temperature corresponds with 0.4 ‰ decrease. But the small number of data points are limiting the result of this study.

The general problem to detect the causes of the correlation between hydrogen and oxygen isotopes is the parallel variation of the environmental parameters of temperature and relative humidity. To avoid this problem a specific situation was used (Roden and Ehleringer 2000). Within this study samples have been collected along the steep slope in a canyon with a strong gradient of temperature. The only water supply was at the top of the canyon. The isotopic composition of this source, and not the local temperature, are represented in the isotopic composition of the plant material. This natural experiment has confirmed that the isotopic composition of the water which supplies the plant determines the isotopic composition of hydrogen and oxygen of the biomass. Beside that specific situation usually the local temperature determines the isotopic composition of the water at a site. But water which originates far away from the site of growth of a tree will impress this extraneous isotopic composition on the biomass, if no local supply is available.

4.2. Anthropogeneous influences

Beside the fact that the stable isotopic composition of hydrogen and oxygen are indicators of the local climate, tree rings were used to study other phenomena too. The dilution of the atmospheric carbon dioxide by the emissions of the industrial burning of fossil fuels has been observed (Leavitt and Long 1984) as a decrease of the $^{13}C/^{12}C$ ratio in tree rings of pine caused since 1900. The curves of different trees follow the same decreasing pattern and therefore reflect the same reason (Figure 6). The variations result from the individual positions of the tree within the stand and therefore become a different mixture of soil and atmospheric carbon dioxide (Leavitt and Long 1988).

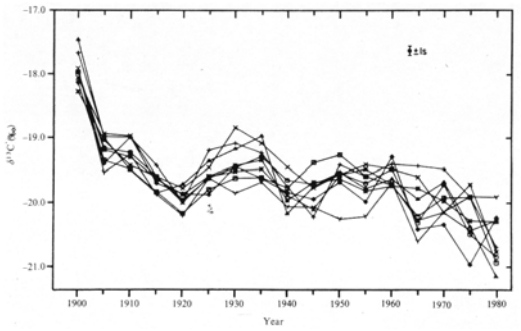



Figure 6:
Decrease of the $^{13}C/^{12}C$-ratio since 1900 as an effect of dilution by the combustion of fossil fuels (Leavitt and Long 1984)

Tree ring cellulose reflects the annual variations. Otherwise one may think about an isotopic tree ring pattern of annual data like a barcode to characterize single populations out of a small region.

Nitrogen has been used to demonstrate long-term tendencies within a forest. Poulson et al. (1995) were interested in the effect of a climatic change. Bukate and Kyser (2005) have investigated the influence of clear-cutting and of the change of the land cultivation. The position of the tree within the stand has a small influence on the $^{14}N/^{15}N$-ratio. This tendency changes after clear cutting areas in the neighbourhood. It is assumed that the local nitrogen cycle was disturbed. Consequently fertilisation also results in a distinct change.

## 5 Conclusions

The natural variation of the stable isotope composition of the “bioelements” hydrogen, carbon, nitrogen, oxygen and sulphur has been intensively used as a tool to reconstruct paleoclimatic changes in the past. Tree rings were used as a calendar. For trees are part of ecosystems the papers about ecological studies summarized by Rundel et al. 1989 may also be considered. The overview reported here may demonstrate that technical improvements have made the application more easy.

The natural variation of the stable isotopes of hydrogen and oxygen are the most promising tools for they are fractionated within a global water cycle. Beside some exemptions under

extreme hydrological situations, as arid conditions, the plant biomass reflects the isotopic composition of the water at the site of growth.

Additional information can be obtained from the stable isotopes of carbon, but variations within the forest stand have to be taken into account. The trees take up a mixture of carbon dioxide from the air and from the soil which differ in their $^{13}C/^{12}C$-ratio.

Only a limited number of papers concerning the stable isotopic variation of nitrogen in wood have been published. They also point to a variation within the tree and the wood also.

Nevertheless the isotopic fingerprint is a property of the material itself and cannot be easily changed or falsified. In future studies one should investigate not only the possibility to compare isotope ratios of complete trunks, but analogous to the measurements of the tree rings an isotopic pattern could be as characteristic as the use in dendrochronological methods.

## 6 References


Brenningmeyer CAM, van Geel B, Mook WG (1982) Variations in the D/H and the 18O/16O ratios in cellulose extracted from peat. Earth Planet Sci Lett 61:283-290

Bowen GJ (2002) Spatial distribution of $\delta^{18}O$ in meteoric precipitation. Geology 30:315-318

Bukata AR, Kyser TK (2005) Response of the nitrogen isotopic composition of tree-rings following tree-clearing and land-use change. Environ Sci Technol 39: 7777-7783

Burk, Stuiver (1981) Oxygen isotope ratios in trees reflect mean annual temperature and humidity. Science 211:1417-1419

Craig H (1961) Isotopic variations in meteoric waters. Science 133:1702-1703

Craig H Gordon LI (1965) Deuterium and oxygen 18 variations in the ocean and in the marine atmosphere. In: Tongiorgi (ed) Stable Isotopes in Oceanographic Studies and Paleotemperatures; Proceedings Spoleto Conferences in Nuclear Geology, Pisa, pp 9–130

Dongmann G, Förstel H, Wagener K (1972) $^{18}O$-rich oxygen from land photosynthesis. Nature (New Biology) 240:127-128

Dongmann G, Nürnberg HW, Förstel H, Wagener K (1974) On the enrichment of $H_2{}^{18}O$ in the leaves of transpiring plants. Rad Environm Biophys 11:41-52

Epstein S, Yapp CJ, Hall JH (1976) The determination of the D(H ratio of non-exchangable hydrogen in cellulose extracted from aquatic and land plants. Earth Planet Sci Lett 30:241-251

Epstein S, Yapp, CJ (1976) Climatic implications of the D/H ratio of hydrogen in C-H groups in tree ring cellulose- Earth Planet Sci Lett 30:252-261

Epstein S, Thompson P, Yapp CJ (1976) Oxygen and hydrogen isotopic ratios in plant cellulose. Science 198:1209-1215

Farquhar GD, Lloyd J (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In: Ehleringer JR, Hall AE, Farquhar GH (eds) Stable isotopes and plant carbon-water relations. San Diego : Academic Press, pp 47–70

Friedman (1953) Deuterium content of natural waters and other substances. Geochim. Cosmochim. Acta 4:89-103

Förstel H, Putral A, Schleser G, Lieth H (1975) The world pattern of oxygen-18 in rainwater and its importance in understanding the biogeochemical oxygen cycle. In: IAEA (Ed.) FAO/IAEA Joint Symposium on Isotope Ratios as Pollutant Source and Behaviour Indicators, IAEA, Wien, pp 3-20

Förstel H (1978) The enrichment of $^{18}O$ in leaf water under natural conditions. Rad Environm Biophys 15:323-344

Förstel H, Hützen H (1982) $^{18}O/^{16}O$-Verhältnis im Grundwasser der Bundesrepublik Deutschland. JÜL Report 1784

Förstel H, Hützen H (1983) Oxygen isotope ratios in German groundwater. Nature 303:614–616

Förstel H (2007) The natural fingerprint of stable isotopes – use of IRMS to test food authenticity. Anal Bioanal Chem 388:541-544

Haneca K, Verheyden A, Beeckman H, Gärtner H, Helle G, Schleser GH (2006) TRACE – Tree Rings in Archaeology, Climatology and Ecology. Schriftenreihe des Forschungszentrums Jülich, Reihe Environment, Vol 74

International Atomic Energy Agency IAEA (1995) Reference and intercomparison materials for stable isotopes of light elements. IAEA-TECDOC-825, Vienna

IAEA (ed) (1969-1994) Environmental isotope data – World survey of isotope concentration in precipitation. IAEA, Vienna, No. 1-10

Leavitt SW, Long A (1984) Sampling strategy for stable carbon isotope analysis of tree rings in pine. Nature 311:145-147

Leavitt SW, Long A (1988) Intertree variability of the $\delta^{13}C$ in tree rings. In: Rundel PW, Ehleringer JR, Nagy KA (eds) Stable isotopes in ecological research. Berlin NY Heidelberg : Springer, pp 95-104

Libby LM, Pandolfi LJ (1974) Temperature dependence of isotopic ratios in tree rings. Prod Nat Acad Sci USA 71: 2482-2486

Libby LM, Pandolfi LJ, Payton PH, Marshall III J, Becker B, Giertz-Sienbenlist V (1976) Isotopic tree thermometers. Nature 261:284-288

Poulson SR, Chamberlain CP, Friedland AJ (1995). Nitrogen isotope variation of tree rings as a potential indicator of environmental change. Chem Geol 125: 307-315

Roden JS, Ehleringer JR (2000) There is no temperature dependence of net biochenmical fractionation of hydrogen and oxygen isotopes in tree-ring cellulose. Isotop Environm Health Stud 36:303-317

Rundel PW, Ehleringer JR, Nagy KA (Eds.) (1989) Stable Isotopes in Ecological Research. Springer, NY

Schiegl WE (1974) Climatic significance of deuterium abundance in growth rings of *Picea*. Nature 251:582-584

Timmell TE (1963) Effect of deacetylation and nitration on normal glycosidic linkages in cellulose. J Polym Sci C2:109

Weidner K, Helle G, Löffler J, Neuwirth B, Schleser GH (2006) In: Haneka K, Verheyden A, Beeckmann H, Gärtner H, Helle G, Schleser GH (eds) TRACE – Tree Rings in Archaeology, Climatology and Ecology. Schriften des Forschungszentrums Jülich, Reihe Environment 74:148–158

## Stable isotope databases for European food products

Andreas Roßmann[1]

**Abstract**

Stable isotope ratio analysis (SIRA) of the so-called bioelements (H,C,N,O,S) and the heavy element strontium is applied for food authenticity control and for geographical origin assignment of food products since about 30 years. The first official methods eg for honey and fruit juices have been acknowledged in 1978 and 1981 by AOAC. In 1990 the EU decided to install an official database for wine stable isotope parameters from all wine producing member states, at the same time accepting the $^{2}$H-NMR (Nuclear Magnetic Resonance Spectroscopy) analysis of wine ethanol as official method. This database now contains the stable isotope data for hydrogen, carbon and oxygen (measured by IRMS= Isotope Ratio Mass Spectrometry) from wine ethanol and wine water of about 1000 authentic samples per year, which have now been collected for 17 years from several countries.

The same stable isotope methods as for wine are applied for fruit juices as well, therefore the Schutzgemeinschaft der Fruchtsaftindustrie (SGF) e.V. in Nieder-Olm compiled a fruit juice database since 1991, which has been further extended during an EU project ("PURE JUICE") from 2002-2005 and is now accessible via internet by the members of SGF, PURE JUICE participants and public control laboratories (www.purejuice.org). The AG Stabilisotopenanalytik of the GDCh has recently installed a stable isotope database for fruit juices as well (www.agstabilisotopen.org), which should be extended to other products, but is at present only accessible for members of this group.

In a recent EU project "TRACE" (www.trace.eu.org) stable isotope data are collected for a variety of food commodities, as honey, cereals, lamb, beef, olive oil, and mineral water. At the same time surface water and soil samples are taken and their stable isotope (and other) parameters analysed as well.

The aim of this project is to model and finally to predict stable isotope data for food commodities from the geographical, climatic, geological, botanical and hydrological information available even for regions where no authentic samples are accessible. If this works, the efforts for compiling databases of stable isotope data could be remarkably reduced.

In case of wood stable isotope data for several regions have been collected since a long time by climatologic researchers, as they use hydrogen and oxygen isotopes of tree ring cellulose to obtain information about the climate of the past. The substance analysed usually is pure $\alpha$-cellulose, which has to be prepared from raw wood by laborious preparation methods. A recent paper has indicated that it might be possible to replace this procedure by a less time consuming method applying the hydrogen isotope ratio of methyl groups instead of the cellulose. Finally, it should be mentioned that hydrological research has produced data collections of water stable isotope data (precipitation and ground water) from many regions and over about 40 years, especially in Europe, but for other regions of the world, too.

The information gathered in those databases could be very useful for geographical origin assignment of wood sample.

### 1 Basic information about stable isotope analysis

Chemicals elements occur in nature as mixtures of two or more stable isotopes. Especially for the light elements H,C,N,O and S, the so called bio elements, as they form the basis of all living matter, the light isotope is by far more abundant as the heavy isotope(s). In case of carbon, the isotope $^{12}$C has about 98.9 %, the heavier isotope $^{13}$C about 1.1 % natural abundance. The mean natural isotopic ratio (eg $^{13}$C/$^{12}$C) of these bio elements is not absolutely constant, it shows distinct and systematic variations due to physical and biochemical isotopic fractionation, which can be used to conclude about the history and provenance of biochemical and/or biological material from different sources, even if it is chemically pure and/or identical (Kelly et al., 2005, Rossmann 2001). This possibility has been used for questions of geology, archaeology, forensics, environmental sciences and food authenticity.

[1] isolab GmbH, Laboratorium für Stabile Isotope, Woelkestraße 9/I, 85301 Schweitenkirchen, and TU München, Center of Food and Life Sciences Weihenstephan, 85350 Freising, Germany
isolab_gmbh@t-online.de

As the absolute isotopic ratio are difficult to handle in practise, stable isotope data are generally used as "delta-values", which are the deviation of the stable isotope ratio of a substance from that of the commonly accepted reference material, as e.g. ocean water for hydrogen and oxygen isotopes or air nitrogen for nitrogen isotopes. In the same way, some heavier elements, which have been applied in geological sciences for dating purpose, as one of their isotopes is formed by radioactive decay (so called geo elements), can provide complementary information especially for geographical origin assignment of food commodities (Hölzl et al. 2004, Horn et al. 1998, Kelly et al. 2005).

Table 1:
Elements and their stable isotopes, which are important as components of living matter (bioelements), their relative mean natural abundance F, the name of the International Isotopic Standard and its isotopic ratio R for the two most abundant stable isotopes**.**
F = cIsotope/Sum cIsotope. R = cIsotope a/cIsotope b
For oxygen and sulfur there are in addition a third or a third and a fourth, but less abundant stable isotope, from which the isotopic ratio(s) is (are) usually not applied for analytical purpose (Hoefs, 1981; Winkler and Schmidt, 1980)

| **Element** | **symbol** | **Isotop F [atom-%]** | **standard name** | **R** |
|---|---|---|---|---|
| hydrogen | $^{1}H$<br>$^{2}H$=D | 99.9855<br>0.0145 | Standard Mean Ocean Water (=SMOW) | 0.00015576 |
| carbon | $^{12}C$<br>$^{13}C$ | 98.892<br>1.108 | Pee Dee Belemnite (=PDB) | 0.011237 |
| nitrogen | $^{14}N$<br>$^{15}N$ | 99.6337<br>0.3663 | Air (Air nitrogen) (=AIR) | 0.0036765 |
| oxygen | $^{16}O$<br>$^{17}O$<br>$^{18}O$ | 99.7587<br>0.0375<br>0.2039 | Standard Mean Ocean Water (=SMOW) | 0.00200520 |
| sulfur | $^{32}S$<br>$^{33}S$<br>$^{34}S$<br>$^{36}S$ | 95.018<br>0.750<br>4.215<br>0.02 | Canyon Diablo Troilite (=CDT) | 0.0450045 |

$$\delta\text{-value} = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000\ [‰]$$

## 2 Some examples for application of stable isotope data banks in food control

Stable isotope analysis of the light elements, especially of hydrogen, carbon and oxygen isotopic ratios is now applied for food authenticity control since more than 20 years. The aims of those investigations are preferably to check if products as wine, fruit juices, honey or spirits had been extended or adulterated by illegal addition of water and/or sugars (Bauer-Christoph et al. 1997, Christoph et al. 2004, Engel et al. 2007, Kelly et al.2005, Rossmann 2001). For this purpose several official methods have been adopted by European or US authorities or institutions (EU Commission, CEN, AOAC).

One of the well-known fields of stable isotope work is the European stable isotope Wine Databank and the application of the $^{2}H$-NMR analysis of wine ethanol and the $^{13}C$ and $^{18}O$ IRMS analysis of wine ethanol and wine water,

respectively, for the control of sugar addition and water addition in wines (Christoph et al.2004). For stable isotope methods used in food authenticity control it is necessary to have a database of results for authentic samples available, which can be used for comparison of data from commercial products with them. That wine databank is built since 1990 for all wine growing EU countries, and a number of about 1600 authentic samples is added to this databank per year. The total number of currently more than 15000 datasets is collected and stored at the JRC of the EU in Ispra (Italy).

Table 2:
Elements and their stable isotopes, which are important in Geology for dating purpose and which can be used for geographical origin assignment of foodstuff, their mother nuclides (radioactive), halflife times and natural abundance ratios R for the radiogenic vs. the primordial stable isotopes (atom-% abundance) given as range observed on earth.
R = cIsotope a/cIsotope b

Isotopic ratios of those elements are usually measured by Thermal Ionisation- (TI-MS)- or Inductively Coupled Plasma- (ICP-MS)-Mass Spectrometry
According to: Horn et al., 1998

| **Element** | **symbol** | **mother nuclide** | **halflife time** | **R** |
|---|---|---|---|---|
| strontium | $^{87}Sr$<br>$^{86}Sr$ (9.87 atom %) | $^{87}Rb$ | 48.8 x $10^9$ years | 0.702 - > 1 |
| neodymium | $^{143}Nd$<br>$^{144}Nd$ (23.8 atom-%) | $^{147}Sm$ | 106 x $10^9$ years | 0.510–0.514 |
| lead | $^{208}Pb$<br>$^{204}Pb$ (1.4 atom-%) | $^{232}Th$ | 14.1 x $10^9$ years | 33.0 – 52.0 |
| lead | $^{207}Pb$<br>$^{204}Pb$ (1.4 atom-% | $^{235}U$ | 0.7038 x $10^9$ years | 14.0 – 24.0 |
| lead | $^{206}Pb$<br>$^{204}Pb$ (1.4 atom-%) | $^{238}U$ | 4.468 x $10^9$ years | 13.0 – 25.0 |

Table 3:
Officially acknowledged methods for food quality control based on stable isotope ratio analysis by isotope ratio mass spectrometry = IRMS or $^{2}$H nuclear magnetic resonance spectroscopy = $^{2}$H-NMR. 1 = $^{2}$H-NMR; 2 = year of official acknowledgement. S = sugar-; W = water-addition, CEN = European Commission for Normalization
AOAC = Association of Official Analytical Chemists (USA)

| Foodstuff | detection of | isotopic ratio | country/year2 institution |
|---|---|---|---|
| fruit juice | S | $^{13}$C/$^{12}$C (sugars) | EU - CEN 1995 |
| fruit juice | S | $^{13}$C/$^{12}$C (sugars) | USA - AOAC 1981 |
| fruit juice | S | $^{13}$C/$^{12}$C (sugars and pulp) | EU - CEN 1998 |
| fruit juice (concentrate) | S | $^{18}$O/$^{16}$O (water) | USA - AOAC 1992 |
| fruit juice | S | $^{2}$H/$^{1}$H (ethanol)1 | EU,USA;CEN/AOAC 1996 |
| fruit juice | W | $^{18}$O/$^{16}$O (water) $^{2}$H/$^{1}$H (water) | EU - CEN 1995 |
| honey | S | $^{13}$C/$^{12}$C (honey) | USA - AOAC 1978 |
| honey | S | $^{13}$C/$^{12}$C (honey and protein) | USA - AOAC 1991 |
| wine | S | $^{2}$H/$^{1}$H (ethanol)1 | EU 1990 |
| wine | W | $^{18}$O/$^{16}$O (water) | EU 1997 |
| wine | S | $^{13}$C/$^{12}$C (ethanol) | EU 2003 |
| maple sirup | S | $^{2}$H/$^{1}$H and $^{13}$C/$^{12}$C (ethanol)1 | USA-AOAC 2001 |

The parameters as mentioned above cannot only be applied to control a wine for illegal additions of compounds, they can also be useful to check if a not adulterated wine complies with its labelled geographical origin or year of vintage, as there are climatic and environmental differences, eg mean temperature, amount of precipitation, air humidity, between different wine growing regions, or in one region between different vintages.

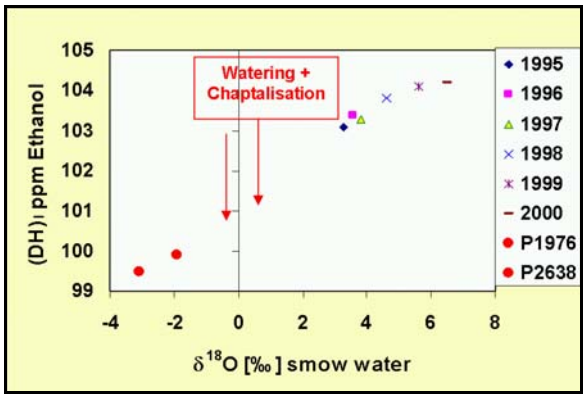



Figure 1:
Examples for the seasonal variability of stable isotope data for authentic wines (mean values) and for adulterated (sugar and water addition) wines labelled to originate from the same region

In cases where the stable isotope data for commercial products are clearly outside the total range for any authentic product (outside the so called cut-off values, e.g. $\delta^{18}$O value of wine water from Southern Europe with –2 or –4 ‰), it is not necessary to have a detailed database at all to prove the adulteration.

In other cases, and especially for the control of the labelled geographical origin and/or year of vintage this cannot be done without the database.

Unfortunately the EU wine database is until now only accessible for the laboratories contributing to the national wine databanks (the so-called member state labs = MSL), and even those laboratories have no access to the EU wine databank results from other countries. The only institution having all results available is until now the Joint Research Center of the EU (JRC) in Ispra (Italy), which has to be asked in cases where the data are required for food control, customs investigation, or court case requirements.

Within the last years, additional stable isotope parameters have been analysed and found applicable and useful for geographical origin assignment, not only of wine and fruit products, but even of animal food commodities as milk, butter, cheese, and meat (Boner and Förstel 2004, Brescia et al., 2005, Camin et al. 2004, Camin et al. 2007, Crittenden et al. 2007, Manca et al. 2006, Pillonel et al. 2005, Rossmann et al. 2000, Rossmann 2001, Schmidt et al. 2005). Especially

for proteins, but even for plant materials the stable isotope ratios of elements nitrogen and sulphur have been used, and several heavy elements, preferably strontium have been found to be very useful for detection of the geographical provenance, too.

On the basis of the experiences with the EU wine databank, the Schutzgemeinschaft der Fruchtsaftindustrie e.V. (SGF e.V.) in 1991 started to build a database for fruit juice stable isotope results as well. This was done in the frame of an EU project from 1991 to 1993, and continued in a second EU project "PURE JUICE" from 2002-2005. This database is now accessible for public laboratories and EU control laboratories via internet as well (www.purejuice.org), and it contains data for all light elements' stable isotope ratios and for strontium isotopic ratios for the commercially most important fruit juices (citrus, pineapple, apple) from the relevant provenance regions. With this interactive databank, own stable isotope results for a product can be plotted in the graphics build with the database values and can be compared in that with the results for all samples available in that database. As an example for its application, the strontium isotopic ratios for authentic orange juice (single strength juices) and commercial products labelled as single strength juices from a certain region are given (figure 2). It is obvious, that the authentic juices exhibit a close correlation of strontium isotope ratios for liquids and solids (pulp), while commercial products sometimes show remarkable deviations from that correlation, due to mixing of single strength juices from one provenance with rediluted concentrates from another region.

In 2005 the Arbeitsgruppe (AG) Stabilisotopenanalytik of the Gesellschaft Deutscher Chemiker e.V. (GDCh) decided to build their own database, starting with carbon and oxygen isotopic ratios of sugars and waters, respectively, from single strength apple juices of relevant European provenance regions, which is accessible at www.agstabilisotopen.org until now for the members of this group. An example for its application for differentiation of commercial single strength apple juice and rediluted apple juice from concentrate is given in figure 3.

Additional private or national databanks of stable isotope parameters exist eg for Grana Padano, parma ham, German and British and Austrian beef, honeys, German and Austrian asparagus, German fruit and grain spirits (Bauer-Christoph et al., 1997, Engel et al., 2007), Italian grape brandies. In general, the use of databases is necessary for stable isotope analyses, especially for the question of geographical origin assignment, but it would be nearly impossible to build up databases for all products which are or might be investigated by stable isotope methods. Furthermore, due to the global trade it must be possible to evaluate even products from very distant regions, from where authentic sample material is not available or only very difficult to obtain. This was one of the reasons for initiating the current EU project "TRACE" (www.trace.eu.org), which finally aims on the prediction of stable isotope data for products from a certain region on the basis of known isotopic data of precipitation, or climatic information, information about geography, geology and soil composition, and agricultural practises. This will be done by producing prediction models for products from European regions (Iceland to Greece, Ireland to Sicily, Spain to Poland) investigated with regard to their stable isotope content in TRACE, and being related to the relevant geological, climatic, hydrological, and geographical information, which includes the stable isotope data e.g. for ground waters, surface waters, soils and rocks. Supposed that such a system finally can be used for practical work, the same would be possible to be applied to products from other regions of the world, even if only climatic and geological /geographical information is accessible, but no authentic samples can be taken.

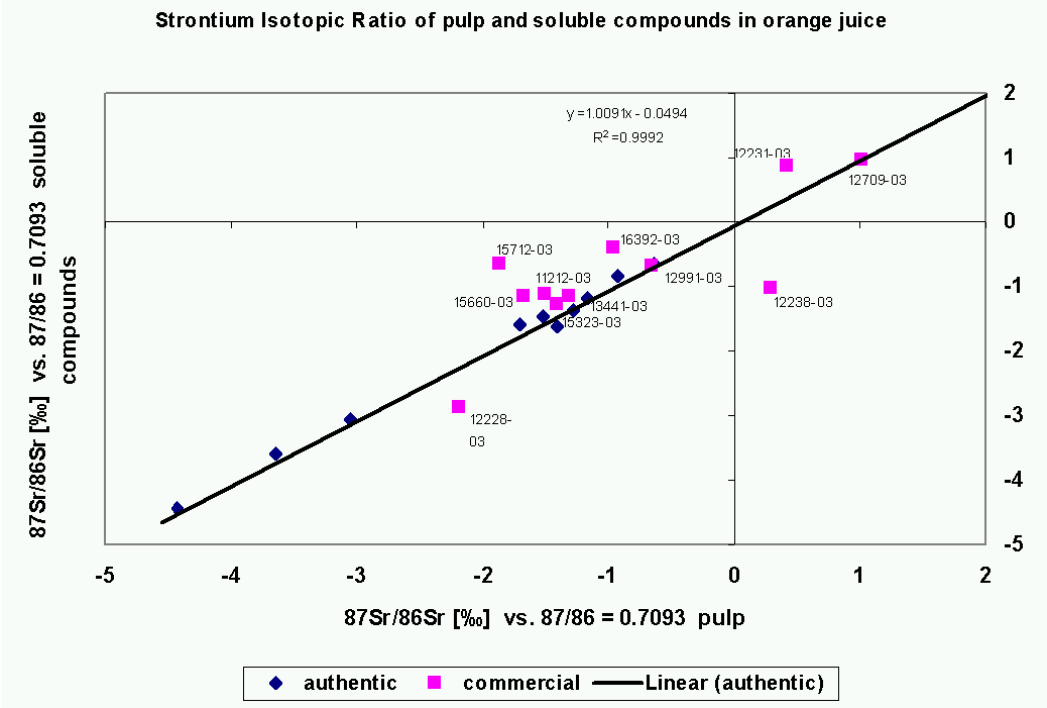



Figure 2:
Strontium isotope correlation of solids (pulp) and liquids in authentic single strength orange juice and in commercial products labelled as single strength juices from the same provenance region

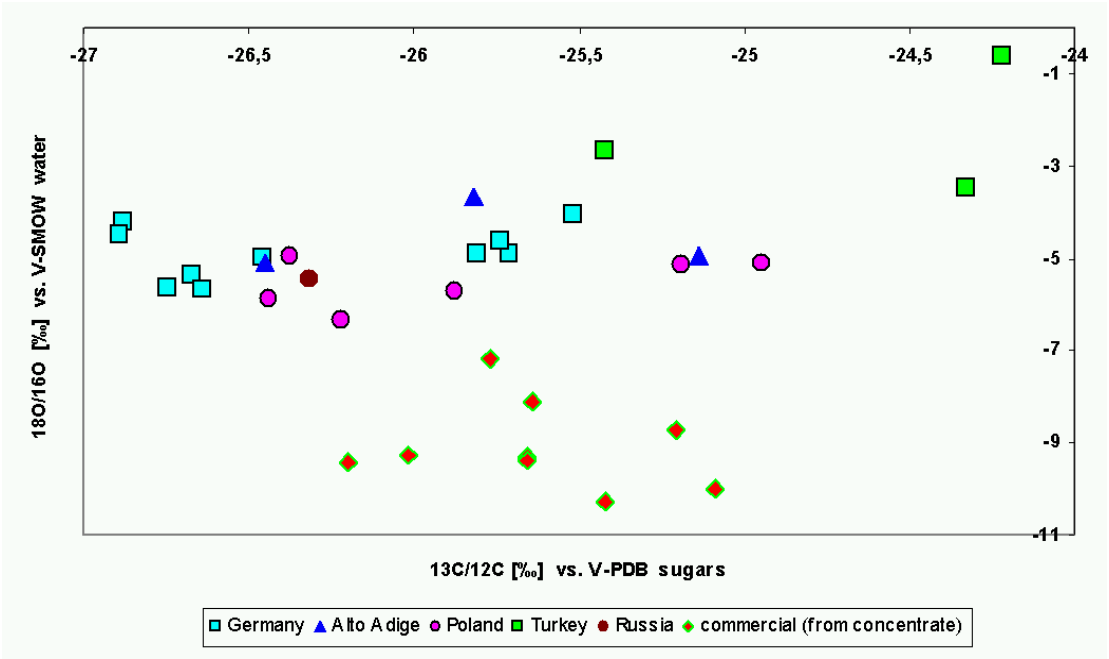



Figure 3:
Carbon and and oxygen isotopic ratios of sugars and water of authentic single strength apple juices and commercial apple juice made from redilution of apple juice concentrates

## 3 Stable isotope analyses for wood geographical provenance control

For the purpose of geographical origin assignment of wood, especially for wood from tropical regions, the experience and data as obtained for food products, namely for fruit juices, which come from tropical regions, too, can be very useful. As an example, the stable isotope characteristics for orange from Brazil or for pineapple, mango, and other tropical fruits from India or South-East Asia can indicate the expectation range for wood from the same regions very well. From experience with fruit juices, the combined application of hydrogen (or oxygen), carbon, sulphur, and strontium isotopic ratios, and the data evaluation using discriminant analyses is recommended (Camin et al., 2007). In the fruit juice business it is well-known, that most reliable results can be obtained by combining two or three different analytical methods, which would in case of wood support the use of genetic and stable isotope analyses together for origin determination.

As the standard method for preparing a suitable probe from wood, the alpha-cellulose, which is applied for oxygen isotope and (after derivatisation) for hydrogen isotope analysis, is very laborious, recently another procedure has been proposed for the hydrogen isotope analysis of wood, aiming on relating the hydrogen isotope data of the organic compounds of wood to the precipitation water of the provenance region (Keppler et al., 2007). This method is based on the measurement of hydrogen isotopic ratios of lignin methoxyl groups as methyl iodine, which is easily prepared from dried and fine grind wood by addition of hydrogen iodine, and can be measured using GC-Pyrolysis-IRMS. As an example of the results obtained with that method, the correlation of the hydrogen isotopic data for methyl iodine prepared from wood and for water of the provenance regions are given in comparison with the hydrogen isotopic data for bulk wood from the same samples (figure 4).

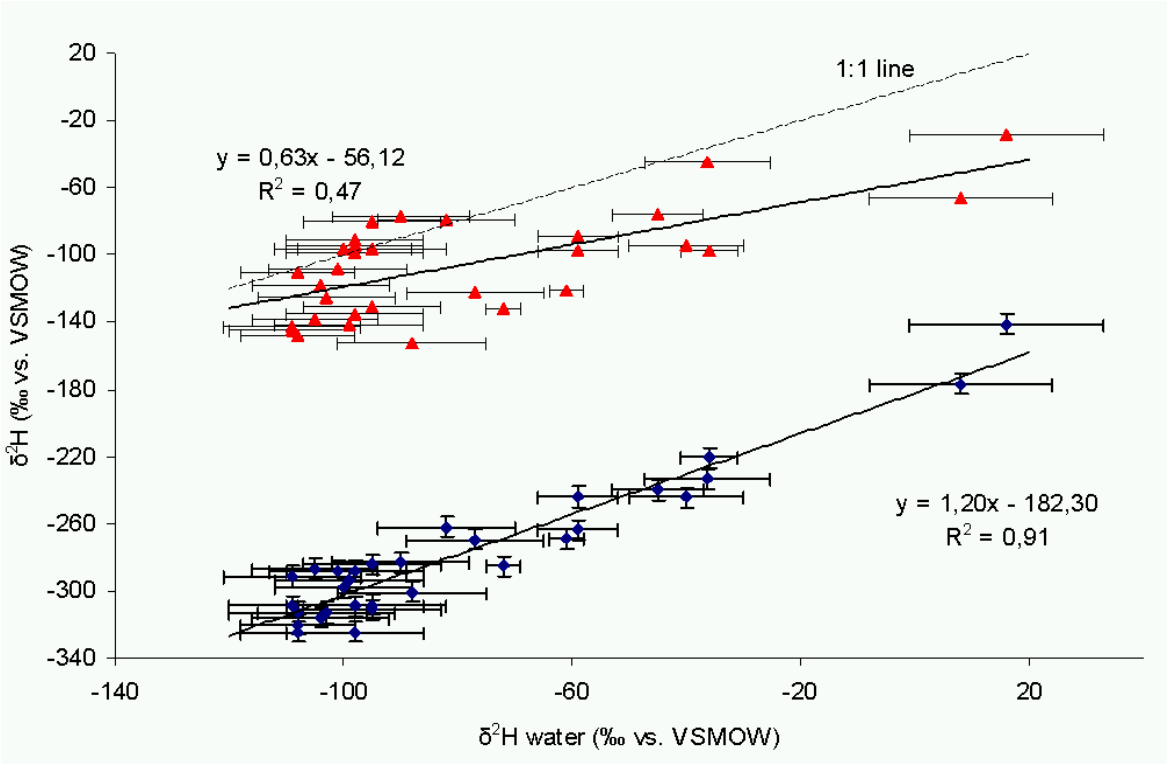



Figure 4:
Hydrogen isotopic data for wood lignin methoxyl groups (blue marks) and bulk wood (red marks) relative to the hydrogen isotope data of meteoric water and respective correlation coefficient

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## 5 References

Bauer-Christoph C, Wachter H, Christoph N, Rossmann A, Adam L (1997) Assignment of raw material and authentication of spirits by gas chromatography, hydrogen and carbon isotope measurements. Part 1. Analytical methods and results of a study of commercial products. Z Lebensm Unters Forsch 204:445-452

Boner M, Förstel H (2004) Stable isotope variation as a tool to trace the authenticity of beef. Analytical and Bioanalytical Chemistry 378:301-310

Brescia MA, Monfreda M, Buccolieri A, Carrino C (2005) Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. Food Chemistry 89:139-147

Camin F, Wietzerbin K, Blanch Cortes A, Haberhauer G, Lees M, Versini G (2004) Application of multielement stable isotope ratio analysis to the characterization of French, Italian, and Spanish cheeses. JAFC 52, 6592-6601

Camin F, Bontempo L, Heinrich K, Horacek M, Kelly SD, Schlicht C, Thomas F, Monahan F, Hoogewerff J, Rossmann A (2007) Multi-element (H,C,N,S) stable isotope characteristics of lamb meat from different European regions. Anal Bioanal Chem 389:309-320

Christoph N, Baratossy G, Kubanovic V, Kozina B, Roßmann A, Schlicht C, Voerkelius S (2004b) Possibilities and limitations of wine authentication using stable isotope data and traceability. Part 2: Wines from Hungary, Croatia and other European countries. *Mitteilungen Klosterneuburg* 54:144-158

Crittenden RG, Andrew AS, LeFournour M, Young MD, Middleton H, Stockmann R (2007) Determining the geographic origin of milk in Australasia using multi-element stable isotope ratio analysis. International Dairy Journal 17:421-428

Engel KH, Baudler R, Adam L, Rossmann A, Versini G, Christoph N, Bauer-Christoph C (2007) Assignment of the regional origin of cherry brandies by stable isotope analysis. In: Authentication of Food and Wine, ACS Symposium Series 952, Ebeler SE, Takeoka GR, Winterhalter P (eds.), chapter 17, 254-272

Hölzl S, Horn P, Rossmann A, Rummel S (2004) Isotope-abundance ratios of light (bio) and heavy (geo) elements in biogenic tissues: methods and application. Anal Bioanal Chem 378: 270-272

Horn P, Hölzl S, Todt W, Matthies D (1998) Isotope abundance ratios of Sr in wine provenance determinations, in a tree-root activity study, and of Pb in a pollution study on tree rings. Isot Environ Health Stud 34:31-42

Kelly S, Heaton K, Hoogewerff J (2005) Tracing the geographical origin of food: the application of multi-element and multi-isotope analysis. Trends in Food Science & technology 16:555-567

Keppler F, Harper DB, Kalin RM, Meier-Augenstein W, Farmer F, Davis S, Schmidt H-L, Brown DM, Hamilton JTG (2007). Stable hydrogen isotope ratios of lignin methoxyl groups as a palaeoclimate proxy and constraint of the geographical origin of wood. New Phytol 176:600-609

Manca G, Franco MA, Versini G, Camin F, Rossmann A, Tola A (2006) Correlation between multielement stable isotope ratio and geographical origin in peretta cow's milk cheese. J Dairy Sci 89:831-839

Pillonel L, Badertscher R, Casey M, Meyer J, Rossmann A, Schlichtherle-Cerny H, Tabacchi R, Bosset JO (2005) Geographic origin of European Emmental cheese: Characterisation and descriptive statistics. International Dairy Journal 15:547-556

Pillonel L, Bütikofer U, Rossmann A, Tabacchi, R. and Bosset JO (2004) Analytical methods for the detection of adulteration and mislabelling of Raclette Suisse® and Fontina PDO cheese. Mitt Lebensm Hyg 95:489-502

Rossmann A, Haberhauer G, Hölzl S, Horn P, Pichlmayer F, Voerkelius S (2000) The potential of multielement stable isotope analysis for regional origin assignment of butter. Eur Food Res technol 211:32-40

Rossmann A (2001) Determination of stable isotope ratios in food analysis. Food Rev Internat 17(3): 347-381

Schmidt O, Quilter JM, Bahar B, Moloney AP, Scrimgeour C, Begley IS, Monahan FJ (2005) Inferring the origin and dietary history of beef from C,N and S stable isotope ratio analysis. Food Chemistry 91 (3):545-549.

Hoefs J (1981) Stable isotope geochemistry. 2. Auflage Springer-Verlag Berlin.

Winkler F-J, Schmidt H-L (1980). Einsatzmöglichkeiten der 13C-Massenspektro-metrie in der Lebensmitteluntersuchung. Z Lebensm Unters Forsch 94, 85-93.

# Stable isotopes as a tool to trace back the origin of wood

M. Boner, Th. Sommer, C. Erven, Hilmar Förstel

## Abstract

The natural variation of stable isotopes can be used to trace back the origin of wood. Especially the D/H and the $^{18}O/^{16}O$ ratio are suitable tools. This results from the isotopic fractionation of the stable isotopes of hydrogen and oxygen within the global water cycle. Each location has a characteristic isotopic composition of the precipitation and consequently of soil and ground water. Both isotopic ratios are closely correlated. Additionally in the needles as well as leaves the isotopic composition is enriched due to the fractionation during the transpiration of plants. Therefore reference samples from the regions of intensive timber logging are preferred, such as from Northern Europe or South East Asia. The WWF has supplied Agroisolab GmbH with 1651 samples of slices from trunks across Northern Europe, and with 487 core drills from South East Asia. In Northern Europe especially the D/H ratio of organically bound hydrogen can be used to trace back the origin of timber, but together with the $^{18}O/^{16}O$ ratio the regional origin can be examined more exactly. In the tropics the conditions seem to be more difficult. Other elements as sulphur have been therefore included successfully. To apply such elements as nitrogen or sulphur new methods have been developed and have to be improved in future. Beside tracing back the geographic origin the method can also be applied to control the declaration, e.g. whether the timber has been cut under legal conditions. Reference samples and data bases are necessary to fulfil this request. The composition of stable isotopes of biomass is mainly the result of that observed in the vicinity. This isotopic composition is stable and varies between different locations. Therefore the method is called “isotopic fingerprint”.

*Keywords: stable isotopes, wood, origin, tracing, Europe, South East Asia*

Agrisolab GmbH, Technology Centre Jülich, Karl-Heinz-Beckurts-Strasse 13, 52428 Jülich, Germany
m.boner@agroisolab.de

## 1 Introduction

The basic idea to localize a sample of unknown origin stems from the observation of the fractionation of the both stable isotopes of hydrogen and oxygen in the global water cycle. From the distribution of temperature and from the movement of air masses results a distinct and in recent times constant pattern. Both mechanisms are often closely connected one to the other. The resulting so called “meteoric water line”, first published by Craig 1961 (see the paper of Förstel within this issue), and afterwards subsequently confirmed (Daansgard 1964), is a result of this correlation. But a model which could explain this very close correlation completely has not been offered until now. Each deviation from the meteoric water line results form specific situations as intensive evaporation of closed basins. The isotopic enrichment of the stable isotopes of water reservoirs which have a slow flow through the basin will be governed by the fractionation during evaporation.

Tracing back the geographical origin of a sample the so-called continental effect plays an important role. As a result from subsequent precipitation and evaporation two opposite effects result in a decrease of the content of the heavy stable isotopes of water vapour crossing the land masses. During the formation of precipitation, a condensation process, the heavy isotopes are preferred to form the rain droplets. The subsequent evaporation at the other hand is a fraction step which leaves back mainly the heavier stable isotopes. Consequently the water vapour from evaporation has a decreased content of the heavy stable isotopes of hydrogen and oxygen. The continental effect is a result of the incomplete balance of precipitation against evaporation.

Water, and also its stable isotopic composition, is the substrate where biomass is formed. Building up biomass needs the material from its direct environment. That means also, that the process of biosynthesis has to use and therefore to reflect the isotopic composition of its environment. The law of mass balance is not only valid for elements, but includes their stable isotopes also. But the formation of some materials like lignin needs the atmospheric oxygen also. The $^{18}O/^{16}O$ ratio of cellulose is assumed to result mainly

from an equilibrium between water and the carbonyl groups which happens during biosynthesis (Sternberg 1989, Sternberg and DeNiro 1990). Therefore the proportion of cellulose and lignin may determine finally the $^{18}O/^{16}O$ ratio of the wood (Gray and Thompson 1977, Keppler et al. 2007). The big reservoir of air oxygen is isotopically homogeneous and its $^{18}O/^{16}O$ ratio is enriched (DOLE effect).

Wood is a well-studied material in the past and today because of two reasons. The first reason is the stability of this material whereas in the past the focus has been set on cellulose, may be taking into account the experiences and techniques from the pulp and paper industry, as demonstrated by the historical publications. The second interest has arisen from the fact that wood in the temperate and cold areas is formed during the annual growth season and stored in visible and separate tree rings like a calendar. This fact is used as a tool of dendrochronology. Additionally the variations of the stable isotope composition of oxygen and hydrogen were used to reconstruct palaeoclimate.

One main problem of the methods to trace back materials to their origin, valid not only for the stable isotope method, is the availability of reference samples. The WWF has enabled this pilot study by an intensive support of material covering large areas of interest. From northern hemisphere forests slices of wood were supplied, from the South-East-Asian region core drills. The sampling strategy was adapted from a study of the $^{18}O/^{16}O$ ratio in German groundwater (Förstel and Hützen 1983). The precipitation is deposited unchanged into the soil and finally into the groundwater. Tap water usually originates from groundwater and can be used as a representation of the isotopic composition of local precipitation. Exceptions are samples from river bank filtrate if the river originates from an area far away from the site of observation, mostly in mountains of high altitude. i.e. of low isotopic content of precipitation.

In the case of wood the local tap water is not sufficient to serve as an indicator of the isotopic composition of cellulose. The biochemical precursors of cellulose are synthesized in the water of needles or leaves. During the uptake of water through the roots and the mass transport in the stem no fractionation can be observed, but in contact with the air the transpiration is a typical step of fractionation. The basis is the local D/H- and $^{18}O/^{16}O$-ratio of the precipitation, but an effect of enrichment is added. The fractionation depends on the physical conditions at the needle or leaf surface, mainly governed by the relative humidity of the air. Thus, two physically driven processes determine the final isotopic composition of the biomass formed: the global climatic conditions of groundwater and the local conditions of the contact of the plant with the environment. This rapid exchange between leaf and air water vapour can be seen by the observation the diurnal cycle of the isotopic composition of leaf water (Förstel 1978). The CRAIG-GORDON model of the exchange between a liquid surface and the wet air in contact gives a first approximation but is not sufficient to describe the enrichment exactly. One reason can be that the small-scale physical conditions at the boundary layer between needle or leaf and the air are not exactly known. These conditions cannot be measured exactly, e.g. the temperature of the leaf surface or the complicated geometry of stomates.

An important argument for the necessity of reference samples results from the experience that the officials and the courts are more convinced by direct comparison with real samples instead of data which have been calculated from models. This has been one reason to initiate this first screening.

## 2 Material and methods

### 2.1. Material

#### 2.1.1. Northern European samples

Discs from stems or strong branches were collected covering nearly the whole area from forests of the Northern Europe, wooded mainly by coniferous forest. From each site about 5 samples were taken, in some cases not only from one species. In Schweden the sample sites were labelled on maps, the other ones by GPS. The use of GPS allows a very rapid and exact localisation of the site of collection and should be preferred as a method to report the data. In combination with Google Earth® an overview of the data points can be obtained quickly and even in some cases the scale can go down to single trees in plantations.

The Swedish samples have been wrapped in plastics which unfortunately have allowed the growth of fungi at the wet surface. Therefore the samples were cleaned by removing down the upper surface. The other samples have been dried and wrapped in paper bags for mailing. Most of the samples were collected from spruce (*Picea abies* (L.) KARST), some from pine trees (*Pinus sylvestris* L.) and others from birch (*Betula sp.*) at the same site. Therefore the

number of samples and of sites does not correspond to each other multipliying the number of sites with the factor 5 (Table 1).

Table 1:
Number of samples collected across Northern Europe, mostly spruce

| Country | Number of samples | Number of sites |
|---|---|---|
| Finland | 199 | 23 |
| Sweden | 305 | 25 |
| The Baltic | 119 | 19 |
| Poland | 144 | 17 |
| White Russia | 69 | 15 |
| Northwest Russia | 815 | 134 |
| Sum | 1651 | 233 |

Annual variations between the tree rings are a common tool of paleoclimatology, but previous tests have demonstrated that at least 6 tree rings are sufficient to get a representative value of the isotopic composition of a sample. In routine analysis the material was obtained by sampling sawmill dust across the whole cleaned disc, drilling a radial trace across the whole surface of the sample. If a sample did not include at least 10 annual rings it was omitted.

The sawmill dust is ground to a fine powder by a ball mill and an aliquot afterwards cleaned by extraction with first methylen chloride followed by methanol in a Soxhlet extraction procedure, each step lasting about 4 hours. After drying overnight at 70º C the samples are ready for further treatment.

### 2.1.2. South East Asian Samples

In South East Asia samples from the Western islands of Indonesia, from Kalimantan, from Vietnam and from Western Malaysia and were collected on the behalf of the WWF (Table 2). The samples from Vietnam were kindly supplied by Prof. Finkeldey University of Göttingen. They were used to study the effect of different species at one location.

Table 2:
Summary of the samples collected in South East Asia.

| Region | Number of samples | Number of sites |
|---|---|---|
| Kalimantan | 203 | 39 |
| Brunei/Sarawak | 32 | 8 |
| Western Malaysia | 34 | 7 |
| Sabah | 78 | 16 |
| Sumatra | 80 | 18 |
| *Kalimantan** | *20* | *10* |
| Vietnam | 40 | 4 |
| Sum | 487 | 102 |

** Two samples were drilled at a close distance from the same trunk to test the variability within the wood.*

From south East Asia only 10 cm long cores from drilling were available. Two cores collected from the same tree at a short distance from the trunk vary only within the methodological and statistical scattering.

### 2.2. Methods

A special technique compares a sample with an internal reference during each measurement, the isotopic ratio mass spectrometry IRMS. Additionally each set of samples is compared not only at the beginning and at the end with a laboratory internal reference material, but also within each run.The laboratory standards are calibrated against internationally distributed standard materials and reported as deviation from there data as δ-values as

$$\delta = \frac{{}^{18}O/{}^{16}O_{(\text{Probe})} - {}^{18}O/{}^{16}O_{(\text{Standard})}}{{}^{18}O/{}^{16}O_{(\text{Standard})}} \cdot 1000$$

$$\delta = \left( \frac{{}^{18}O/{}^{16}O_{(\text{Probe})}}{{}^{18}O/{}^{16}O_{(\text{Standard})}} - 1 \right) \cdot 1000$$

The water of timber may be changed during the transportation. Therefore cellulose will be isolated and used as a memory of the local isotopic composition. To avoid effects of an isotopic exchange between foreign water and the hydrogen of –OH groups these groups are deleted by nitrification (Epstein et al. 1976, Dubois 1984, Brendel et al. 2000, Loader et al. 2003). Agroisolab GmbH has developed a specific furnace (silicon carbide) which can be run at high temperatures above 1500º C without an increasing background.

Carbon and nitrogen are measured by a conventional elemental analyser which converts the biomass to the simple gases carbon dioxide and nitrogen. To avoid problems resulting from the different content of carbon and of nitrogen within the biomass the inflow of carbon dioxide has to be reduced by a diluter. Nowadays the connection between sample preparation and mass spectrometer is achieved by an open split system.

The measurement of the isotopic ratio of sulphur $^{34}S/^{32}S$ is more difficult because of the very low concentration of this element in the matrix. The sulphur has to be converted to a form which enables the concentration and extraction from a bulk of material. Sulphur is an indicator of fertilisation and immission situations as well of the close distance to the sea. A first application is presented for samples in Borneo.

Figure 1 gives a general overview of the methods which have been applied for this study. Latest results have shown that for recent wood nitration is not necessary.

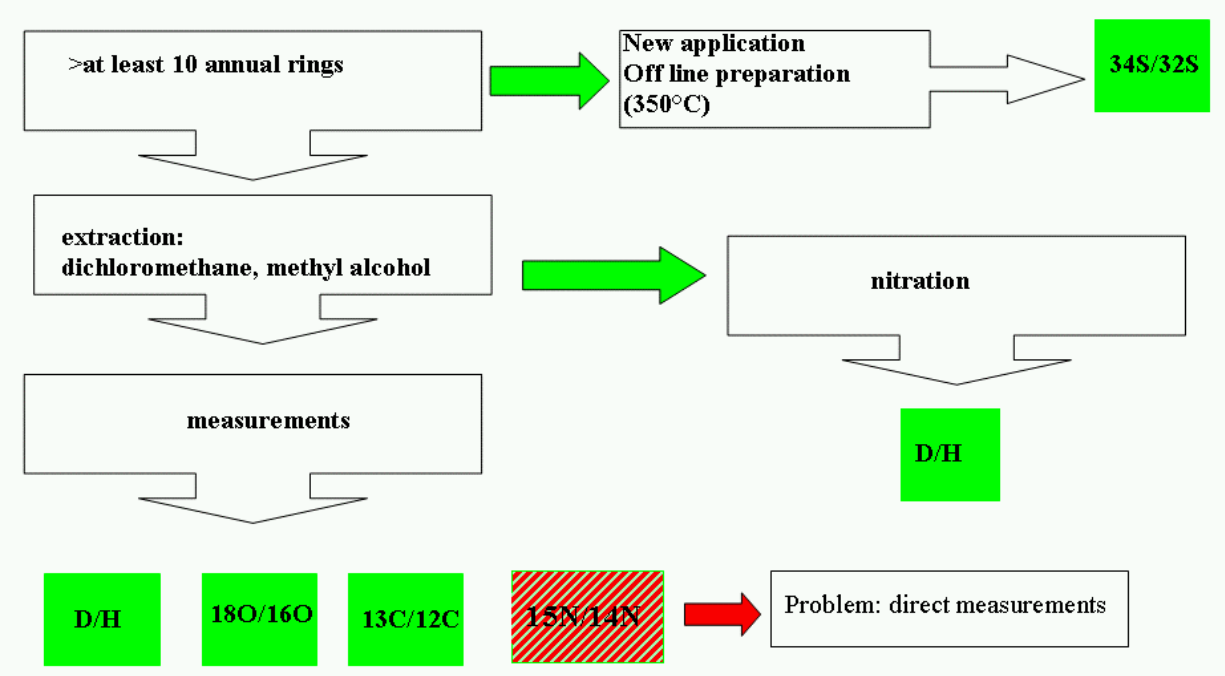


Figure 1:
Overview of the methods used for this study

## 3 Results

### 3.1. Northern European data

Northern Europe is originally covered by coniferous forests, so-called boreal eco-systems. Spruce and other species are the dominating species. This results from the en-vironmental conditions of cold winter seasons including a big amount of snow. The trees grow more slowly compared to the plants at warmer climates more south.

The $^{18}O/^{16}O$ ratios show only minor tendencies across Northern Russia. In contrast to that result a distinct pattern of the D/H ratios in wood will be seen (Figure 2) Two tendencies overlap each other, a decrease from southern towards northern regions due to the decrease of the temperature and from the west to the east as a result of the preferred direction of the movement of air masses across the continent (continental effect). A north-south profile is given for the data along the western border of Russia (Figure 3). The $^{13}C/^{12}C$ ratios may be useful for the identification of a certain batch controlling the paper documents.

Taking into account our experience with food political units may be too limited to catch larger tendencies. Figure 4 and 5 summarize the data of North East Europe and demonstrate the tendencies of the D/H and $^{18}O/^{16}O$ ratios: a decrease to the north and to the east, for $^{18}O/^{16}O$ this is symbolized by the colour of the dots from dark blue to yellow, for the D/H ratio from yellow to red dark. Both of the parameters, the temperature and the distance from the ocean determine the local isotopic composition.

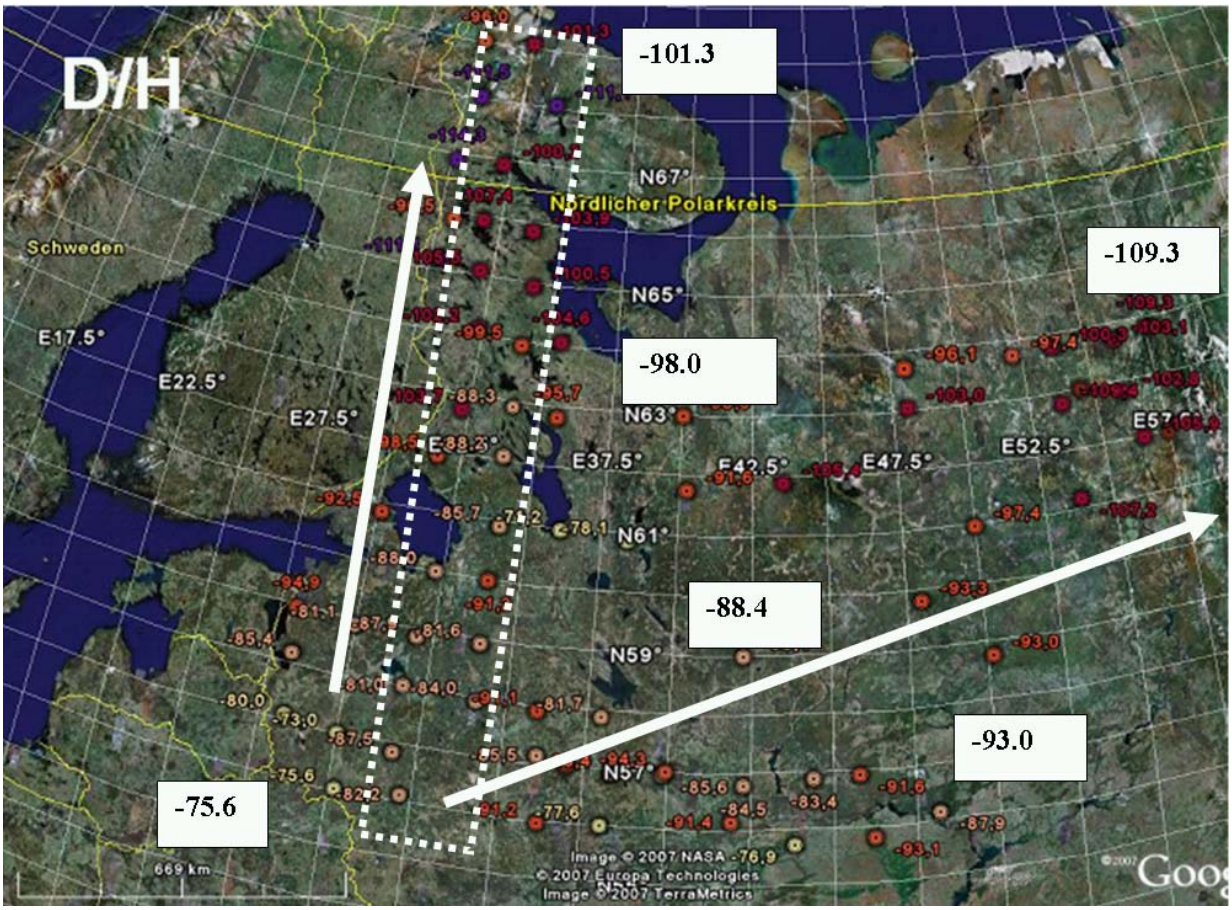


Figure 2:
Tendencies of the D/H ratio across Northern Russia. The dotted rectangle labels approximately the region of data which were used to demonstrate to south-north tendency (see Figure 3)

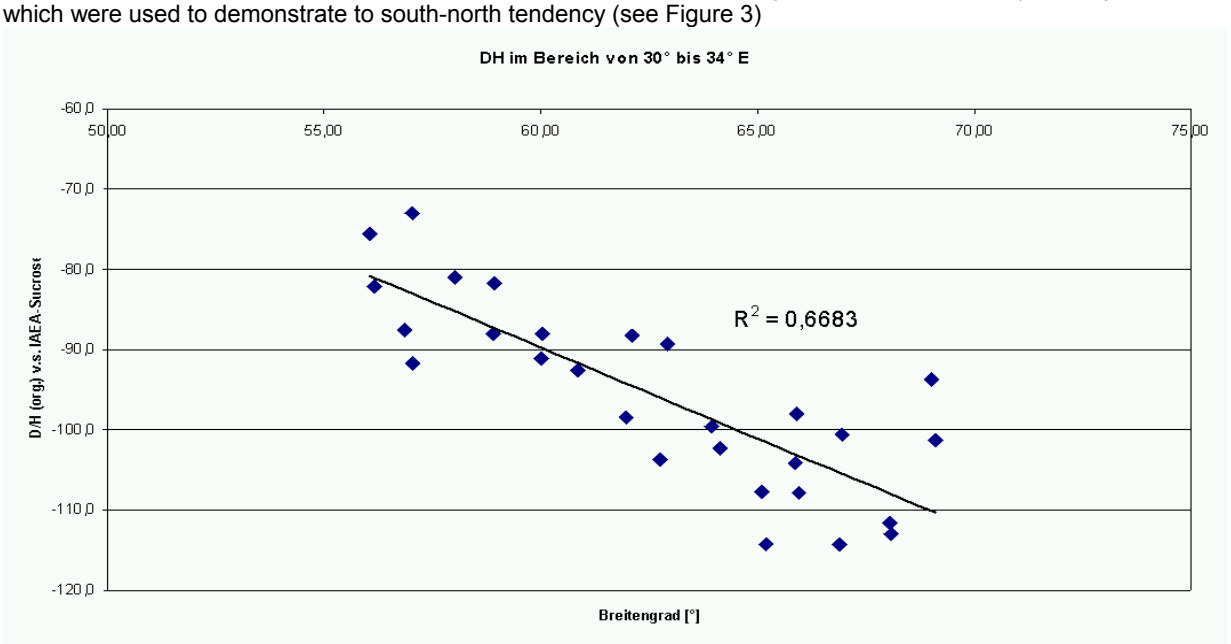


Figure 3:
D/H ratios along a north-south profile close to the western border of Russia (see Figure 2 dotted rectangle)

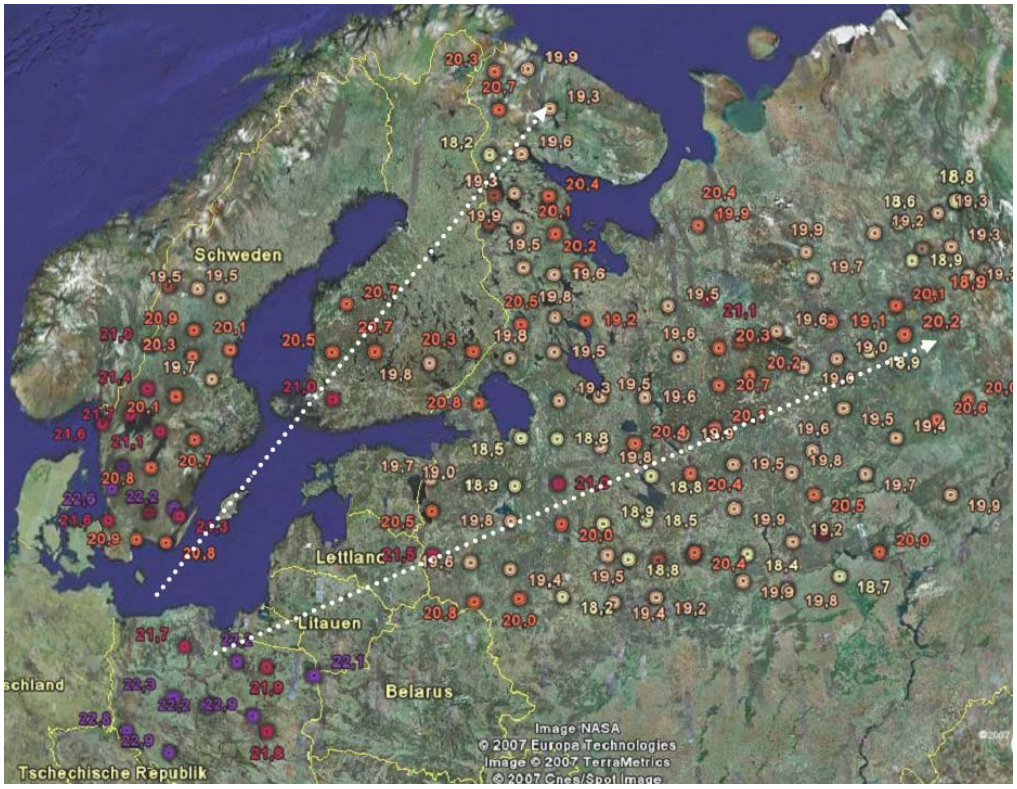


Figure 4:
Overview of the $^{18}O/^{16}O$ data from North East Europe. The decrease of the value of the $^{18}O/^{16}O$ ratio is symbolized by coloured points from dark blue to yellow. Map is generated by Google Earth®

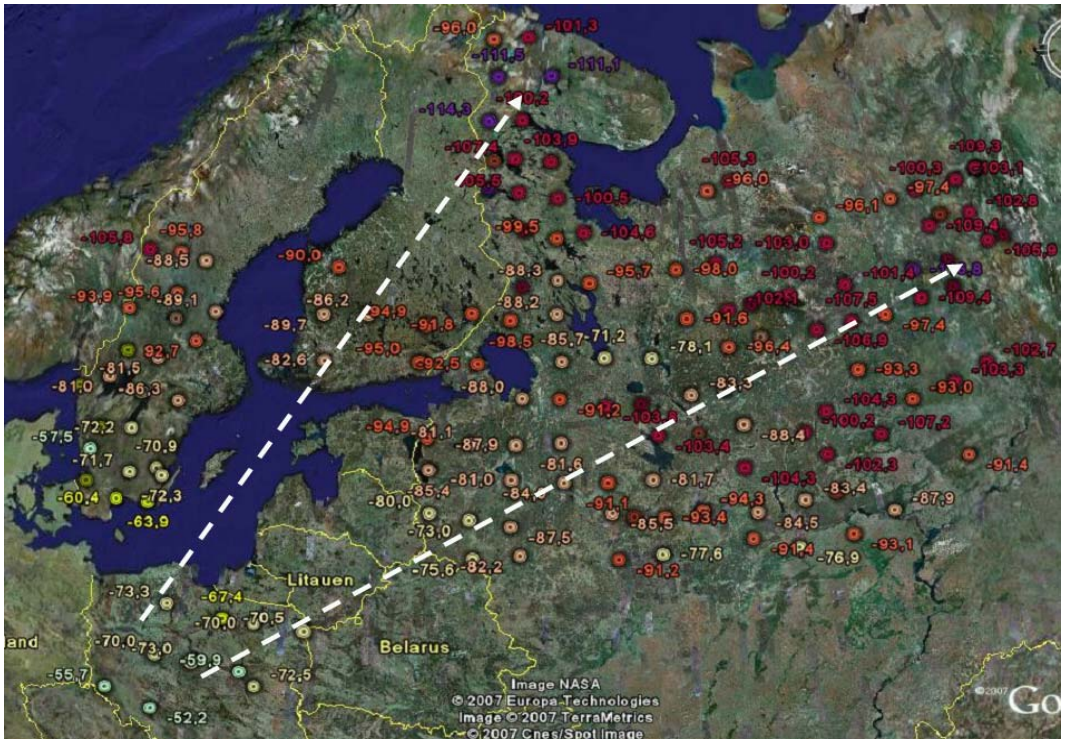


Figure 5:
Overview of theD/H data from North East Europe. The decrease of the value of the D/H ratio is symbolized by coloured points from white to dark purpleMap is generated by Google Earth®

From some locations samples of different species were collected. The number of samples is too small to make general conclusions, but the tendency fits well into the experiences with the isotopic fractionation of plants. The $^{18}O/^{16}O$ data of organically bound oxygen seem not to differ between the species (Figure 20a). The reason should be the use of atmospheric oxygen for some biosynthetic pathways. The D/H ratio originates from the tissue water itself and consequently a difference between coniferous and deciduous trees can be seen (Figure 20b).

Beside the general tendencies many exceptions will appear. But the combination of the D/H and the $^{18}O/^{16}O$ ratio is a successful tool to distinguish between different sources of timber. Combining the D/H and $^{18}O/^{16}O$ ratio of samples from different sources which may show the same value of one isotope, but differ in the corresponding other measurement (compare D/H and $^{18}O/^{16}O$ from Poland with Western Russia).

First observations of the influence of the species were possible if samples of different species were collected from the same site. Despite of the limited amount of data the differences of the water management between needle and deciduous trees can be seen. Both types of trees differ in the enrichment of D/H and $^{18}O/^{16}O$ and must be treated separately.

The physical and local conditions in North East Europe allow a clear differentiation between the various origins of samples collected or better cut in various regions. Both effects, the fall of temperature towards the north and the so-called continental effect, respectively, produce a pattern of the stable isotopes in the local precipitation and groundwater which finally is reflected by the biomass like wood. Local influences can be observed: Trees growing near a river from the high altitude of the Ural mountains show lower values.

Considering the biochemical details of the formation of biomass hydrogen isotopes shall be the most promising and independent parameter. No influence of the atmospheric oxygen pool have to be taken into account.

The spatial variation especially of the $^{18}O/^{16}O$ ratio may at a first glance limit the application. At the other hand this effect allows a good separation of different batches of products. The D/H ratio can be used as a geographical guidance. The other isotopic pairs reflect the local situation and can confirm the general declaration of the geographic origin of a specimen, and will be included more detailed in future work.

### 3.3 South East Asian data

A spatial collection of samples in the tropics has two disadvantages: the systematic determination of species is difficult, additionally the next tree of the same species may be found only some kilometres distant. The number of species is very high compared to the Central European flora. Therefore the number of species was larger, and consequently more botanical families are included in this study. Figure 6 shows the percentage of the different families. The family of *Dipterocarpaceae* is the most frequent one of the botanically identified species and plays also an important role in timber trade from the tropics.

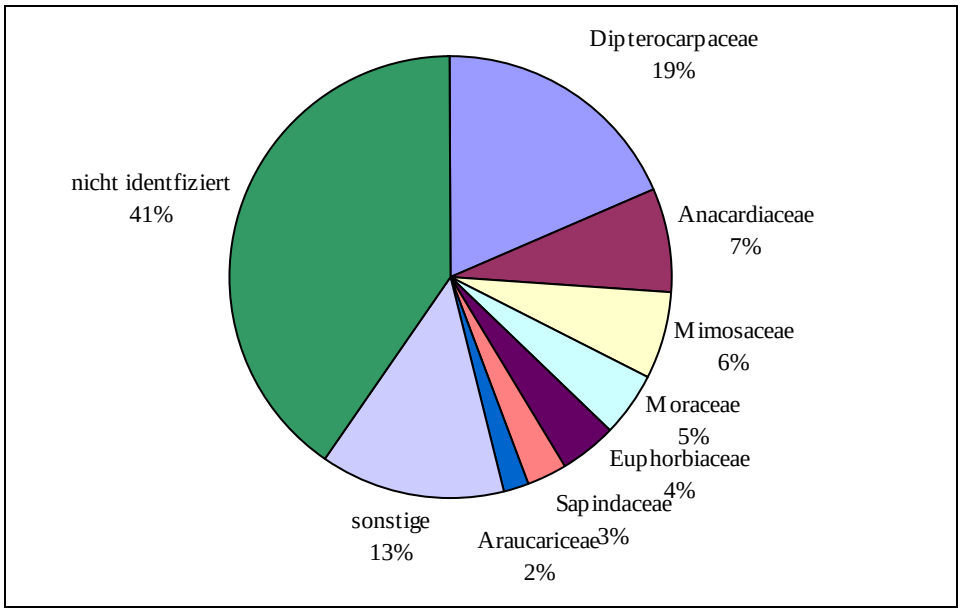


Figure 6:
Portion of the different botanically identified and not identified species assigned to its family

Cores from 4 collection sites in Vietnam, all from *Dipterocarpaceaea*, did not show significant differences between each other. The variations of the D/H ratios (between ± 4.5 and ± 8.2 ‰) and of the $^{18}O/^{16}O$ ratio (between ± 0.3 and ± 0.5 ‰) at one location are higher compared to the technical scattering of the method, but within the range which is usually observed for measurements under natural conditions. The largest standard deviation for the D/H ratios is generated by one extreme value only. The same general statement can be given for the $^{13}C/^{12}C$ ratios (between ± 0.4 and ± 0.7 ‰).

This consideration is a first screening only, for the number of samples is limited. The $^{18}O/^{16}O$ ratios of trees of different botanical classification at a certain location differ not more than ± 0.8 ‰. This means that the $^{18}O/^{16}O$ ratio seems not depend on the species. Half of the D/H ratio show a standard deviation of more than 0.8 ‰ and therefore seem to be more sensitive to systematic classifications, i.e. more depend on the physiological behaviour of the species. The standard deviations of the $^{13}C/^{12}C$ ratios are below ± 0.8 ‰ and not depend on the species, assuming that all samples are from $C_3$ plants only.

Table 3 summarizes the results and demonstrate the broad scattering. The data of $^{18}O/^{16}O$ and $^{13}C/^{12}C$ seem to contain only limited information- More promising are the D/H and $^{34}S/^{32}S$ ratios.

Table 3:
Range of the results from the drill cores of East Asia

| | $^{18}O/^{16}O$ | D/H | $^{13}C/^{12}C$ |
|---|---|---|---|
| Westmalaysia | 17.9 / 20.7 | -87.0 / -71.3 | -29.7 / -27.1 |
| Sumatra | 17.1 / 20.3 | -90.8 / -50.3 | -30.9 / -25.3 |
| Ostmalaysia | 16.9 / 19.5 | -82.8 / -58.7 | -29.0 / -25.1 |
| Kalimantan | 15.4 / 18.9 | -101.4 / -56.1 | -30.0 / -26.6 |

In the tropical region the gradients shall not be as distinct as across the big northern continents. Generally, the local situation may play a dominating role. More details concerning the sampling should be available to interpret the data and to understand the reasons of these scattering (local climate, situation within the local ecosystem, etc.).

The situation in South East Asia differs from that of North East Europe. Water alone seems not to be the governing factor. This can be seen when the altitude effect is considered. Usually the content of the heavy stable isotopes of water decreases with increasing altitude above sea level. In the area of observation such effect could not be observed.

To differentiate between samples other isotopic pairs have to be added. This has been tested for Borneo where the political structure does not follow physical borders. Figure 7 shows that the sulphur results can be used to differ even between the regions of Borneo. Including these results sample can be distinguished beside the irregular shape of the political borderlines (Figure 8).

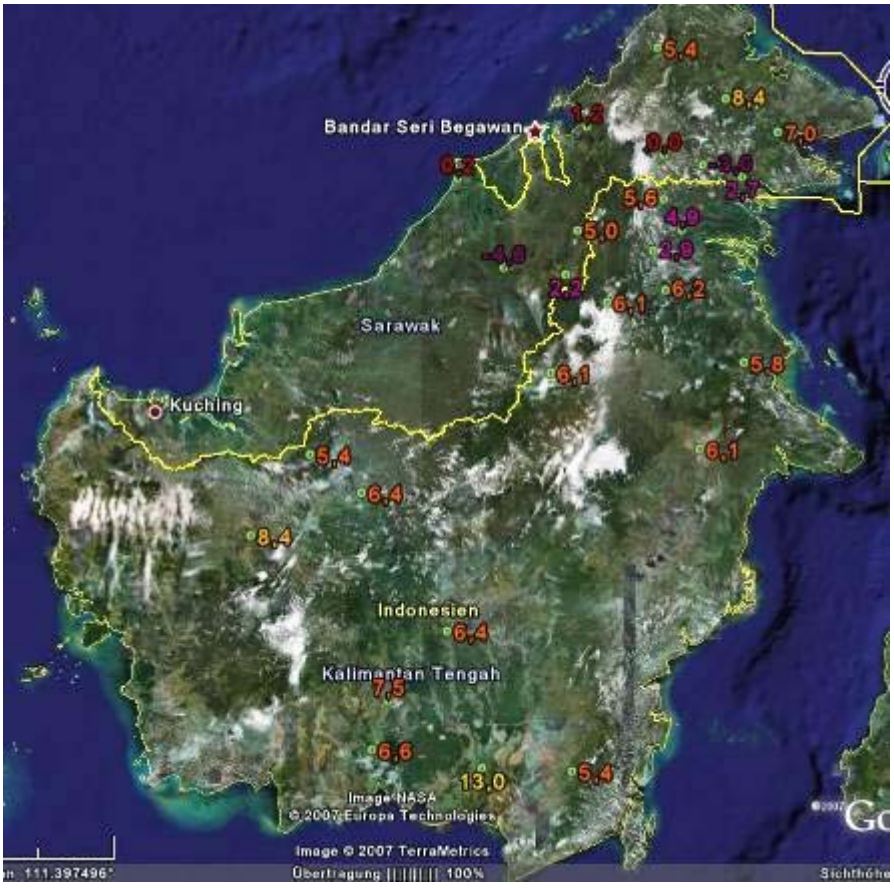


Figure 7:
$^{34}S/^{32}S$ ratios from Borneo (all political units included).Map is generated by Google Earth®

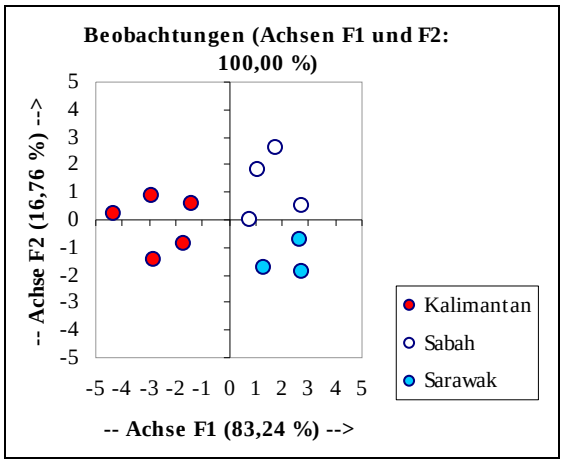


Figure 8:
Discrimination analysis including the $^{34}S/^{32}S$ ratios

## 4 Final discussion and conclusions

The pilot study of the stable isotopic composition of wood compares two important regions of timber trade. The isotopic composition is one of the best criteria to trace back the origin of a product. The origin and the declaration respectively can be controlled. No paperwork is needed, for water is fractionated within the global hydrological cycle. Therefore the isotopic ratios of hydrogen D/H and oxygen $^{18}O/^{16}O$, in liquid or in organically bound form respectively, are useful tracers. Two effects govern the isotopic composition at a certain location: the temperature and the distance from the sea. As a third effect the altitude above sea level is the important parameter, but may be not important in the tropics.

To obtain a useful spatial pattern of the isotope ratios of hydrogen and oxygen a gradient of one of the both parameters is necessary. This is the case for Northern Europe. The isotopic composition of water has a distinct pattern, a decrease of the heavier isotopes of hydrogen and oxygen from south to north and simultaneously from west to east. The incomplete local balance of precipitation and evapotranspiration results in a clear continental effect. Neglecting some local exceptions this general tendency has been observed for the D/H ratio of wood. The pattern of the $^{18}O/^{16}O$ ratios does not show a similar distinct pattern, probably caused by different biochemical pathways of the synthesis of biomass (Schmidt et al. 2001).

The $^{18}O/^{16}O$ ratio of cellulose is assumed to result mainly from an equilibrium between water and the carbonyl groups which happens during biosynthesis (Sternberg 1989, Sternberg and DeNiro 1990). The proportion of cellulose to lignin may determine finally the $^{18}O/^{16}O$ ratio of the wood (Gray and Thompson 1977, Keppler et al. 2007). Nevertheless the $^{18}O/^{16}O$ ratio is a tool to discriminate between timber of different origin.

In South East Asia the situation is different from that of North East Europe. The land is divided in a group of islands and peninsulas. The distances of locations at land are not far from the sea. Surprisingly the altitude above sea level seems to have no influence on the isotopic ratios of hydrogen and oxygen, even up to altitudes higher than 1500 m. The region under observation is situated around the equator, the zone of intensive hydrological turnover and the border of northern and southern atmospheric circulation. There the local water turnover seems to be intensive enough to minimize geographical tendencies. Additionally the ecological situation of each tree is not known. The tropical forest is divided into subsequent layers from the top to the bottom. Only a part of the species has been identified.

The first observations from North East Europe point to a difference between needle and deciduous trees. In the tropic forest the deciduous trees are the overwhelming portion of species, but differ in the physiological behaviour. Nevertheless a first comparison between species collected at the same location does not show too many differences within one botanical family, except for the D/H ratios. If the isotopic ratios would not depend on the kind of species this will be an important simplification for the practical use of the stable isotope fingerprinting.

The method is improved by adding other isotopic pairs like $^{14}N/^{15}N$ and $^{34}S/^{32}S$. The content of both elements in wood is low. Specific methods have to be developed. The $^{87}Sr/^{86}Sr$ ratio can also be included. This parameter is the result of long-time radioactive mother-daughter decay. The equilibrium of this decay is disturbed by geological turnover processes and depends on the local geological situation only. No isotopic fractionation is expected because of the high atomic weight of strontium and the small difference between both isotopes.

The practical work has also demonstrated that the collection of reference material should always include the mapping by a geographically based location system which makes the orientation much easier and in some cases allows a repeated collection of material of the same site or even tree.

The study has tested whether untreated wood, beside a Soxhlet extraction before the measurements, shows a sufficient variation of the stable isotopic composition across large areas. At least for North East Europe the assumption of a clear gradient has been confirmed for D/H ratios. Further studies should test whether the separation of the different pure materials from the wood as well as the addition of other isotopic pairs can improve the method. First measurements of the $^{34}S/^{32}S$ ratio of some samples from Borneo support this assumption.

## 5 Acknowledgement

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*Figures 2,4,5 and 7 are based on Google Earth® and are applied in this scientific study.*

## 6 References

Brendel O, Ianetta PPM, Stewart D (2000) A rapid and simple method to isolate pure alpha-cellulose. Phytochem Anal 11:7-10

Craig H (1961) Isotopic variations in meteoric precipitation. Science 133:1702-1703

Dansgaard W (1964) Stable isotopes in precipitation. Tellus 16:436-467

Degen B, Schmitt U, Boner M (2006) Pilotstudie Holzherkunftsidentifizierung. Bundesforschungsanstalt für Forst- und Holzwirtschaft, Arbeitsbericht des Instituts für Forstgenetik und Forstpflanzenzüchtung 2006/1, pp. 22

Dubois A (1984) On the climatic interpretation of the hydrogen isotope ratios in recent and fossil wood. Bull Soc Belg Geol 93:267-270

Förstel H (1978) The enrichment of $^{18}O$ in leaf water under natural conditions. Rad Environm Biophys 15:323-344

Förstel H, Hützen H (1983) Oxygen isotopes in German groundwater. Nature 303:614-616

Gray J, Thompson P (1970) Climatic information from $^{18}O/^{16}O$ analysis of cellulose, lignin and whole wood from tree rings. Nature 270:708-709

Keppler F, Harper DB, Kalin RM, Meier-Augensten W, Farmer N, Davis S, Schmidt HL, Brown, DM, Hamilton JTG (2007) Stable hydrogen isotope ratios of lignin methoxyl groups as a palaeoclimate proxy and constraint of the geographical origin of wood. New Phytol 176:600-609

Loader NJ, Robertson I McCarroll (2003) Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree rings. Paleogeogr. Paleoclimat Paleoecol 196:395-407

Schmidt HL, Werner RA, Rossmann A (2001) $^{18}O$ pattern and biosynthesis of natural plant products. Phytochemistry 58:9-32

Sternberg LSL, DeNiro MJ (1986) Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. Plant Physiol 82:423-427

Yakir D, DeNiro MJ (1990) Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L. Plant Physiol 93:325-332

Yakir D (1992) Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. Plant Cell Environ 15(9):1005-1020

Yapp CJ (1982) A reexamination of cellulose carbon-bound hydrogen measurements and some factors affecting plant-water D/H relationships. Geochim Cosmochim Acta 46(6):955-965

**Representing isotopic ratio data using state of the art mapping technologies.**

P. Bliznakov[1], M. Boner[1]

**Abstract**

Contemporary web technology, especially the use of geographical position systems, can be easily applied to locate the sampling site as well as to combine different data as isotopic ratios or genetic information. Preliminary examples are demonstrated from the current studies of spruce from Eastern Europe. The exact measurement of the geographic position during the collection of wood is the basis to re-find the point of sampling. This can be used to check the environment of the tree and to estimate other influences on the results as e.g. a position close to a river bank.

*Keywords: stable isotopes, mapping technology, geographic position, data handling, data registration*

## 1 Introduction

One of the most difficult parts of the research is representing the information gathered along the way. Usually when it comes to the geographical analysis of data, the common way of data representation is to gather the GPS coordinates during the study and then place the measured data onto a suitable map.

Nowadays, there are diverse mapping resources on the market. However choosing one of the most popular and cost effective platforms may proof to be an efficient way of representing GPS based databases. Such a system needs to be:

- cost effective
- flexible
- compatible with already established standards
- worldwide accessible
- secure
- user friendly

## 2 Material and Methods

A very important aspect of the project is the minimal data set construction. Giving the users the capability to define their own data ranges, increases the productivity and saves research time and money. For this purpose a suitable combination of several modern Web technologies is offered. Google Maps® for example provides the background mapping, with built in functions for zooming in and out from 100 meters to several kilometres in range, revealing the environment surrounding the sample (lakes, rivers, water reservoirs) (see Figure 1). Customers overlays and specialized views like climatic and soil maps are also possible, guaranteeing the flexibility of the project.

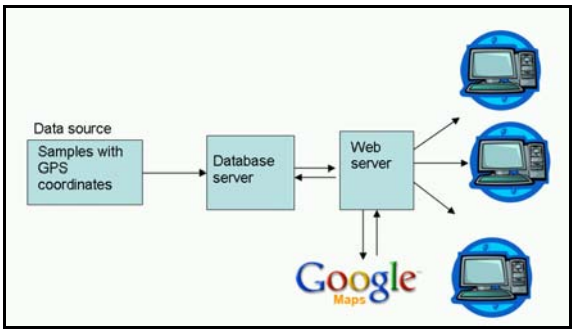



Figure 1:
Data acquisition including a geographical mapping system

The application can work with a every structural data standard (XML, XLS, CSV…), extending the diversity of information offered to the user. A combination of genetic data sets with stable isotopes information is therefore easy to achieve. Other information like images, raw data and user comments can also be integrated into this information system, which utilizes the whole knowledge gathered about the particular problem.

Agroisolab GmbH., Technology Centre Jülich, Karl-Heinz-Beckurts-Strasse 13, 52428 Jülich-Germany
m.boner@agroisolab.de

## 3 Examples

In the following section examples of the integration of stable isotope data into Google Earth® are presented.

In figure 2 data are assigned to the sampling sites. The general orientation of the local conditions can be seen distinctly, e.g. the ecological regime within a river valley, within a larger forest area etc. Specific results may be explained by these observations. To view the local situation zooming presents more details. Depending on the quality of the section presented by Google Earth® even single trees, especially in plantations, can be seen. To achieve this resolving the geographical position must be determined at the site of sampling directly and as exactly as possible. Figure 3 shows an example of a combination of a map with additional information for each data point. The amount of additional data as results, descriptions or pictures may be selected by the user.

## 4 Conclusions

The current web based technology offers simultaneously a high level of security and availability. The infrastructure of the project requires a single investment and regular maintenance. This will guarantee the worldwide accessibility and secure data exchange.

The application uses SSL (Secure Socket Layer) encryption mechanisms combining the newest security standards and algorithms. Additional security measures are also possible. User management and control over restricted areas could be introduced as a further feature along the development of the project.

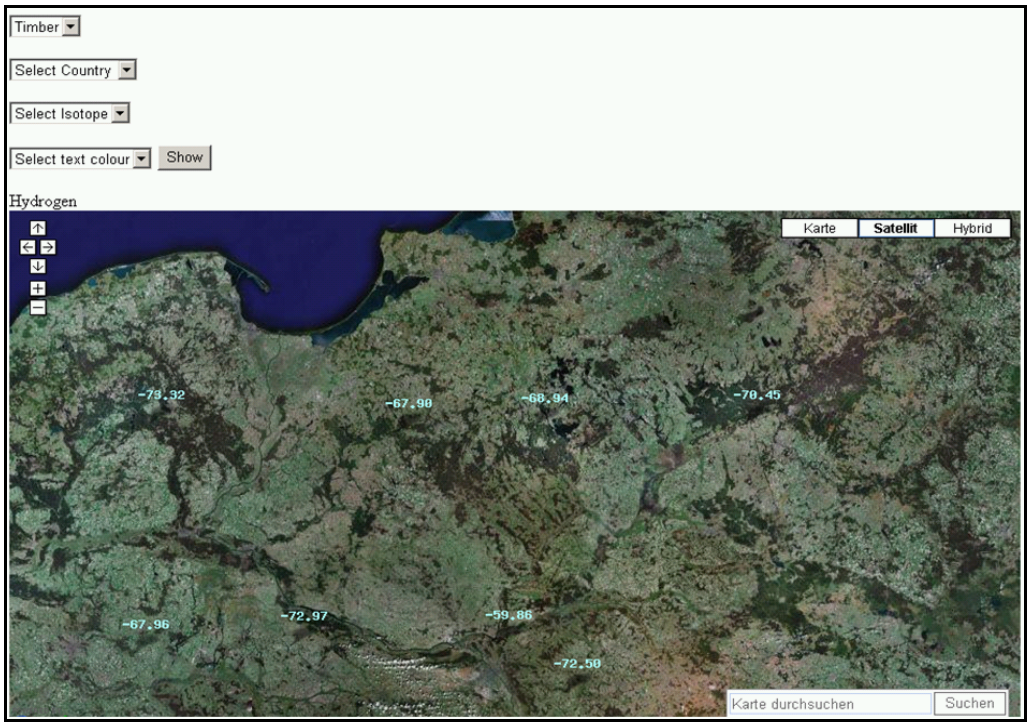



Figure 2:
Assignment of stable isotope data (organically bound hydrogen of wood) to the sampling sites using Google Earth®

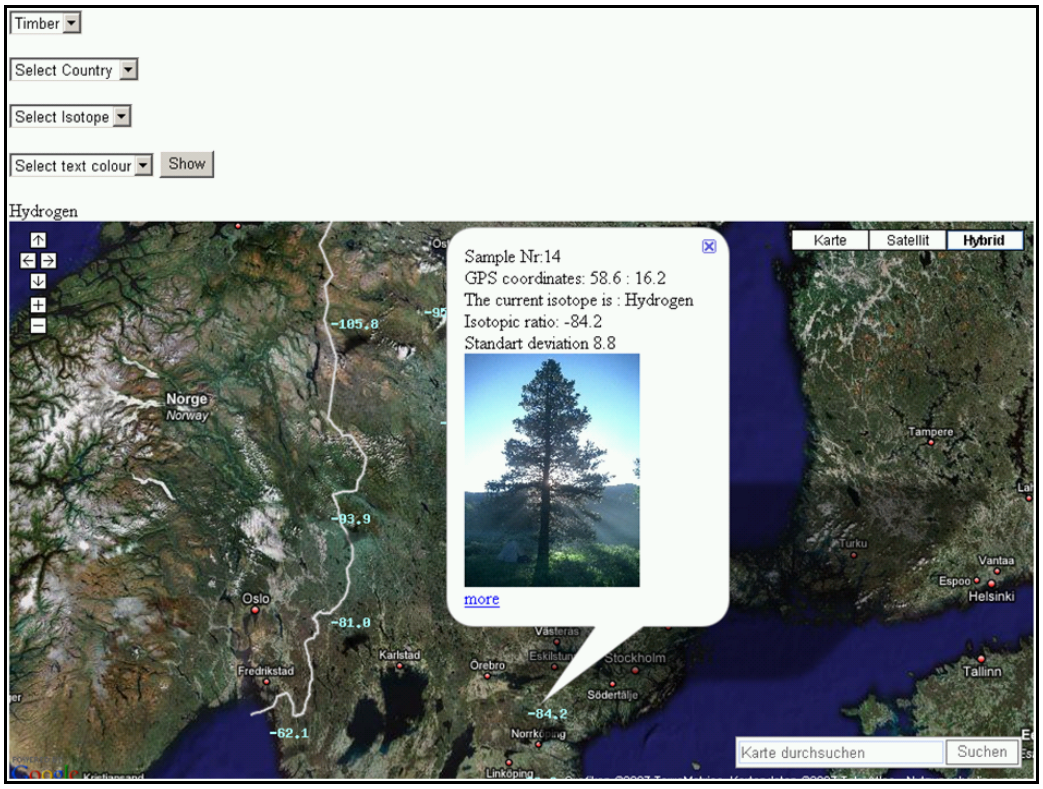



Figure 3:
Example of the combination of a map with additional information

**Cites and Wood Data Bases**
Computer-Aided Identification And Description Of Cites Protected Trade Timbers

Gerald Koch[1], Hans-Georg Richter[1] and Uwe Schmitt[1]

**Abstract**

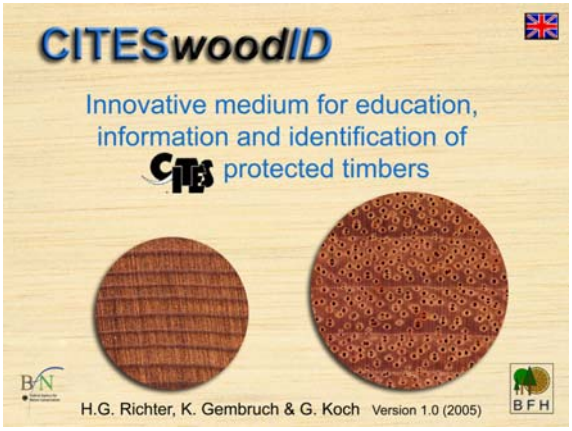
The knowledge about recognition of CITES-protected wood species is of prime importance to control trade with and enforce regulations to protected species. A valuable support for computer-assisted wood identification based on macroscopic features is already available from a database developed in the DELTA-INTKEY-System. This database contains descriptions and an interactive identification system for 8 CITES-protected timbers (7 hardwoods, 1 softwood) known for their potential in the manufacture of lumber and downstream processing into products, and 41 trade timbers which can be easily mistaken for CITES-protected timbers due to a very similar appearance and/or wood anatomical pattern. The database is primarily designed for institutions and persons involved in controlling import as well as export of wood and wood products under consideration of CITES regulations. It also serves to all primary and secondary educational facilities active in teaching wood anatomy and wood identification.

*Keywords: CITESwoodID, database, wood identification*

Johann Heinrich von Thünen Institute (vTI), Institute of Wood Technology and Wood Biology, Leuschnerstr. 91, D-21031 Hamburg, Germany, gerald.koch@vti.bund.de

## 1 Introduction

The computer-aided identification program CITES*woodID* was developed in response to a proposal by the German CITES Scientific Authority at the Institute for Wood Biology and Wood Protection of the Federal Research Centre for Forestry and Forest Products (BFH), Hamburg, and is at present available in the four languages English, German, French and Spanish.




CITES*woodID* enables the user to identify trade timbers controlled under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) by means of macroscopic characters. Macroscopic characters are all those, which can be observed or perceived, respectively, with the unaided eye and a handlens of approximately 10-fold magnification.

Particular interest has been taken to provide high quality photographic illustrations with the database of both the characters used for identification and the timbers comprising the database. The photomacrographs of transverse sections were taken at a magnification commensurate with that of a handlens (ca. 10-fold). Illustrations of wood surfaces are reproduced in natural size (1:1). These illustrations provide an excellent means of visualizing certain character expressions and directly compare the results of an identification run and the unknown object to be identified. Furthermore, nearly all characters used for description and identification are accompanied by explanatory notes with definitions, examples, procedures, etc.

## 2 Objectives of the database

CITES*woodID* serves as a visual (illustrations) and textual (descriptions) identification tool to all institutions and persons involved in controlling import as well as export of wood and wood products under particular consideration of CITES regulations. It also serves to all primary and secondary educational facilities active in teaching wood anatomy and wood identification.

The database contains a) the 8 CITES-protected timbers (7 hardwoods, 1 softwood) known for their potential in the manufacture of lumber and downstream processing into products, and b) 41 trade timbers which can be easily mistaken for CITES-protected timbers due to a very similar appearance and/or wood anatomical pattern. CITES-protected plants/trees utilized for non-wood products are not included.

Table 1:
List of CITES protected trade timbers in the database

| Botanical name | Trade name | CITES Appendix |
|---|---|---|
| *Caesalpinia echinata* | Brazil wood | CITES II |
| *Cedrela odorata* | Cedro | CITES III |
| *Dalbergia nigra* | Brazilian rosewood | CITES I |
| *Fitzroya cupressoides* | Patagonian cypress | CITES I |
| *Gonystylus* spp. | Ramin | CITES II |
| *Guaiacum* spp. | Guaiacum wood | CITES II |
| *Intsia* spp. | Merbau | "observation" |
| *Pericopsis elata* | Afrormosia | CITES II |
| *Swietenia* spp. | American mahogany | CITES II |

Table 2:
Information on trading of CITES protected timbers (data of the German CITES Scientific Authority, 2006)

| Species | Annex | Trading | Quantity [m³/a] |
|---|---|---|---|
| Cedro | CITES III | Bolivia, Peru ⇨ USA | 45.000 |
| Brazilian rosewood | CITES I | *trade is not allowed* | |
| Ramin | CITES II | Malaysia ⇨ Italy, China | 70.000 |
| Guaiacum wood | CITES II | Mexico ⇨ Germany | 100t |
| Afrormosia | CITES II | Congo, Cameron ⇨ Italy, Belgium | 15.000 |
| American mahogany | CITES II | Bolivia, Peru ⇨ USA | none information |
| Merbau | "observation" | Indonesia, PNG ⇨ China, Germany, Netherlands | 660.000 (illegal logging) |

## 3 Basics on macroscopic wood identification

Observations made in these different planes add up to a three-dimensional picture of the gross wood structure. Observed differences in structure between the various timbers can be described, attributed to certain characters, and used for wood identification with the help of reference material for comparison.

### 3.1 Macroscopic structure of hardwoods

Hardwoods are composed of the following main tissues:

- Fibres (mechanical support),
- Parenchyma (storage and transport of nutrients),
- Vessels (conduction of water) and, of rather rare occurrence,
- Resin canals (secretory tissue)

In simple terms, fibres impart mechanical strength. They are responsible for resisting the many dynamic and static stresses in the living tree and in wood under load. Fibres usually make up the largest part of the wood volume. They are among the smallest diameter cells and, because of their often thick walls, appear as darker areas when seen en masse in cross section. At the macroscopic level they simply form a (usually darker) background for pores, rays and parenchyma.

The vessels of hardwoods constitute the principal passageways in the living tree for axial transport of water from the roots to the crown. On cross sections they are visible as pores (openings) arranged in a variety of distinctive patterns, on longitudinal sections as shallow grooves (vessel lines). Vessels are the only cells, which expand to dimensions (diameter, length) visible to the unaided eye or with a handlens.

Figure 2:
Distribution of vessels in hardwoods. (left): ring-porous structure of oak; (middle): semi-ring-porous structure of walnut, (right): diffuse-porous structure of American mahogany

Figure 3:
Various pattern of axial parenchyma in hardwoods. (left): aliform and confluent parenchyma in Afzelia/Doussie, (middle): reticulate pattern with rays in Pterygota, (right): marginal parenchyma bands in American mahogany

Parenchyma cells are responsible for axial and radial transport and storage of nutrients in the living tree and serve as depositories of accessory compounds during heartwood formation. Parenchyma tissues are orientated axially (parallel to the stem axis = axial parenchyma) or horizontally (perpendicular to the stem axis = rays). Parenchyma cells are nearly always thin-walled and become visible macroscopically only when forming larger agglomerates. In hardwoods, the axial parenchyma can be very conspicuous and its various expressions are of high diagnostic value.

The rays also have an important role in macroscopic wood identification, particularly as regards size (width and height) and arrangement on tangential surfaces (storied vs. not storied).

Few hardwoods also possess longitudinal and/or radial (forming part of a ray) resin canals, tubular passages in wood, which are actually intercellular spaces surrounded by specialized secretory cells. Resin canals are a characteristic feature of some tropical timbers, for instance of the large Dipterocarp family. Presence vs. absence, size and arrangement of axial resin canals are often highly diagnostic features. On

cross sections, resin canals are difficult to distinguish from the vessels/pores unless still exuding resin (dark irregular patches around the openings) or containing crystallized dry resin of a brilliant white colour.

3.2 Macroscopic structure of softwoods

Conifers, here referred to as softwoods, evolved prior to the angiosperms (hardwoods), and retain a relatively primitive wood structure compared with the more specialized and complex structure of hardwoods. Because macroscopic identification of softwoods is much more difficult due to the lack of distinctive features they are discussed here last. Essentially, softwoods are characterized by only three cell elements or tissues:

- Tracheids (combined mechanical and conductive functions),
- Parenchyma (storage tissue), and
- Resin canals (secretory tissue).

The tracheids of softwoods serve the combined functions of mechanical strength and conduction. Their diameter is highly variable and rarely large enough to become visible under a 6x—12x magnifying lens. Nevertheless, tracheids produced early and late in the growing season of a tree may differ in size and, particularly, cell wall thickness, thus forming lighter coloured earlywood and darker coloured latewood. Latewood width and the appearance of the transition of earlywood to latewood within a growth ring is, in some instances, a very useful feature in macroscopic softwood identification.

Axial parenchyma cells, though present in many softwoods, never form large enough agglomerates to become macroscopically visible. Rays in all softwoods, composed of radial parenchyma cells, are generally uniseriate (narrow) and low, and therefore cannot contribute to the distinction of individual softwood timbers. Rays containing radial resin canals ("fusiform rays") are the exception to the rule and, when large enough, may also be a useful feature in softwood identification.

Resin canals occur in all species of several genera within the pine family (*Pinaceae*), among them pine (*Pinus* spp.), spruce (*Picea* spp.), larch (*Larix* spp.) and Douglas fir (*Pseudotsuga* spp.), which contain both axial and radial resin canals. The presence of resin canals thus provides an initial basis for separating pine, spruce, larch and Douglas fir from the remaining conifers. Size, frequency and arrangement of axial resin canals can be helpful for distinction between and within these four taxonomic groups.

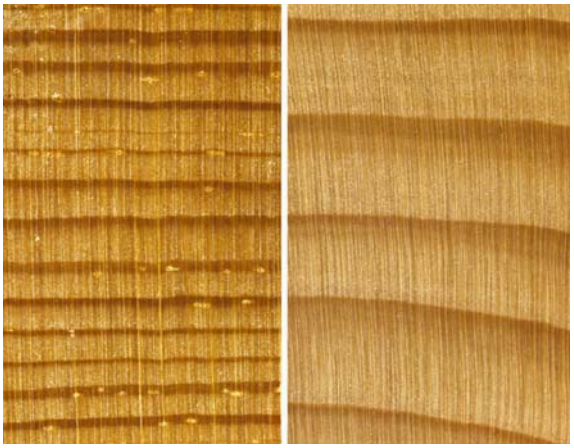


Figure 4:
Macroscopic structure of softwoods. (left): transverse plane of spruce with the occurrence of resin canals. (right): transverse plane of white fir without resin canals

## 4 How to use CITESwoodID for wood identification

When initiating the identification process, the user has several options to follow. The program starts in the *normal working mode*, i.e., the available features are listed in a sequence of "best characters" with the character on top of the list which, by definition, is best suited for separating the (remaining) taxa in the database. Following these suggestions is the approach recommended for inexperienced users. If a more experienced user is sure of the detection of a certain character it can immediately be chosen. The search function in the toolbar facilitates locating the desired character. A further option is using the natural order of the characters as represented in the original character list.

Each character is accompanied by notes with information on definitions, explanations as to how observations can be correctly interpreted, procedures concerning specimen preparation for certain purposes, examples of timbers with a very typical expression of the character in questions, cautionary notes on how to guard against misinterpretation, information on specific wood characters not covered by the character list, etc. In addition, characters and the timbers in the database are accompanied by high quality colour images illustrating important macroscopic features on both transverse and longitudinal

Figure 5:
American mahogany = *Swietenia* spp. (CITES Annex II, left) are very similar to khaya = *Khaya* spp. (right) in external appearance. The two timber groups differ significantly by the lack of macroscopically visible axial parenchyma in *Khaya* spp.

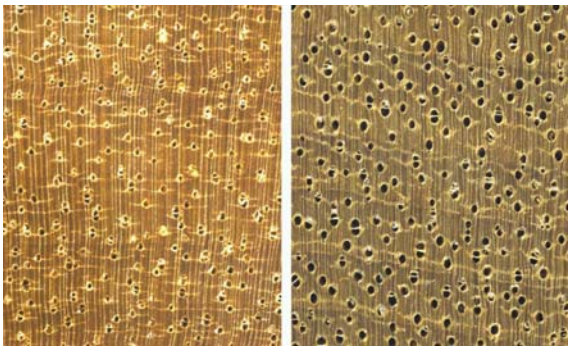


Figure 6:
Ramin = *Gonystylus* spp. (CITES Annex II, left) and limba = *Terminalia superba* (right) are similar in appearance and structure. However, limba has much larger and fewer vessels often filled with tyloses. Furthermore, the wood of limba is darker with a greenish hue

Figure 7:
Alerce = *Fitzroya cupressoides* (CITES Annex I, left) and Western Red Cedar = *Thuja plicata* are virtually indistinguishable given their similar structure. The aromatic odour and yellowish hue of Western Red Cedar, however, is of much help in separating the two timbers

faces. These images can be of considerable help in finding a character and using it in an appropriate way. Images are also very useful when it comes to confirming identification results by visual comparison.

CITES*woodID* also offers complete program-generated wood descriptions. These encompass all relevant information contained in coded form in the database, converted into a natural language text and saved in a single file. The individual wood description can be used interactively for consultation and/or printing at any point of an identification run.

At least it must be quite clear to the user that the possibilities of macroscopic wood identification are much more limited than those of microscopic study. Firstly, the number of characters available for observation is considerably smaller. Secondly, in macroscopic identification one has to rely quite often on characters subject to a high variability due to different growth conditions of the tree (viz. formation of growth rings) or exposure to oxygen and UV radiation (viz. wood colour). This may lead to subjective judgement on behalf of the user, and errors which might result in wrong decisions. In fact, in cases of closely related trade timbers, the use of macroscopic characters will end with a choice of several likely matches whose safe separation must be left to microscopic study performed by any of the scientific institutions in Europe with the necessary equipment and experienced staff, in Germany the Federal Research Institute for Rural Areas, Forestry and Fisheries with its Institute of Wood Technology and Wood Biology, Hamburg (www.vti.bund.de/en/institutes/htb/).

## 5 References

CITES*woodID* Descriptions, illustrations, identification, and information retrieval. In English and German. Version: November 2005

Koch G (2005) Holzanatomie - Anatomischer Aufbau und chemische Zusammensetzung des Holzes. In: Müller J (ed) Holzschutz im Hochbau. Fraunhofer IRB Verlag Stuttgart, pp 15-23

Koch G (2007) Eigenschaften und Verwendung von Austauschhölzern für den Außenbereich - Markt-entwicklungen und CITES-Schutzmaßnahmen. In: Deutsche Gesellschaft für Holzforschung München (ed) Proceedings 25. Holzschutz - Tagung „Wege zu geschütztem Holz im modernen Holzbau“. pp 21-27

Koch G, Richter H-G, Schmitt U (2006) Topochemical investigation on phenolic deposits in the vessels of afzelia (*Afzelia* spp.) and merbau (*Intsia* spp.) heartwood. Holzforschung 60:583-588

Schmitz-Kretschmer H-J, Koch G, Krämer, G (2006) Lupe, Notebook und die Software CITESwoodID. Holz-Zentr.bl. 132:484-485

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