Miljø- og Planlægningsudvalget (2. samling)
MPU alm. del - Svar på Spørgsmål 112
Offentligt

DANISH MINISTRY
OF THE ENVIRONMENT

Forest and Nature Agency

Tracing timber
from forest
to consumer
with DNA markers

BY : DANISH CENTRE FOR FOREST, LANDSCAPE AND PLANNING. UNIVERSITY OF COPENHAGEN

This report is prepared by Lene Rostgaard Nielsen and Erik Dahl Kjær, Department for Management of Forest Genetic Resources, Danish Centre for Forest, Landscape and Planning, Faculty of Life Sciences, University of Copenhagen. These assessments are not necessarily identical with those of the Danish Ministry of the Environment, Forest and Nature Agency. However, the Agency does consider the report as an important contribution to clarifying options for tracing timber through DNA-technology.

Danish/dansk:
Denne rapport er udarbejdet af Lene Rostgaard Nielsen og Erik Dahl Kjær fra Center for Skov, Landskab og Planlægning, Det Biovidenskabelige Fakultet, Københavns Universitet for Miljøministeriet, Skov- og Naturstyrelsen. Rapportens vurderinger og konklusioner er alene forfatternes ansvar, og er ikke nødvendigvis identiske med Skov- og Naturstyrelsens vurderinger. Styrelsen finder imidlertid, at rapporten er et vigtigt bidrag til afklaringen af mulighederne for at spore træ ved hjælp af DNA-teknologi.

Preface

DNA testing has been an important part of forensic methods for decades, but can similar DNA based methods be applied for enforcing legislation or control regimes in timber trade? In early 2007, the Danish Forest and Nature Agency approached us with a number of interesting questions in relation to this issue:

- What kind of DNA based techniques can be relevant to apply for tracing the orgin of timber?
- What kind of answers can such techniques be expected to provide, and with what precision?
- How easy can they be implemented for operational testing?
- What kind of experience has been gained so far?

In order to address these questions we prepared the present report based on a review on available literature with the objective of providing an overview of the available options for tracing timber based on DNA techniques. The final version of the report was completed after the workshop in Bonn (8–9 October 2007) on »Fingerprinting methods for the identification of timber origins« organised by the German Federal Ministry of Food, Agriculture and Consumer Protection and the World Wildlife Fund. This was a unique chance to discuss these options with researchers involved in this kind of studies. Discussions and recommendations from the workshop are therefore incorporated into the present report.

Hørsholm, 2007

Lene Rostgaard Nielsen and Erik Dahl Kjær

Content

Danish summary

Efter drøftelser af forslag til folketingsbeslutning om forbud mod import og salg af illegalt træ (B17/2005-2006) opfordrede Folketingets Miljø- og Planlægningsudvalg miljøministeren til

»... at arbejde for at fremskynde tilvejebringelsen af grundlaget for en nærmere vurdering af, om tømmer er fældet lovligt eller ulovligt i oprindelseslandet...«

For at kunne vurdere om træ i et givet træprodukt er fældet lovligt eller ulovligt i oprindelseslandet er det relevant at 1) kunne spore, hvor træet oprindeligt er fældet og 2) kunne vurdere under hvilke omstændigheder træet er fældet, herunder særligt om de relevante love er overholdt. Nærværende rapport beskæftiger sig alene med del 1) mulighederne for at spore træet.

Der er på dette område udviklet forskellige certificerings- og mærkningsordninger, som på grundlag af forskellige regler og kontrolprocedurer sikrer at der 'holdes styr på træet' på hele dets vej fra skoven til slutbrugeren gennem alle bearbejdnings- og handelsled. Certificeringssystemernes krav til skovdrift såvel som kontrol af forarbejdningsled og distribution varierer, og der er forskellige regler for mærkning.

I projektet *»Sporing af oprindelsen af træ ved hjælp af DNA-analyse«* har vi på opfordring af Skov- og Naturstyrelsen vurderet de eksisterende muligheder for at kunne spore træet primært via DNA-teknologi – uanset om det er certificeret eller ej. Vi har også forsøgt at vurdere under hvilke forudsætninger det vil være realistisk at implementere sådanne metoder på kort eller lang sigt. Med henblik herpå har vi gennemgået relevant international litteratur og indsamlet andre tilgængelige erfaringer på området. Resultatet heraf – sammen med sammendrag af diskussionerne fra den internationale workshop i EU *»Fingerprinting methods for the identification of timber origin«* (som holdtes 8.-9. oktober 2007 i Bonn) – har dannet udgangspunkt for nærværende rapport. Vi mener på baggrund af arbejdet, at det er relevant at drage følgende fem konklusioner:

1) Tilgængelighed af DNA og DNA-teknikker
Der findes en række tilgængelige DNA-baserede metoder, som hver har deres særlige styrker og svagheder i forhold til sporing af træ. Overordnet kan metoderne dog opdeles i to grupper: De universelle og de artsspecifikke. Markører, der er universelle, kan bruges henover mange forskellige arter. De specifikke metoder skal modsat udvikles specielt til én konkret art, selvom de i mange tilfælde kan bruges på nærtbeslægtede arter. Ulempen ved brug af universelle markører er, at der er en risiko for at genotype DNA fra noget andet end selve træet. En sådan uopdaget kontaminering er især kritisk ved brug af DNA-ekstraktioner med relativ lav koncentration af DNA, som det typisk vil gøre sig gældende for DNA fra træprøver (se nedenfor). De artsspecifikke markører er dyre og tidskrævende at udvikle, men til gengæld vil risikoen for kontaminering med DNA fra andre arter være uden betydning.

En stor del af importeret træ ankommer til EU i form af savskårede emner eller forarbejdede produkter (f.eks. havemøbler el. lign). Grundlaget for at kunne anvende DNA-teknikker til bestemmelse af oprindelsessted for tømmer afhænger derfor i høj grad af muligheden for at kunne udtrække brugbart DNA fra det tørre træ. Dette er vanskeligt, bl.a. fordi DNA i dødt væv nedbrydes igennem tid og pga., at træ indeholder indholdsstoffer, som hæmmer de kemiske processer bag de nødvendige DNA-teknikker. Det har vist sig muligt at ekstrahere korte DNA-stykker især fra mitokondrier og kloroplaster, som er organeller i cellen med egne genomer, der findes i mange kopier i hver celle. Desværre er DNA-markører fra kloroplast og/eller mitokondrie ikke så variable som markører der stammer fra kerne-DNA (såkaldte nukleare markører). Metoder udviklet med henblik på at undersøge fossilt materiale er muligvis brugbare til rutinemæssigt at kunne ekstrahere DNA fra tørt træ af en så høj kvalitet at også nukleare markører kan anvendes. Vores konklusion er indtil videre at det sandsynligvis vil være muligt at ekstrahere DNA fra tørt træ i mange, men ikke nødvendigvis alle, tilfælde. Der er derfor et afgørende behov for at undersøge fra hvilke typer træprodukter (og under hvilke betingelser) DNA kan ekstraheres og benyttes til relevante analyser.

2) Træets geografiske oprindelse

Kloroplast markører kan i visse tilfælde afsløre stærk geografisk struktur, hvilket kan bruges til at stedfæste oprindelsen af visse vedprøver til større regioner (f.eks. eksempler fra eg og spansk ceder). Hvis man ønsker at operere på en finere skala (på populations- eller individniveau) er der typisk ikke opløselighed nok i disse markører og man må i stedet anvende nukleare markører, som f.eks. nukleare *mikrosatellitter*. En mikrosatellit er et område på DNA-strengen, hvor en kort DNA-sekvens (motiv) er gentaget få til mange gange (feks. ATT ATT ATT...). Variationen ligger i antallet af gange motivet er gentaget. Inden for hver træart har de enkelte bestande af træer over tid udviklet sig til at blive forskellige fra hinanden i antallet af motiver. Ud fra analyser af sådanne mikrosatellitter kan man derfor få et mål for, hvor sandsynligt det er at en given træprøve stammer fra en given population. Testens sikkerhed afhænger af stikprøvestørrelse, tilgængelig variation, samt hvor store genetiske forskelle man finder mellem populationerne inden for en given art. For at kunne gennemføre en sådan test kræver det desuden, at man har en database over referencepopulationer, som man så kan sammenligne sin prøve med. Brugbarheden til kontrol af oprindelse må derfor afgøres baseret på en vurdering/pilotanalyse art for art.

At påvise fra hvilken plantage en given prøve kommer fra kan være meget vanskeligt, fordi plantager kan stamme fra frø med meget forskellige oprindelse. De enkelte træers DNA-profiler vil reflektere frøenes oprindelse frem for træernes nuværende voksested. Man kan imidlertid forstille sig at et DNA-baseret kontrolsystem til plantager kan effektueres, hvis der benyttes et veldefineret plantemateriale i en given plantage, og der foreligger et veldokumenteret kendskab hertil. Dette kan tænkes at blive en del af et fremtidigt certificeringssystem

Metoder, der ikke baserer sig på DNA, har været forsøgt anvendt på biologisk materiale med det formål at bestemme materialets geografiske oprindelse. For

træers vedkommende har man forsøgt sig med analyser af træets kemiske sammensætning samt isotopindhold ud fra den devise at planter optager sådanne elementer fra den omgivende jord og luft. Indholdet i træet skulle således være relateret til dets voksested. Ifølge den sparsomme, tilgængelige litteratur på området har disse metoder en usikkerhed idet den kemiske profil ser ud til at variere med typen af anvendt væv (hjerteved, kerneved, bark o.s.v.). Hvis prøvetagningen kan sikres således, at man altid anvender samme type væv, er det muligt at man med fordel kan kombinere en af disse metoder med en DNA-baseret metode i forbindelse med stedbestemmelse. For eksempel kunne man ved brug af kloroplast-markører fastsætte region og derefter anvende isotopanalyse til at præcisere hvor i regionen prøven kunne tænkes at komme fra.

Samlet konkluderer vi at DNA-analyser sandsynligvis vil kunne bidrage med vigtig viden i forhold til at stedfæste en given træprøves geografiske oprindelse. Dette må dog udvikles og afprøves på art til art basis, og for de fleste arter og geografiske oprindelser forventer vi der vil kunne blive tale om en sandsynliggørelse af en oprindelse snarere end et decideret bevis herfor.

3) Sporing af en stamme fra fældning til kunde
DNA-baserede metoder kan være særligt anvendelige til at undersøge om et stykke formodet illegalt fældet tømmer kan stamme fra en given stub i en given skov. Teknisk set vil det være relativt let at opbygge et system, hvor et givent firma skal indsamle små prøver fra alle de træer som fældes, og at disse prøver så kan bruges til at matche de stammer (eller savskårede emner) som efterfølgende kommer på det europæiske marked. Ved at bruge et passende højt antal nukleare mikrosatellitter, SNP[1] loci (en anden type markør) eller lign. vil man kunne konkludere med meget høj sandsynlighed om to prøver har samme DNA-profil, og dermed kan stamme fra samme træ. Man kan muligvis også benytte universelle metoder (f.eks. AFLP[2] m.fl.) til dette formål, men her vil risikoen for DNA-kontaminering (som nævnt ovenfor) være større.

Samlet set konkluderer vi, at brug af DNA-teknikker til en sådan type analyse er lovende, og at der – under forudsætning af et passende organisatorisk setup – vil kunne blive tale om analyser med egentlig beviskraft, som man f.eks. kender det fra human retsgenetik.

4) Hvilken art er der tale om – Genetiske stregkoder
Der foregår en del arbejde med udvikling af særlige DNA-markører til at kunne fortage en entydig artsbestemmelse ud fra en vævsprøve (såkaldt genetic barcoding). Idéen bag disse metoder er at analysere DNA-sekvenser på nogle særligt udvalgte, meget variable, dele af DNA'et på tværs af (på sigt et meget stort antal) arter. Arbejdet er generelt mere fremskredent på dyr end på planter, men der knytter sig en del forventning til at sådanne metoder vil kunne udvikles til et fornuftigt redskab til artsbestemmelse inden for visse grupper af dyr og planter i løbet af en (kortere) årrække.

I relation til afsløringen af ulovligt træ vil en sådan metode være interessant i forhold til at afsløre import af arter, som er omfattet af hugstforbud. Enten globalt, eller lokalt i det land de importeres fra. Samlet vurderer vi at

1 Single nucleotide polymorphism

2 Amplified length polymorphism

genetic barcording har et spændende potentiale i forhold til at kunne udvikles til et værdifuldt redskab til en relativ enkel og objektiv artsbestemmelse. Det samlede antal af træarter, som er omfattet af hugstforbud, er relativ begrænset, så det vil være en overkommelig opgave at få disse ind i en arts-reference database. Der udestår dog stadig noget forsknings- og udviklingsarbejde før et sådan system kan forventes at være på plads.

5) Om at komme i gang
Det vil være relativt omkostningskrævende at udvikle og afprøve de ovenstående DNA-teknikker på et bredt spektrum af arter og problemstillinger. For eksempel vurderer vi, at implementering af DNA-analyser til sporingen af oprindelsen af én træart kan forventes at beløbe sig til ca. 1 million DKK. Dette dækker udviklingen af markører, udarbejdelse af referencemateriale, indsamlingsarbejde, praktisk implementering samt lønomkostninger. Da der er mange arter, og flere interessante metoder at inddrage, kan der således samlet set blive tale om en stor opgave. Omvendt mener vi, at der her er tale om en opgave, hvor man på europæisk niveau kan drage stor fordel af et samarbejde. Import af ulovligt træ er et fælles europæisk problem og med DNA-baserede metoder kan der udvikles fleksible og reproducerbare redskaber, der i EU kan bidrage til at reducere importen af ulovlig høstet og/eller ikke-bæredygtigt produceret træ.

Abstract

The main objective of the present report is to explore the abilities and potentials of available DNA based techniques for tracing timber to origin and species. The report is based on a literature survey and benefits further from discussions at a newly held workshop on fingerprinting methods for timber identification. Practical experiences with implementation of appropriate techniques are also discussed as well as involved costs.

DNA markers have different features that make them more or less useful in terms of tracing wood. Some markers are universal, meaning that they amplify across species while others are species-specific, and can only be used for one species and perhaps its close relatives. Universal primers have the drawback of potential amplification of non-target DNA whereas specific markers need to be developed for each species. It is also important to differentiate between chloroplast, mitochondrial and nuclear DNA markers. Chloroplast and mitochondrial markers are, in broadleaved trees, maternally inherited (spread via the seed) and often show strong geographic structure but relative low overall variation. In contrast, nuclear markers are bi-parentally inherited (spread via pollen and seed) and often show less geographic structure but higher variation.

Extracting DNA from timber samples for genotyping purposes is difficult. The most valuable part of the trunk is the central part, the heartwood, which consists of dead cells with DNA that is partly degraded and occurs in low concentrations. The wood further contains compounds that inhibit the necessary PCR reactions. Despite these obstacles, several papers have been published showing that it is possible to extract and amplify smaller pieces of DNA from dry wood, including the heartwood. The success rate is generally higher for chloroplast and mitochondrial DNA where the genes are found in many copies (in contrast to the nucleus). By adding a PCR reagent that neutralizes PCR inhibitors it has further been possible to amplify DNA from wood that has been heat treated. This is important in relation to plywood and other wood products that go through high temperatures during the manufacturing process.

Tracing the geographic origin of wood samples requires a priory sampling and genotyping of reference populations throughout the distribution area of the target species. The ability of being able to track wood samples to geographic origin highly depends on sample size, number of polymorphic markers, and the genetical divergence of the reference populations. Even though it may be difficult to track down individuals to their population of origin based only on DNA marker information, the system is likely to be useful for controlling purposes. The objective is then to test if the stated origin may be false rather than to identify correct origin. DNA analysis may therefore be much more relevant if combined with a system where the importers are requested to state the origin of the timber, and the DNA markers then applied for validating this stated origin.

It may be interesting to combine DNA analysis with tests based on ratios between stable isotopes (SIRA analysis) of H, O and C in order to provide more

power in a test of declared origin. This aspect is therefore also discussed briefly in the report.

Highly polymorphic DNA markers can provide evidence in cases of suspected stolen timber logs. By comparing the genotypes of both logs and stumps, it will be possible to determine whether the presumed stolen timber originates from one of the investigated stumps with high confidence. Another interesting potential application of genetic fingerprinting is to develop a DNA register of sustainably logged trees from one or more logging concessions. Potentially, each log can be sampled and the genotypes entered into a database. Hereafter, any wood sample can be tested and compared with the database to confirm the origin. Hereby the timber can be traced 'from-logging-to-customer'.

Ongoing research seeks to define short regions of plant DNA sequences that effectively divide species and thus function as so-called 'DNA barcodes'. Such a system will be useful for discriminating among morphologically similar species of which some are illegal to export (e.g. CITES species). Easy identification at the species level may be an important tool for preventing illegal trade with the protected species. The barcoding technique may not only be relevant for CITES species, but could also prove relevant to prevent that high value species are falsely declared to be low value timbers and sold as such.

Development and implementation of a DNA based system will require an initial investment. We expect that the costs for developing an operational system for testing the origin of timber logs of one species would be approximately 1 million DKK, which includes sampling and travel expenses, laboratory work and salary. A DNA barcoding project for the correct identification of a given species is estimated to be less than 50,000 DKK. The costs for a single sample for either origin or species identification is believed to be around 500 DKK with the present techniques. This price is expected to be lowered in the future, especially if a fairly high number of samples are to be tested.

Overall, we conclude that no simple DNA technique is available to identify any species and origin of any timber imported to Denmark. However, we also conclude that the DNA based methods may prove very useful in many aspects of preventing trade with illegal timber. This is especially the case if DNA methods are applied to verify/falsify a species and/or origin declared by timber traders.

1. Introduction

The degradation of tropical forests with corresponding loss of global biodiversity and environmental services has called for concern for decades, and a number of international actions have been initiated in order to reduce this problem. Still, unsustainable management of forest resources continues. Decreasing forest area in large parts of the World (FAO, 2005; FAO, 2006) is a clear indicator of this unsustainable development. Besides loss of natural resources and environmental values, unsustainable and illegal logging practices are associated with a number of negative effects such as violation of indigenous peoples rights and public or private ownership, violation of local financial and tax regulation, and corruption of civil servants (Tacconi, 2007).

Degradation of the environmental, social and forest values is a global challenge. Timber is an international commodity, and wood consumers in one part of the world therefore influence the logging activities in other parts of the world. The potential role of international timber trade in driving unsustainable logging has lead to development of certification systems such as FSC[3], PEFC[4] and others. The certification system shall ensure the consumers that the purchased timber originates from sustainable managed forest. Forest area certified by one of the major certification schemes has increased from a few million ha in 1998 to more than 250 million ha in 2006. This process has probably to a large extent been driven by requirements of public procurement policies (Kraxner *et al.*, 2006), where the requirement of wood from certified suppliers is increasingly being specified.

The majority of the certified forest areas are located in North America and EU/EFTA where problems with deforestation and illegal logging are less compared to logging in the tropics. Trustworthy certification schemes that can be easily and effectively controlled are not easy to implement in large parts of the world. However, such systems are highly called for if the consumers are expected to pay a premium for certified wood. Also, public procurement policies and/or legislation can only be expected to contribute to increased sustainability if the origin of wood products can be controlled in a reliable way. It is on this background that the interest for using molecular methods for controlling the origin of internationally traded wood products arises.

3 Forest Stewardship Council

4 Programme for the Endorsement of Forest Certification

2. The DNA tool box for tracking timber: general considerations

Molecular DNA technology has been rapidly developing over the last decade. This has raised the hope that it will be possible to use molecular markers to trace the origin of imported timber to clarify a potential illegal origin and/or illegal logging practices. At present several kinds of DNA based techniques are available, however, with very different properties.

2.1 Reflecting genetic history or growth conditions?

Common for most DNA based techniques is that they focus on genetic markers expected to be *selectively neutral*. This refers to the explicit expectation that observed variations do not have an influence on the fitness of the individual carrying them; rather, the markers are expected to reveal patterns formed by the joint effects of migration, mutation and random genetic drift. Often the genetic patterns have been generated from bottlenecks during glaciations in which case the patterns may depend heavily on the location of refugia combined with routes and speed of subsequent re-colonisation (Lascoux *et al.*, 2004). These patterns are homogenised by gene flow either by seed or pollen. The markers can thus be expected to reveal patterns that correspond to areas carrying genetic pools of a given species that have 1) similar genetic history, and 2) are separated areas with limited gene flow between them. The selectively neutral markers will not reflect areas having ecologically similar conditions.

The issue of selective neutrality of the markers is important. For instance, timber originating from forests with similar growth conditions could cluster together, if analysed by markers that are not selective neutral. This can be the case even if the forests are located far apart, and this is obviously an undesirable feature in relation to tracing the origin of timber. However, for some species (mainly belonging to the genera *Pinus*, *Populus* and *Eucalyptus*) there is on-going research aiming at identifying DNA polymorphisms that *reflect adaptive clines*. If clear variation along clines (e.g. reflecting adaptation to day-length) is successfully identified, such polymorphisms *in combination* with neutral markers can become a powerful tool in the future.

2.2 Types of DNA markers and their suitability for tracing timber

Two categories of markers are accessible: DNA sequence markers and DNA fragment markers. DNA sequence markers are based on comparing the actual sequence of base pairs in a selected DNA region between individuals. DNA fragment markers are based on comparing the length of DNA fragments formed by either cutting the DNA by restriction enzymes, or by amplifying special so-called microsatellite sites on the DNA. *DNA sequence markers* are in general less variable than DNA fragment markers and are thus chosen when

the aim is to differentiate at species level or in large geographic regions. *DNA fragment markers* are used when a higher degree of resolution is needed as when discriminating at the population or individual level. The array of markers is elaborated more in section 5 below.

There are also significant differences between DNA markers regarding sensitivity to the quality of the wood samples on which the analyses are to be performed. Some methods require high-quality DNA whereas other methods tolerate DNA that is degraded to a certain point. Some types of DNA markers will therefore be more suitable for analysis of processed wood samples than others.

Also, some DNA techniques are universal, meaning that they can easily be applied to an array of different species (maybe in principle all tree species) whereas others must be specifically designed to each species in question.

Thus, the suitability of a given DNA marker highly depends on the question in mind and on the quality of the available material. In relation to the tracing of timber one should therefore consider three key questions related to the required specificity of the marker, the precision, and to the type of samples, respectively:

Specificity: How broadly must the DNA tool work: across species or within a single species?

Precision: What scale(s) to be targeted: geographic regions, specific population, or single trees?

Samples: What kind of plant material (wood) is available for DNA extraction: Fresh tissue from the cambium of the felled log, dry tissue from the cambium, sawn wood, oven dried sawn wood, plywood?

A common feature for basically all relevant DNA marker techniques is that they utilize the PCR-amplification technique (Polymerase Chain Reaction), where DNA fragments or longer gene sequences are multiplied in many copies and hereafter visualized by the use of gel-electrophoresis. The reaction is initiated by primers that are short DNA sequences (about 20 base pairs long), which work as starting point for the synthesis of the new DNA strands. This technique allows analyses of fairly low levels of DNA, which is important for their use for traceability studies.

3. Some important features of plants in relation to DNA based traceability

When using molecular markers for timber traceability purposes we apply the techniques to species from the plant kingdom. For several reasons this is more difficult than studying humans or animals. Also, some timber species will probably be significantly more difficult to trace than others. This is because species differ in many respects, some of which are important to take into consideration when rolling out the molecular technology. Below we will therefore highlight a few important genetic features.

First of all, the breeding system of plants varies from species that are almost totally self-pollinating (e.g. cheatgras (*Bromus tectorum*); Ramakrishnan *et al.*, 2004) to totally outcrossing species (e.g. Big-leaf Mahogony (*Swietenia macrophylla*); Loveless & Gullison, 2003). Species that are almost totally self-pollinating are only able to spread their genes via seed while species that outcross experience gene flow from both pollen and seed. Thus, we must expect that outcrossing species show less genetic structure than self-pollinating species. Many of the important timber species in the temperate and subtropical region are wind-pollinated species (e.g. conifers, oak, beech, ash), which mostly are outcrossing (Vogler & Kalisz, 2001). Hence, in many of the timber species we may not be able to detect high levels of genetic differentiation to discriminate among populations based on genes spread though pollen. This is of course important in relation to the *precision* aspect mentioned above.

3.1 Maternally, paternally or bi-parentally inherited DNA markers

Unique for all plants compared to animals is that they contain three genomes in their cells, (i) the nucleus, (ii) the mitochondrion and (iii) the chloroplast. The three genomes of plants are inherited differently. Offspring of flowering plants; to which the broadleaved trees belong, generally inherit their chloroplast and mitochondrion genomes from their mother (Corrieveau & Coleman, 1988). Therefore, these genomes are transmitted only via the seeds. There are some exceptions, where the chloroplast genome is paternally inherited. Conifers are different from broadleaved species in the sense that the chloroplast genome in general is paternally inherited through the pollen only, but mitochondrion is still inherited from the mother.

In contrast to the mitochondrion and chloroplast genomes, the nuclear genome is bi-parentally inherited, meaning that the offspring inherits one copy from the father and one copy from the mother. Hence, nuclear genes are dispersed through both pollen and seeds. The consequence of these differences is that the chloroplast and mitochondrial DNA variation is often more highly geographically structured than the variation found by the use of nuclear markers. This feature is therefore also important in relation to the required *precision*. Mitochondrial

(and/or chloroplast) DNA markers can therefore be considered if it is sufficient to know the seed origin of a given tree, which is often the case for timber tracing purposes.

The chloroplast and mitochondrion have only one set of genes (haploid) each as they are inherited uni-parentally. This means that (unlike nuclear genes) different genes do not recombine. Specific haplotypes are thus inherited unchanged through generations, and this can be valuable in some types of analyses as will be shown in section 5 below.

Another characteristic of the chloroplast and mitochondrion is that the DNA in these regions is found in many copies. Thus, if the DNA in the material of origin is more or less degraded—as likely to be the case with dry wood—the probability of PCR amplification success is likely to be higher for chloroplast and mitochondrial DNA than for nuclear markers. This is obviously very important in relation to the issue of available *samples*.

Table 1 summarizes some of the properties of the genetic markers from the three genomes.

Table 1. Important differences between mitochondrion, chloroplast and nuclear markers (for broadleaved trees)

Important properties	Mitochondrion	Chloroplast	Nucleus
Size (base pairs)	Small	Small	Large
Level of ploidy	Haploid	Haploid	Diploid-polyploid
Marker variability	Low	Low	High
Inheritance	Maternal	Maternal	Bi-parental
Geographic structure	High	High	Low
Gene copies	Many	Many	Two (to many)
Recombination	No	No	Yes

Another important feature of plants is that the nuclear genome of plants may have different levels of polyploidy (i.e. contain many sets of chromosomes). Diploid organisms (such as e.g. humans) always have two sets. Estimates on the proportion of polyploid species within the flowering plants is ranging from 30-35% (Stebbins, 1947) and up to 80% (Masterson, 1994). Polyploidy gives problems in interpreting some types of nuclear markers, and most standard software packages are not able to analyse polyploid data. Polyploidy can therefore be a severe complication, especially if a specific technique shall be used simultaneously for many different species of which some are polyploid and others diploid.

4. The challenge of getting DNA from wood samples

The first fundamental challenge for using genetic markers in timber identification (species or geographical origin) is to successfully retrieve DNA from dry processed wood. While DNA extraction from fresh material (typically leaves or buds) by now is a matter of routine in modern molecular laboratories, extracting DNA from dry wood is by far more complicated.

Wood contains compounds that inhibit the PCR technique (Lee & Cooper, 1995). In living trees, it should be possible to obtain suitable DNA from cambium (growth layer underneath the bark) and perhaps also the sapwood, which constitutes both living and dead cells (Deguilloux *et al.*, 2002). The heartwood (central part of the trunk) is less valuable as DNA-source as the cells here are dead and substantial chemical alterations often have taken place during the heartwood formation. Still, the heartwood is the most valuable products, and processed wood pieces of high value timbers may consist of only heartwood by the time it reaches the European marked.

Fortunately, extraction of DNA from even heartwood may be possible when applying appropriate protocols. Deguilloux *et al.* (2002) tested nuclear, chloroplast and mitochondrial markers on DNA extracted from different regions of 10 oak (*Quercus petraea*) logs. Though the DNA in general was degraded, it was successfully amplified from all parts of the wood (cambium; outer and inner sapwood; transition wood; outer, central and inner heartwood) except for the bark. Even DNA from the dead heartwood could be amplified though with a lower success rate compared to amplifications conducted on DNA from outer parts of the trunk. Thus, it seems that though the heartwood is dead, short DNA fragments may become adsorbed on the cell walls as suggested by Cano (1996).

Deguilloux *et al.* (2002) further found that short sequences had higher amplification success than long sequences as well as sequences found in numerous copies compared with single copy genes (e.g. chloroplast DNA markers versus nuclear markers). Another important result from this paper is that DNA availability in dry wood is highly reflected by the conservation of the felled trees. DNA isolated from trunks kept outside for half a year amplified with a lower success rate than DNA isolated from a trunk kept indoors for three years. It seems that the DNA is better preserved when the tissue is quickly dried (as when kept indoors) as also seen in fossil materials (Cano, 1996).

Three years after the work by Deguilloux *et al.* (2002) was published, a new method was presented by Asif & Cannon (2005). They explored the efficiency of DNA technology for identifying an endangered timber species (*Gonystylus bancanus*). Two established extraction methods (CTAB and DNAesy Plant Mini Kit, Qiagen) were compared with a new method based on *N*-phenacylithiazolium bromide (PTB). PTB extraction has previously been used for isolating DNA from ancient bone in paleobiological studies as for instance from Neanderthal human remains (Krings *et al.*, 1997). The method has now been modified to

DNA extraction from plant tissue. Dry wood samples resulted in DNA extractions of both high quality and quantity. Also, the PCR amplifications were successful (from one mitochondrial and two chloroplast regions) and produced long fragments indicating that long strands of DNA were still intact in the dry wood.

Asif & Cannon (2005) further tried to extract DNA from a 50 year old wooden desk, which however did not succeed. Although the methodology introduced by Asif & Cannon (2005) seems promising, a test of whether the quality of the DNA is appropriate for amplification of nuclear regions is missing.

Rachmayanti *et al.* (2006) extracted DNA from wood of Dipterocarpaceae, an important timber family in Southeast Asia. DNA was also extracted from leaf material and the genotypes from the corresponding wood and leaf DNA from each individual was compared. The genotypes were identical, thus indicating that wood is a reliable DNA-source.

A recent paper of Yoshida *et al.* (2007) tested whether sequences from the three genomes (chloroplast, mitochondrion and nucleus) could be amplified with PCR on DNA extractions from wood from ten timber species. In most of the cases PCR amplification was possible for all genes even the nuclear one. The addition of a reagent that should neutralize PCR inhibitors released from for instance heat-treated wood improved the outcome so much that even DNA-samples that did not amplify now resulted in distinct PCR products (*Quercus crispula, Juglans mandshurica*, and samples from the outer parts of the trunk of *Salix udensis* and *Gingko biloba*) (only tested for the chloroplast gene). Another aspect the paper examines is how heat treatment of wood affects the ability to extract DNA. This knowledge is important as raw wood is treated at high temperatures in the manufacturing process of wood products such as plywood and laminated wood. Yoshida *et al.* (2007) tested this question on heat treated sapwood of *Larix gmelinii var japonica* and *Quercus cripula*. In both species the DNA became degraded at temperatures of 140°C and above. PCR was however reproducible in *L. gmelinii var japonica* when heated up to 160°C. The addition of the PCR reagent that neutralizes PCR inhibitors again improved the PCR reactions such that amplification succeeded in both species at all tested temperatures (tested up to 180°C).

The main and very important conclusion is that it is possible to retrieve DNA from dry wood for further DNA analysis, also even after treatment at high temperatures. In fact, it has been possible to obtain DNA from 1000 year old *Fagus* (beech) wood (Liepelt *et al.*, 2006).

5. Geographic patterns and precision in identifying site of origin

As mentioned previously, chloroplast DNA (cpDNA) data has an advantage when the aim is to differentiate among different origins of broadleaved species.

A clear example of geographically structured chloroplast DNA variation is seen in the European oaks. In 2002, the results from a cooperation of 16 laboratories throughout all Europe were presented in a joint paper on the chloroplast DNA variation of European oak species (Petit *et al.*, 2002). The data showed a strong phylogeographic structure characterized by a differentiation of eastern versus western populations. Related haplotypes had approximately the same geographic distribution. Deguillloux *et al.* (2004) tested whether this strong geographic structure may be usable for oak wood traceability purposes in the context of the cooperage industry. According to Deguilloux *et al.* (2004) the need for oak wood for barrel production in France is growing (15% per year), which has forced the French coopers to import oak wood from the United States and Eastern Europe. This has lead to an open discussion on whether the origin of the wood has an effect on the wine. The diversification of oak wood origins in France has led to a need for clear provenance identification. Based on this knowledge, the goal of Deguilloux *et al.*'s (2004) paper was to test traceability on different types of wood samples that could be used for provenance control in the French cooperage industry. These samples were barrel staves that were either less than a year old, two years old, or staves collected from finished barrels (meaning that they had been toasted). By the use of chloroplast DNA markers it was tested whether the haplotypes of these samples were French of origin and whether they were in agreement with the provenances announced by the coopers. In a few cases the samples did not correspond to the proposed provenance. Some of these samples had haplotypes that either had never been detected in France or only very rarely. The haplotypes were instead widespread in Eastern-Central Europe to Russia. The results thus strongly indicated the existence of woods from Eastern Europe sold as French wood. Deguilloux *et al.* (2004) conclude from this that the barrel industry could avoid mislabelled wood by the use of DNA analysis.

Though it seems possible to trace oak wood to provenance regions by the use of chloroplast DNA markers, the resolution of these markers is not high enough for separating between smaller regions e.g. within France. A similar picture is seen in populations of Spanish cedar (*Cedrela odorata*) studied by Cavers *et al.* (2003). They studied the phylogeography of Spanish cedar throughout Mesoamerica and found a total of five haplotypes in a total of 29 populations (each of approximately 20 individuals). Almost all populations were fixed for one single haplotype. In Mexico, all populations were fixed for the same haplotype (denoted Haplotype 2 in Cavers *et al.*, 2003). All populations from Honduras and Nicaragua were fixed for another haplotype (Haplotype 3). Thus, in this case even though there is strong geographic structure, the resolution is not even high enough to assign individuals to a specific Mesoamerican country. It should also be mentioned that even though it is possible to assign individuals

of oaks down to geographic origins, it is not possible to distinguish between the two species *Quercus robur* and *Q. petraea* by the use of the very same markers as the species hybridize (Petit *et al.*, 2002). Thus, only in some cases are chloroplast DNA markers a potential tool for tracing purposes.

Nuclear markers typically possess higher resolution than markers constrained to the chloroplast DNA. An example hereof is microsatellite markers. Microsatellites are regions of the DNA sequence where nucleotides are tandemly repeated several or many times (e.g. ATT ATT ATT ...). The polymorphism is found in the number of times the sequence is repeated. Unfortunately, these markers are often quite species-specific meaning that a single set of microsatellite markers cannot be used as a general tool for testing multiple species. Instead, a specific set of microsatellite primers must be developed for each target species in question, which is time-consuming and expensive.

Although microsatellite markers often show prominent variation, the resolution may in some cases not be adequate for individual-based population assignment (Campbell *et al.*, 2003). A potential alternative to microsatellites could then be AFLP's (amplified fragment length polymorphism's). These are DNA fingerprints composed of many bands that are visualized by electrophoresis. AFLP´s are dominant markers and polymorphisms are seen as presence/absence of particular fragments. Many markers are relatively easily generated by the use of the AFLP technique. In contrast to microsatellite markers, the same set of primers used for AFLP studies can be applied to many plant species. This, however, can potentially cause problems. As the primers used for genotyping individuals of any focal species are universal, there is a probability of amplifying contaminant DNA as well. This may especially become critical in cases of low-quality and quantity DNA extractions. In this sense the microsatellite markers seem more robust.

To test if the genotype of a specific individual (tree) is likely to originate from a given population (forest), a so-called assignment test can be applied. This can be done based on different assignment criteria. A simple approach is based on the allele frequency distributions of the candidate populations (Paetkau *et al.*, 1995). For a set of reference populations, the likelihood of a genotype belonging to a given population is calculated based on the genotype frequencies in the specific populations, and the individual is then assigned to the one population where its genotype has the highest likelihood to occur from. There are other assignment approaches (cf. Piry *et al.*, 2004), but it is a common prerequisite for all these assignment approaches that a fairly comprehensive set of reference populations must have been sampled and analysed.

Until present, most assignment tests have relied on the use of nuclear microsatellite loci. The resolution obtained with these markers is often high due to a high number of alleles at each microsatellite locus, but it can still be difficult to assign a specific individual genotype to a given population with significant statistical power. This is of course especially the case if population differentiation is weak. The level of differentiation is given by the F_{ST} value, which is the proportion of total genetic variation attributable to the genetic differences

among populations. Limited statistical power in situations of weak differentiation between putative source populations (e.g. $F_{ST} < 0.05$) reduces the ability to back trace the population membership of a set of individuals. Examples of F_{ST} values in commercially valuable tree species are: Black walnut (*Juglans nigra*), $F_{ST} = 0.017$ (Victory *et al.*, 2006), Common ash (*Fraxinus excelsior*) $F_{ST} = 0.076$ (Heuertz *et al.*, 2004) and Bigleaf mahogany (*Swietenia macrophylla*) $F_{ST} = 0.097$ (Lemes *et al.*, 2003). These three studies are all based on microsatellite markers.

5.1 Identification of origin versus exclusion of origin

Improvement of assignment methods in order to enhance actual resolution in population assignment studies may lead to better results. However, even the present assignment techniques may prove valuable because the question in relation to control of certification often is 'did the tree actually come from the announced geographic site?' rather than the more general question 'where did the tree come from?' With a sufficient sampling of reference populations (including the claimed origin), it may be possible to obtain significant power in a test of excluding a given origin—especially if the test involved a number of trees that are certified to originate from the same site. In the latter case, the challenge is to examine if a number of wood samples (trees) with an observed genotypic distribution, are likely to originate from a specified reference population with a pre-established genotypic distribution.

Heinze *et al.* (2006) distributed four seed lots from three Austrian origins of *Fraxius excelsior* (two seed lots came from the same site) to six different laboratories in Europe. The labs were asked to compare the seed lots with a set of microsatellites, and based on their results to identify which of the two seed lots that had the same origin. Even though the F_{ST} was low, all labs easily identified the two similar seed lots (Heinze *et al.*, 2006).

The precision of the assignment test will depend on the possible sample size, the number of loci, number of alleles per locus, and the $F_{ST.}$ However, the present experience suggests that it is likely that a comprehensive set of nuclear microsatellite markers can provide an effective tool in testing origins of timbers logged in natural forests. It will require that the genetic structure of a set of reference populations are determined *a priori*, and the power will be much higher if the origin of a number of logs (rather than a single log) is tested. The geographical scale at which test of origin is possible will depend on the differentiation level of the populations. The expected precision given different combinations of these factors (sample size, resolution level and F_{ST}) can be studied by fairly simple simulation experiments. We have not done so in the present report, but it is valuable in order to identify threshold values for which a testing system is likely to work with sufficient precision. Also, pilot studies of selected timber species would shed more light on the efficiency and practical limitations (if any) involved.

5.2 What about plantations?

Planted trees obviously present a challenge in the present context, because their genetic origin is determined by the origin of the seed, not the plantation site. Seed of many commercially important plantation species are—and have been—moved substantially around the world. This is very pronounced for teak (*Tectona grandis*) that is planted extensively outside its natural range (e.g. Kjær & Siegismund, 1996).

In many tree plantations, the plantings from different years may be composed of material from dissimilar origins, because seed can be procured from many different locations. Seed availability often varies from year to year, and there is often limited focus on choosing the most appropriate genetic origin (Kjær *et al.*, 2005). A single certified plantation may thus be based on a variety of mixed origins.

Planted forests composed of a mixed genetic background will be very difficult to track by the use of DNA markers with a standard set of reference populations. However, if a suitable number of trees at the plantation site are sampled, logs from the plantation appearing on the international marked can in principle still be compared to logs from the plantation. However, that means that the scope goes from geographic regions to specific plantation estates or co-operatives. Also, it requires that the plantations have a suitable and well documented seed procurement system as this is a prerequisite for selecting appropriate samples.

In general, it must be concluded that planted forests provide an extra challenge in terms of tracking timber from forest to costumer by the use of DNA markers. However, if the plantation is sufficiently large to justify a specific sampling, and the plantation management has a well documented seed procurement system, we expect it should be possible to implement a DNA based control system. This would be easier for plantations that base there plantings on genetically improved planting material, because this material in general will be genetically more well defined than 'random seed sources'.

6. Fingerprinting: Tracking a single log from forest to customer

Microsatellite markers are valuable when the aim is to test whether two DNA samples come from the same individual (that is whether they are identical). This approach is relevant if one wishes to determine whether specific wood has been illegally harvested from particular forests by using a similar approach as seen in human forensic genetics. Attempts have in fact been made for the purpose of matching DNA profiles from illegally harvested tree stumps to suspected stolen wood (White *et al.*, 2000). According to White *et al.* (2000) theft of timber is a major problem in forests of British Columbia, Canada. The thieves often steel the most valuable trees, and the tree theft activities are judged to cost the Canadian province as much as $20 million per year. One of the most treasured trees is Red cedar (*Thuja plicata*). White *et al.* (2000) tried to develop microsatellite markers from DNA extracted from wood of Red cedar; however only one usable microsatellite locus was obtained. Nevertheless, this can be used for illustrative purposes. The sequences among individuals differed by multiples of $(CT)_2$. After surveying 102 trees a total of 16 alleles were found. With 16 alleles, their frequencies are relatively low. Therefore, the probability that one individual harbours two specific alleles is proportional to the product of their frequencies in the population (bearing in mind that Red cedar is diploid, each individual carrying two alleles at a given locus). It is clear that when many markers are analyzed the probability of a perfect match between two randomly chosen genotypes will be very low. So-called exclusion probabilities of $P > 0.999$ can thus be obtained when working with a number of highly variable loci (e.g. 5-10 suitable microsatellite loci). Thus, if the genotype of suspected illegally cut wood matches DNA profiles from the remaining stump it is strong evidence for stolen wood.

A similar approach could be applied to particularly treasured timber species as a routine validation technique. By the use of microsatellite markers it is possible to test if the wood at the saw mill corresponds to the stumps in the certified forest as proposed by the foresters. A drawback is, however, that microsatellite markers would need to be developed for each species in question.

AFLP markers can be used for the same purpose. They are not species-specific, and can therefore be applied to a broad set of species. Also, a few AFLP primers in general provide a large number of markers, which result in high exclusion probabilities. However, AFLP markers can be sensitive to DNA contamination (as described above), in which case microsatellites are to be preferred.

In general it must be concluded that DNA markers are highly suitable for tracking a given timber log from stump to consumer. The techniques can be very precise, and the only serious obstacle would be if DNA has become too degraded in the processed wood.

7. Systems that target many species simultaneously

As mentioned earlier, we operate with molecular markers with primers that are either species-specific or universal, meaning that they easily amplify across species. The major concern when using universal primers for wood traceability purposes is the risk for contamination. Due to difficulties when extracting DNA from dry wood the DNA concentration may be relatively low. This increases the possibility for alien DNA to be amplified together with the target DNA at perhaps equal proportions. We may thus reach false results. If we use universal markers that amplify chloroplast DNA rather than nuclear DNA, the risk for amplifying contaminant DNA is not as critical simply because there are many copies of the organisms DNA in the chloroplast genome.

Below in Table 2 we list the primer requirements for the most common fragment markers

Table 2. Primer requirements

Fragment marker	Specific or Universal primers
PCR-RFLP (PCR-restriction fragment length polymorphism)	Specific
RAPD (random amplification of polymorphic DNA)	Universal
AFLP (amplified fragment length polymorphism)	Universal
SSCP (single strand conformation polymorphism)	Specific
Microsatellites (SSR)	Specific
SNP (single nucleotide polymorphism)	Specific

In many cases, however, it is possible to use primers that have been developed for one particular species on other closely related species (e.g. microsatellites in species of elm, Whiteley *et al.*, 2003, Collada *et al.*, 2004). In such cases the priming sites are intact despite species differentiation. DNA sequence data is gathered by the use of universal primers.

It is outside the scope of this report to go through all the available techniques from the above list. We have chosen to concentrate on those methods that have been related to timber traceability purposes. If further knowledge is wished on the various markers and their individual properties we can refer to Vignal *et al.* (2002), or Vendramin & Hansen (2005).

7.1 Species identification

When it comes to using DNA markers to identify morphologically similar species, which e.g. is relevant in order to prevent illegal trade with CITES species, it is obvious that a universal type of marker is required. This is often referred to by molecular taxonomists as 'DNA barcoding', and this technique is likely to become very important in the coming years.

DNA barcoding is a technique developed to identify organisms by the use of short DNA sequences (Kress *et al.*, 2005). If it is possible to define a short region of a DNA sequence, that consistently divides species, the sequence functions as a species identifier; a DNA barcode (Hebert *et al.*, 2003). Thus, the ideal goal is to establish a practical, standardized tool to identify specimens to species-level. A Consortium for the Barcode of Life has been established (*www. barcoding.si.edu*) to promote the technique as a standard for species identification. A DNA barcode database has been initiated to which one can compare unidentified specimens. For the identification of animal species a region of a mitochondrial gene, known as cytochrome c oxidase I (COI), has been used (Hebert *et al.*, 2004a; b). This mitochondrial sequence seems to become the standard barcode region for higher animals. Among the plants, the COI sequence is not appropriate for distinguishing species simply because of the slow evolutionary rate of plant mitochondrial DNA (Kress *et al.*, 2005; Chase *et al.*, 2007). Most of the variation is likely to be found in the nuclear genome, where loci such as ITS (part of a so-called ribosomal internal transcribe-spacer) has proven useful in many studies on phylogenetic relationships among species. However, because of difficulties in producing universal primers and problems, due to polyploidy and partial duplications of the genome, the plastid genomes (chloroplast and mitochondrion) are in general favoured for barcoding purposes. Several suggestions have been made on suitable genomic areas for barcoding land plants (Chase *et al.*, 2005; 2007; Kress *et al.*, 2005; Newmaster *et al.*, 2006). In all cases sequences from more than one region are suggested as no single plastid sequence possesses enough variation. Even when using regions of three genes there are many genera that do not possess enough variability to separate closely related species. For example in *Hordeum* and *Crocus* only approximately 50% of the species can be separated by using three regions (Gitte Petersen, pers. comm.). Another problem is the fact that the chloroplast is only inherited from one parent (in most species from the mother). This means that first generation hybrids will group together with their mother. Thus, you cannot distinguish between hybrids and the maternal species by the use of the proposed plant barcoding genes. Polyploid species may in some cases also cause problems.

Although the barcoding technique is yet under development (at least when talking about plants) and not as straight forward as desirable, it may become relevant to detect whether or not specific logs of wood belong to a CITES regulated species. To fulfill this, one would need first to sequence several individuals of the target illegal species to establish the level of intra-specific variation. Then also individuals of more or less related species must be sequenced to determine the level of inter-specific variation. These sequences function as the reference material to which the putative illegally harvested timber is compared. At the Royal Botanic Gardens, Kew, they are in fact involved in a project that seeks to detect certain timber species associated with CITES (*Aquilaria* and *Cedrela* species, Mark Chase pers. comm.). For this purpose they use markers from portions of three plastid genes (*rpoC1*, *rpoB*, and *matK*). The outcome of this project is interesting and should be followed.

8. Methods that do not rely on DNA

Plants tend to incorporate chemical elements such as stable isotopes and inorganic elements from the soil and the air. These elements are hereafter measurable in, for instance, the wood and seem to reflect environmental conditions from the area where the plant originated from. A number of methods, that are not DNA based, have been applied to various biological samples to determine their geographic origin from such organic or inorganic elements (e.g. molecular spectroscopy on extra virgin olive oils, Tapp *et al.*, 2003; analysis of stable isotopes and elemental profiles on strawberries, blueberries and pears, Perez *et al.*, 2006; analysis of elemental profiles in wood, Durand *et al.*, 1999; analysis of isotope ratios in wood, English *et al.*, 2001). Stable isotope ratio analysis (SIRA) of so-called bio-elements (H, C, N, O, S) reflect different conditions (H and O: regional water situation, C: climatic factors, N and S: local geological site) (Boner & Bliznakow, 2007; Förstel, 2007). Also the stable isotope ratio of strontium is a potential source for separating wood due to differences in soil and geological conditions.

Below, we briefly touch upon some of these approaches although it is outside the main scope of this report, and certainly outside our expertise.

A first example illustrates the use of inorganic elements for tracing the origin of wood used for the building of prehistoric great houses in New Mexico around the 11th century. Durand *et al.* (1999) tested 29 elements on ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*) from three bedrock types to reveal a potential bedrock specific chemical profile. If a chemical signature appeared prehistoric samples could then be matched to the surrounding bedrock profiles. The two species differed significantly from each other in mean concentrations of four elements (Ba, K, Mg, Zn) independent of locality and tissue type (sapwood or heartwood). When different stands within the same bedrock were compared, only few elements differed significantly. Most of the differences were found when different bedrocks were compared (12 elements out of 29 tested). The authors thus conclude that most of the variation found in the element data is caused by the substrate (i.e. bedrock differences). Although the results appear promising, there are also serious drawbacks especially when considering wood material. First of all the results seem to be tissue dependent. The pith of the stem reveals elemental profiles that differ from the profiles obtained from sapwood and bark (Hoffmann *et al.*, 1994; Durand, 1999). It is also difficult to determine at which geographical scale the method is usable as it depends on the composition of the underground. It seems important that the focal area is geologically diverse.

Thus, a priori knowledge about the geology seems necessary to distinguish between different bedrock types.

A second example also examines prehistoric wood from New Mexico. English *et al.* (2001) compared $^{87}Sr/^{86}Sr$ ratios from beams from ruins of great houses to ratios in trees growing in the surrounding mountains. They concentrated on species of spruce (*Picea*) and fir (*Abies*). They found differences in live wood from the three explored mountain ranges (0.7143 ± 0.0001, 0.7095 ± 0.0001,

0.7078±0.0001). There was no significant difference in strontium ratio between species at two of the mountain ranges. Regarding origin of wood, the strontium ratios of great house timber revealed that the wood originated from two of the three mountain ranges. However, several of the wood samples had strontium ratios that were just in between the range of the two mountain ranges (around 0.709) why it is not possible to determine from which of the two mountain ranges these have origin. At one of the mountain ranges tree samples had been taken from sites with different substrates (granite, limestone, and sandstone). Even so, the isotopic ratios varied only slightly. Based on this, the authors suggest that most of the strontium incorporated in the trees is from atmospheric dust sources. They further suggest based on the data that strontium ratios in the air vary on geographical-scales closer to tens of kilometres rather than hundreds.

A third example combines stable isotope analysis with analyses of inorganic elements to determine the geographic origin of wood species (*Rubroshorea* spp.) from Southeast Asia (Kagawa *et al.*, 2007). Kagawa *et al.* (2007) examined material from the Philippines and Borneo (Sabah, Sarawak and Brunei) by the use of isotope ratios of three bio-elements and eight inorganic elements. They found significant correlations between the stable isotopes and the longitude/latitude of the wood habitat and could, based on this data, separate the Philippines from Borneo. They were however not able to distinguish between wood from different localities in Borneo (Sabah, Sarawak and Brunei). The inorganic elements showed minor differences that seemed to reflect habitat conditions rather than geographical locations of the habitat.

Although the methodology is interesting—stable isotope analysis in particular—there are some limitations that should be taken into account. First, the technique is not suitable for species identification. Second, within-tree variation may reduce the accuracy of the results. Third, it is uncertain what happens when the geographical scale is increased. Though only one match appears at close range between a potential source area and a given wood sample several matches may be possible when increasing the distance. Also, areas with homogeneous climate situation may show no regional resolution.

Chemical markers may, however, have an advantage over DNA markers in terms of testing the presumed origin from a certain plantation. Where the DNA markers will reveal the genetic origin of the seed rather than plantation site the chemical profile is a reflection of conditions at the site of growth.

A different approach is to study the microbial fauna and flora associated with the logs. A microbial profile can be very specific to the geographic origin, and DNA profiles of the microbial fauna and flora may therefore provide important information on the origin of the timber. The technique is being tested for fish (cf. CIRAD, 2005) and may also be interesting for testing timber origin. A limitation of this technique is probably that the logs are exposed to many microbes during transport, shipment and processing, and that the wood processing includes heating and drying, which also may influence the microbial profile substantially.

9. Ideas on how DNA markers may be applied in practice

As mentioned above, highly polymorphic DNA markers will be effective for fingerprinting a specific tree. Thereby, it will be possible *to track logs from the stump to the saw mill,* and—if the DNA is not too degraded—to the consumer. The fingerprinting systems will probably be most stable if based on markers with species-specific primers as microsatellites or SNP markers. AFLP markers may, however, also be useful. AFLP primers generate a high number of bands but as the primers are universal there is a risk of amplifying contaminant DNA. Thus, the technology requires a high degree of carefulness and high quality DNA, which may be difficult to obtain when the target material is dry, processed wood.

Tracing of timber from forest to consumer can provide evidence in cases of *stolen timber* logs. If both logs and stumps are genotyped and compared, it will be possible to determine with high degree of precision, if a given log originates from one of the investigated stumps.

Another potential application of genetic fingerprinting is the *development of a DNA register of sustainable logged trees* from one or more logging concessions. In principle, a small tissue sample can be collected and genotyped for each log, and the DNA profile entered into a database. Then, any wood suitable for DNA extraction can be tested and compared with the database to test the origin. Such a system will of course involve costs for DNA extraction and genotyping, and therefore seems especially relevant in cases where valuable timber is harvested under conditions where traditional control is difficult. However, as DNA analysis becomes increasingly automatic, the costs of a sample may be minor compared to the price of the valuable log. Such a DNA based control system could be based on sampling by the independent agency at the logging site. Subsequent sample based tests of the wood products could hereafter be compared with the results gathered by the independent agency.

A quite different application also discussed above, is to test *the geographic origin* by comparing wood samples with a reference of potential populations. The precision of this approach will differ between species according to distribution and reproductive systems, and also depends on the number of involved markers. In most cases, it will probably be difficult to identify the correct origin down to population level, but the system may prove valuable for controlling purposes as long as some between-population variation exists. This is because in practice the analysis should actually not identify the correct origin, but rather test if the stated origin may be false. As long as a substantial possibility for such an identification of 'false origin' exists, the methods will be valuable from a verification point of view. We recommend that this idea is investigated further through initiation of a pilot study that—combined with theoretical developments and simulation experiments—will shed light on the practicalities and especially the information power. This approach should most likely be based

on microsatellites or SNP markers, nuclear or from one of the haploid genomes. However, a combination of such neutral markers with one or more markers under selection may prove very efficient within a few years (as discussed above). Although premature today, it is reasonable to expect a rapid development in the DNA tools over the coming few years that may provide such options.

We also conclude from the above that 'Barcoding' may become a very useful tool for identification of specific species (CITES associated species or others). Much work is done in this field at present, and it will therefore be possible to benefit from this in relation to identifying illegal trade with CITES species. Further, the methodology is not only relevant for CITES species. It can also be applied to reveal if high value species are falsely declared and sold as low value timbers.

Unfortunately, at the present stage we do not see an easy way for developing a simple DNA tool that can be used to identify the specific origin of all timber imported to Denmark. Also, we do not see an operational method for using highly degraded DNA. For example, at present it does not seem feasible to extract and analyse DNA from an old chair or likewise. Of course, this may change in the future.

10. Costs required for the implementation of a DNA based system

The costs for development of an operational system based on the above discussed DNA technologies will differ substantially depending on species (availability, size of distribution area, prior genetic knowledge). As an example, the costs of two potential projects are estimated below. The two examples both relate to bigleaf mahogany (*Swietenia macrophylla*) as target species. Bigleaf mahogany is grouped together with two other species (*Swietenia humilis* and *S. mahagoni*) in the genus *Swietenia* and family Meliaceae (Mahogany family). They are all highly treasured hard wood species native to the Caribbean, Central and South America. All three species are regulated under CITES. Bigleaf mahogany is, however, only associated with CITES in the Neotropics, meaning that plantations in Asia are not under CITES regulation.

Example 1: Detecting the origin of timber logs of *Swietenia macrophylla*

For this purpose it is needed to apply markers with high resolution, such as microsatellites or SNPs. Microsatellite markers are already available for this particular species (Lemes *et al.*, 2002), thus saving the costs of having species-specific microsatellite primers developed. Otherwise an extra cost of minimum 10,000 dollars would have been needed (e.g. at SREL DNA Lab, the University of Georgia, USA). The development of a reference library can benefit from existing data, but more reference populations will probably be required. We expect that a sampling of at least 50 populations scattered around in the distribution area of *S. macrophylla* will be required. From each population 30 trees need to be sampled.

Directly involved costs will include sampling, travel expenses, DNA extractions, PCR amplifications (genotyping the sampled trees at 10 available polymorphic microsatellite loci), visualisation, data analysis, salary, other lab costs, likely to be around 1 million DKK. In case existing data for development of the reference database can be utilised, these costs will be reduced substantially.

However, such a project should include cooperation with partners and stakeholders—including a component of technology transfer—in the respective countries. Additional costs may therefore arise from the need for coordination and cooperation.

Once the system has been established the costs of testing whether the presumed origin of one piece of wood is correct would be relatively low—around 500 DKK. The answer could be available after 1-2 working days. Collecting the wood sample could be done by a customs officer following simple instructions and hereafter sent to the laboratory for DNA genotyping.

Example 2: DNA barcoding to support correct identification of *Swietenia macrophylla*

The approach here will be both to sequence individuals of the focal species and individuals of its related species at specific potential barcoding regions to gain information on intra-specific variation relative to inter-specific variation. From a study on the molecular phylogeny of Meliaceae it appears that *Swietenia* group together with other genera in what is recognized as the subfamily Swietenidioideae (Muellner *et al.*, 2003). The closest relatives to *Swietenia* are found in the genera *Khaya* and *Carapa*. We would choose to sequence individuals from not only *S. macrophylla* but also the other species in the genus *Swietenia*. Along with these also individuals from species of *Khaya* and *Carapa* should be sequenced. In addition, we would select other more distant genera in the subfamily, like *Cedrela* and *Toona*. From each chosen species at least four individuals should be chosen to reveal the level of intra-specific variation. In summary the starting point would be to sequence four individuals from for instance 12 species of the subfamily Swietenidioideae. Depending on the variability 1-3 gene regions would be sequenced. We hereby have the reference material to which it is possible to compare sequences of log pieces of potentially CITES regulated species that do not have the relevant papers in order (given that the genes possess enough variation).

Fairly few samples are required, and the DNA samples can most likely be obtained from herbarium material saving cost and time for sampling and morphological identification of the samples. The cost will therefore be substantially lower compared to »Example 1» above; probably less than 50,000 DKK.

When first the system is available the costs of species identification of a single individual would be low, perhaps a few hundred DKK and the result would be available within two working days. As mentioned under »Example 1» collecting the wood sample could be done by a customs officer following simple guidelines and hereafter sent to the laboratory for DNA sequencing.

11. What has been implemented so far?

The problem of illegal logging is a topic of global concern, and work on the application of DNA markers and chemical markers for timber tracking is in progress in a number of countries.

In Japan, a recent project entitled »Development of improved methods to identify *Shorea* species wood and its origin« has been initiated as a joint corporation among the Forestry and Forest Products Research Institute (FFPRI, Tsukuba), the Forest Research Centre, Sabah (FRC) and the Forest Research Institute Malaysia (FRIM). In contrast to other ongoing projects effort has here been put into different disciplines; molecular methods and chemical methods hereunder chromatography and the use of chemical markers such as stable isotopes and inorganic elements. Once a set of identification tools has been established the future perspective is to use the technologies for monitoring and controlling specific trade regulations (Fujii, 2007).

Newspaper articles dated March–April 2007 report that a timber verification company, Certisource, located in Singapore has, together with the University of Singapore, developed a DNA based technique for verifying the legality of Merbau (*Intsia palembanica*). They match samples from a legally allocated concession area to the presumed corresponding wood at the saw mill. For verifying legality the WWF Keep it Legal standards is utilized (Andrew Young, pers. comm.). If they can achieve suitable resolution their longer term perspective is to use the data together with samples taken at other localities to generate a reference database for biogeographic localization. According to the director (Andrew Young) the technology is about to be patented meaning that no further details are available at present.

In Canada, the British Columbian Ministry of Forests (together with the Canadian Biotech Strategy Genomics Fund) put money into the development of microsatellite markers for Red cedar (*Thuja plicata*) for the purpose of tracking illegally harvested timber as mentioned previously. According to an article published in »The Vancouver Province« in august 2006, British Columbia has developed a DNA databank of red and yellow cedars. The reporter interviews a compliance and enforcement officer with the B.C. Ministry of Forests named Jerry Hunter. Jerry Hunter explains that they are waiting for the final approval that confirms the statistical accuracy of matching tree DNA so that the evidence can be used in court. It is also mentioned that if the method proves beneficial the next tree in line is likely to be the Western broadleaf maple (*Acer macrophyllum*). Despite intensive search we have not been able to establish contact to Jerry Hunter, or to Dr. White or to any of her coauthors from White *et al.* (2000) to confirm the progress of their initial work. We do agree, however, that microsatellite markers could prove valuable in detecting illegally cut wood with the approach presented by White *et al.* (2000).

In the U.K., the Royal Botanic Gardens, Kew, is—as already mentioned—involved in a project that seeks to detect certain timber species associated with CITES by the use of the DNA barcoding technique. By employing distinct regions of three plastid genes they aim on being able to detect two or three species from the genera *Aquilaria* and *Cedrela* (Mark Chase, pers. comm.) from Southeastern Asia and subtropical-tropical America, respectively.

12. Recommendations from the workshop entitled »Fingerprinting methods for the identification of timber origins«

The workshop »Fingerprinting methods for the identification of timber origins« organised by the German Federal Ministry of Food, Agriculture and Consumer Protection and the World Wide Fund for Nature, was held in Bonn, October 8–9 2007. The workshop brought together a number of scientists and other professionals working with the issue of timber traceability. The discussions at the workshop on applicability of the DNA and chemical technologies for timber identification led to some general conclusions listed below:

1. Knowledge and technology available for timber fingerprinting are quite advanced with standards available and ready for practical uses; applicability for different types of processed wood needs to be consolidated.
2. Different groups worldwide are working in the field, partly government or NGO-driven. Further networking and platforms for discussions are needed.
3. Further standardization and harmonization is needed to strengthen the effectiveness and increases synergies.
4. Partly cross-fertilisation of work in related areas takes place, e.g. taxonomy, i.e. barcoding, or food analysis, i.e. standard technologies for isotope analyses.
5. The two disciplines, isotope- and DNA-analysis, have been mainly progressing in parallel, both having strengths and weaknesses; scope for synergies and complementary applications has been preliminarily discussed.
6. Need for decision-makers to prioritise future work, i.e. species, regions, legal frameworks.
7. Exclusion scenario should be the guiding principle for future development (instead of direct determination of origin).
8. Development of methods should be targeted for acceptance before court.
9. Potential applications: customs, companies, certification bodies, further stakeholders.

References

Asif MJ & Cannon CH. 2005.
DNA extraction from processed wood: a case study for the identification of an endangered timber species (*Gonystylus bancanus*). *Plant Molecular Biology Reporter* 23, 185-192.

Boner M & Bliznakow P. 2007.
Construction of databases: experience from the food sector / Timber database in cooperation with WWF. Abstract. Workshop on »Fingerprinting Methods for the Identification of Timber Origins«, Bonn.

Campbell D, Duchesne P & Bernatchez L. 2003.
AFLP utility for population assignment studies: analytical investigation and empirical comparison with microsatellites. *Molecular Ecology* 12, 1979-1991.

Cano RJ. 1996.
Analysing ancient DNA. *Endeavour* 20, 162-167.

Cavers S, Navarro C & Lowe AJ. 2003.
Chloroplast DNA phylogeography reveals colonization history of a Neotropical tree, *Cedrela odorata* L., in Mesoamerica. *Molecular Ecology* 12, 1451-1460.

Chase MW, Cowan RS, Hollingsworth P et al. 2007.
A proposal for a standardized protocol to barcode all land plants. *Taxon* 56, 295-299.

Chase MW, Salamin N, Wilkinson M, Dunwell JM, Kesanakurthi RP, Haider N & Savolainen V. 2005.
Land plants and DNA barcodes: short-term and long-term goals. *Philosophical Transactions of the Royal Society B Biological Sciences* 360, 1889-1895.

CIRAD. 2005:
Valorisation, Innovation et Partenariat 2005/2, p. 2. CIRAD, Montpellier, France

Collada C, Fuentes-Utrilla P, Gil L & Cervera MT. 2004.
Characterization of microsatellite loci in *Ulmus minor* Miller and cross-amplification in *U. glabra* Hudson and *U. laevis* Pall. *Molecular Ecology Notes* 4, 731-732.

Corriveau JL & Coleman AW. 1988.
Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *American Journal of Botany* 75, 1443-1458.

Deguilloux MF, Pemonge MH & Petit RJ. 2002.
Novel perspective in wood certification and forensics: dry wood as a source of DNA. *Proceedings of the Royal Society of London Series B Biological Science* 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. *Annals of Forest Science* 61, 97-104.

Durand SR, Shelley PH, Antweiler RC & Taylor HE. 1999.
Trees, chemistry, and prehistory in the American Southwest. *Journal of Archaeological Science* 26, 185-203.

English NB, Betancourt JL, Dean JS & Quade J. 2001.
Strontium isotopes reveal distant sources of architectural timber in Chaco canyon, New Mexico. *Proceedings of the National Academy of Sciences, USA* 98, 11891-11896.

FAO. 2005.

State of the Worlds Forests 2005. FAO, Rome. ISBN 92-5-105187-9.

FAO. 2006.

Global Forest Resources Assessment 2005—Progress Towards Sustainable Forest Management. *FAO Forestry Paper* 147. FAO, Rome. ISBN 92-5-105481-9.

Förstel H. 2007.

Stable isotope history and applications to trace back the authenticity of agricultural products. Abstract. Workshop on »Fingerprinting Methods for the Identification of Timber Origins«, Bonn.

Fuji T. 2007.

Outline of the research project »Methods to identify wood species and the origin of timber of Southeast Asia«. *Proceedings of the International Symposium on Development of Improved Methods to Identify* Shorea *Species Wood and its Origin*, 19.

Hebert PDN, Cywinska A, Ball SL & Dewaard JR. 2003.

Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B Biological Science* 270, 313-321.

Hebert PDN, Penton EH, Burns JM, Janzen DH & Hallwachs W. 2004a.

Ten species in one: DNA barcoding reveals cryptic species in the Neotropical Skipper Butterfly *Astraptes fulgerator. Proceedings of the National Academy of Sciences, USA* 101, 14812-14817.

Hebert, PDN Stoeckle MY, Zemlak TS & Francis CM. 2004b.

Identification of birds through DNA barcodes. *PLoS Biology* 2, 1657-1663.

Heinze B, Arthofer W & Fussi B. 2006.

A ring test for identity and distinctness in forest tree seedlots. In: *Population Genetics and Genomics of Forest Trees: From Gene Function to Evolutionary Dynamics and Conservation*. Joint IUFRO/COST conference. Alcalá de Henares, Madrid, Spain.

Heuertz M, Hausman J-F, Hardy OJ, Vendramin GG, Frascaria-Lacoste N & Vekemans X. 2004.

Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southern European populations of the common ash (*Fraxinus excelsior* L). *Evolution* 58, 976–988.

Hoffmann E, Lüdke C, Scholze H & Stephanowitz H. 1994.

Analytical investigations of tree rings by laser ablation ICP-MS. *Fresenius' Journal of Analytical Chemistry* 350, 253-259.

Kagawa A, Kuroda K, Abe H, Fuji T & Itoh Y. 2007.

Stable isotopes and inorganic elements as potential indicators of geographic origin of Southeast Asian timber. *Proceedings of theInternational Symposium on Development of Improved Methods to Identify* Shorea *Species Wood and its Origin*, 39-44.

Kjær, ED & Siegismund H. 1996.

Allozyme diversity of two Tanzanian and two Nicaraguan landraces of Teak (*Tectona grandis* L.f.). *Forest Genetics* 3, 45-52.

Kjær, ED, Hansen CP, Roulund H & Graudal L. 2005.

Procurement of plant material of good genetic quality. In: *Restoration of Boreal and Temperate forests*. Standturf JA & Madsen P (eds). CRC Press, USA, pp 139-152. ISBN 1-56670-635-1.

Kraxner, F, Hansen E & Owari T. 2006.

Public procurement policies driving certification: certified forest products markets, 2005-2006. In: *Forest Products Annual Marked Review 2005-2006*. In: *Geneva*

Timber and Forest Study Paper 21, 97-110. United Nations, New York & Geneva. ISBN 92-1-116945-3

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA & Janzen DH. 2005.
Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences* 102, 8369-8374.

Krings M, Stone A, Schmitz RW, Krainitzki H, Stoneking M & Paabo Sl. 1997.
Neanderthal DNA sequences and the origin of modern humans. *Cell* 90, 19-30.

Lascoux, M,Palmé AE, CheddadiR & Latta RG. 2004.
Impact of Ice ages on genetic structure of trees and shrubs. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* 359, 197-207

Lee AB & Cooper TA. 1995.
Improved direct PCR screen for bacterial colonies: wooden toothpicks inhibit PCR amplification. *Biotechniques* 18, 225–226.

Lemes MR, Brondani RPV & Grattapaglia D. 2002.
Multiplexed systems of microsatellite markers for genetic analysis of mahogany, *Swietenia macrophylla* King (Meliaceae), a threatened Neotropical timber species. *The Journal of Heredity* 93, 287-291.

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. *Molecular Ecology* 12, 2875–2883.

Liepelt S, Sperisen C, Deguilloux MF et al. 2006.
Authenticated DNA from ancient wood remains. *Annals of Botany* 98, 1107-1111.

Loveless MD & Gullison RE. 2003.
Genetic variation in natural mahogany populations in Bolivia. In: *Big-Leaf Mahogany—Genetics, Ecology and Management.* Lugo AE, Colón JCF, Mildred A (eds). Springer, New York, pp 9-28. ISBN 9780-3879-883-75

Masterson J. 1994.
Stomatal size in fossil plants: evidence for polyploidy in majority of Angiosperms. *Science* 264, 421-424.

Muellner AN, Samuel R, Johnson SA, Cheek M, Pennington TD & Chase MW. 2003.
Molecular phylogenetics of Meliaceae based on nuclear and plastid DNA sequences. *American Journal of Botany* 90, 471–480.

Newmaster SG, Fazekas AJ & Ragupathy S. 2006.
DNA barcoding in land plants: evaluation of rbcL in a multigene tiered approach. *Canadian Journal of Botany* 84, 335-341.

Paetkau D, Calvert W, Stirling I & Strobeck C. 1995.
Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4, 347-354.

Perez AL, Smith BW & Anderson KA. 2006.
Stable isotyope and trace element profiling combined with classification models to differentiate geographic growing origin for three fruits: effects of subregion and variety. *Journal of Agricultural and Food Chemistry* 54, 4506-4516.

Petit RJ, Csaikl UM, Bordacs S et al. 2002.
Chloroplast DNA variation in European white oaks. Phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management* 156, 5-26.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L & Estoup A. 2004.
GENECLASS2: A software for genetic assignment and first-generation migrant detection. *The Journal of Heredity* 95, 536-539

Rachmayanti Y, Leinemann L, Gailing O & Finkeldey R. 2006.
Extraction, amplification and characterization of wood DNA from Dipterocarpaceae. *Plant Molecular Biology Reporter* 24, 45-55.

Ramakrishnan AP, Meyer SE, Waters J, Stevens, MR, Coleman CE & Fairbanks DJ. 2004.
Correlation between molecular markers and adaptively significant genetic variation in *Bromus tectorum* (Poaceae), an inbreeding annual grass. *American Journal of Botany* 91, 797-803.

Stebbins GL. 1947.
Types of polyploids: Their classification and significance. *Advances in Genetics* 1, 403-429.

Tacconi, L. 2007:
The problem of illegal logging. In: *Illegal Logging: Law Enforcement, Livelihoods and Timber Trade.* Tacconi L (ed) Earthscan, London, pp 1-16. ISBN 1-84407-348-3.

Tapp HS, Deferes M & Kemsley EK. 2003.
FTIR spectroscopy and multivariate analysis can distinguish the geographic origin of extra virgin olive oils. *Journal of Agricultural and Food Chemistry* 51, 6110-6115.

Vendramin GG & Hansen OK. 2005.
Molecular markers for characterizing diversity in forest trees. In: *Conservation and Management of Forest Genetic Resources in Europe.* Geburek T & Turok J (eds.), Arbora Publishers, Zvolen, pp 337-368. ISBN 80-967088-1-3.

Victory ER, Glaubitz JC, Rhodes Jr OE & Woeste KE. 2006.
Genetic homogeneity in *Juglans nigra* (Juglandaceae) at nuclear microsatellites. *American Journal of Botany* 93, 118-126.

Vignal A, Milan D, SanCristobal M & Eggen A. 2002.
A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution* 34, 275-305.

Vogler DW & Kalisz S. 2001.
Sex among the flowers: the distribution of plant mating systems. *Evolution* 55, 202-204.

White E, Hunter J, Dubetz C, Brost R, Bratton A, Edes S & Sahota R. 2000.
Microsatellite markers for individual tree genotyping: application in forest crime prosecutions. *Journal of Chemical Technology and Biotechnology* 75, 923-926.

Whiteley RE, Black-Samuelsen S & Clapham D. 2003.
Development of microsatellite markers for the European white elm (*Ulmus laevis* Pall.) and cross-species amplification within genus *Ulmus*. *Molecular Ecology Notes* 3, 598-600.

Yoshida K, Kagawa A & Nishiguchi M. 2007.
Extraction and detection of DNA from wood for species identification. *Proceedings of the International Symposium on Development of Improved Methods to Identify* Shorea *Species Wood and its Origin,* 27-34.