DoubleHELIX
TRACKING TECHNOLOGIES

Practical experience

DNA applications for wood

THE UNIVERSITY OF ADELAIDE
AUSTRALIA

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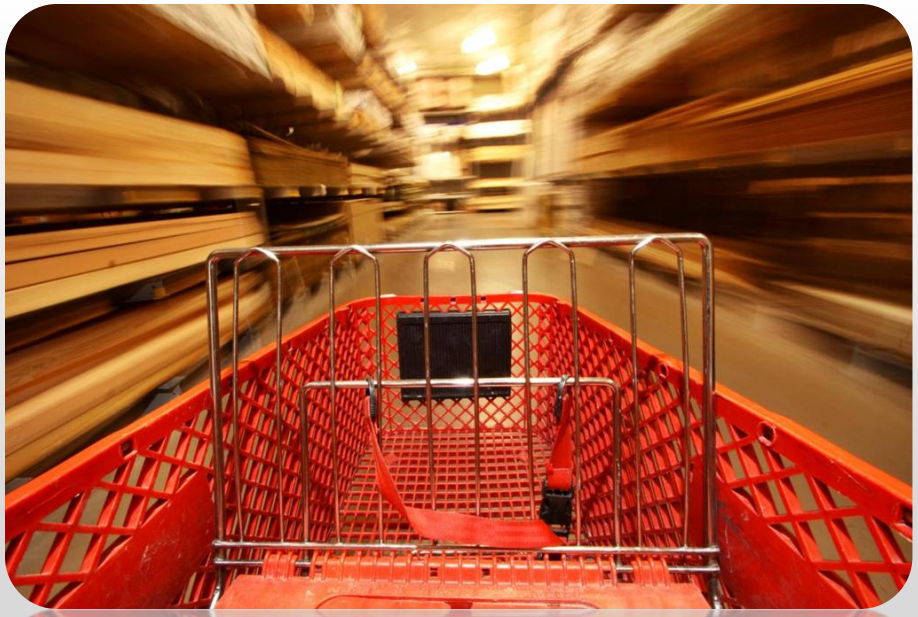
First to commercialise
DNA testing of timber

- Exclude illegal timber from global supply chains by **verifying trade documentation**
- **Reduce** costs of legal compliance
- **Minimise** cost of monitoring and enforcement



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Range of levels of DNA differentiation

- Individual log tracking
  - Verify integrity of supply chain
- Concession origin
  - Verify sustainable source
- Regional origin
  - Verify country source
- Species origin
  - Verify species

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- DNA Fingerprinting
- Population genetics
- Phylogeography
- DNA barcoding

Application to date

Timber Tracking with Certisource

With funding support from the
International Tropical Timber Organisation

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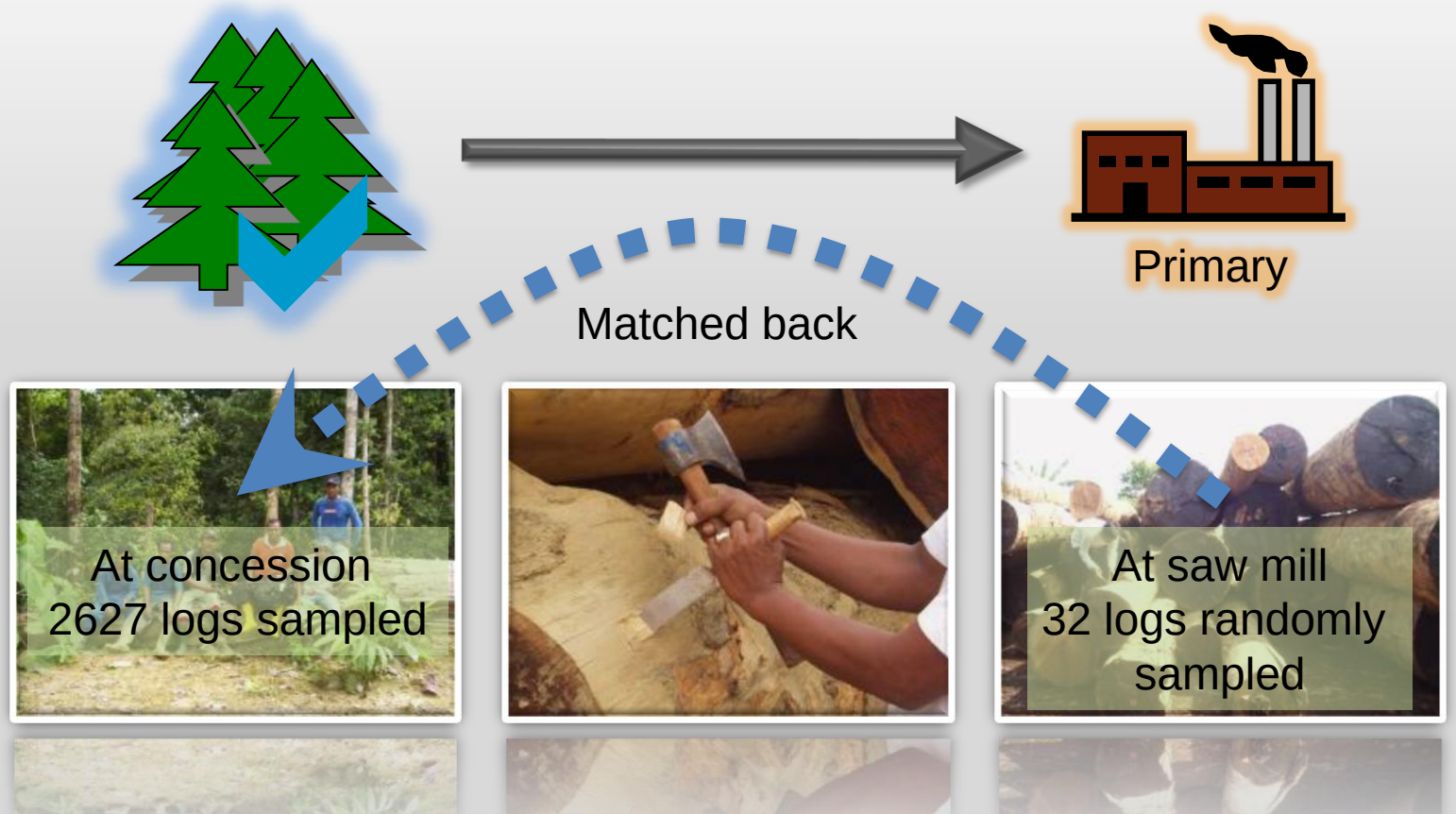

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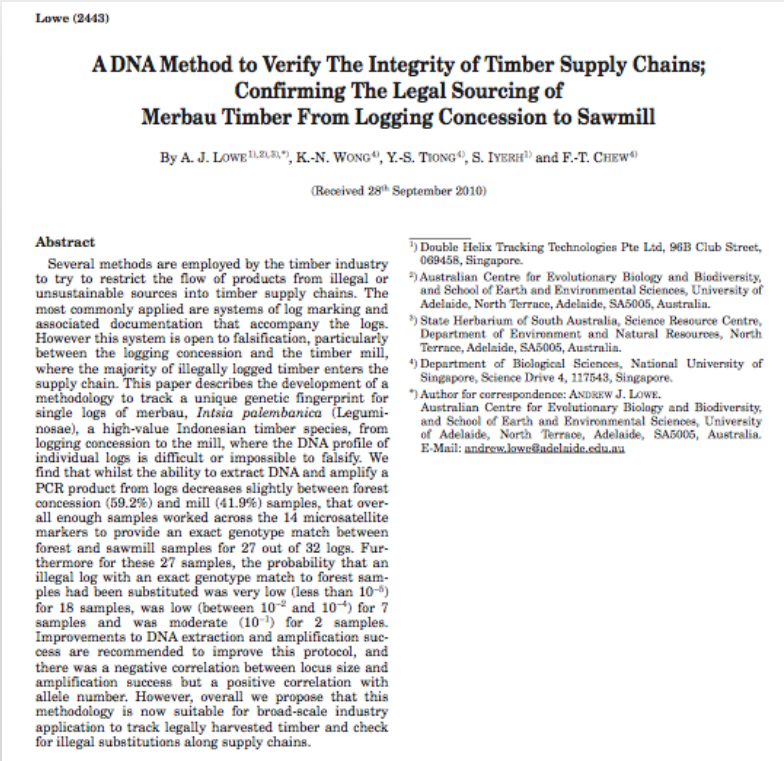
Timber Tracking
ITTO project results

Forest and sawmill samples profiled with 14 microsatellites

Example	Test 1	Test2
Forest sample	236, 238	240,248
Sawmill sample	236, 238	238,246

	No. loci	match?	Substitution?
Sample 1	6	exact	1 in 50 million



Lowe (2443)

A DNA Method to Verify The Integrity of Timber Supply Chains; Confirming The Legal Sourcing of Merbau Timber From Logging Concession to Sawmill

By A. J. Lowe[1),2),3),*)], K.-N. Wong[4)], Y.-S. Tiong[4)], S. Iyerh[1)] and F.-T. Chew[4)]

(Received 28th September 2010)

Abstract

Several methods are employed by the timber industry to try to restrict the flow of products from illegal or unsustainable sources into timber supply chains. The most commonly applied are systems of log marking and associated documentation that accompany the logs. However this system is open to falsification, particularly between the logging concession and the timber mill, where the majority of illegally logged timber enters the supply chain. This paper describes the development of a methodology to track a unique genetic fingerprint for single logs of merbau, *Intsia palembanica* (Leguminosae), a high-value Indonesian timber species, from logging concession to the mill, where the DNA profile of individual logs is difficult or impossible to falsify. We find that whilst the ability to extract DNA and amplify a PCR product from logs decreases slightly between forest concession (59.2%) and mill (41.9%) samples, that overall enough samples worked across the 14 microsatellite markers to provide an exact genotype match between forest and sawmill samples for 27 out of 32 logs. Furthermore for these 27 samples, the probability that an illegal log with an exact genotype match to forest samples had been substituted was very low (less than 10^{-6}) for 18 samples, was low (between 10^{-2} and 10^{-4}) for 7 samples and was moderate (10^{-1}) for 2 samples. Improvements to DNA extraction and amplification success are recommended to improve this protocol, and there was a negative correlation between locus size and amplification success but a positive correlation with allele number. However, overall we propose that this methodology is now suitable for broad-scale industry application to track legally harvested timber and check for illegal substitutions along supply chains.

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4) Department of Biological Sciences, National University of Singapore, Science Drive 4, 117543, Singapore.
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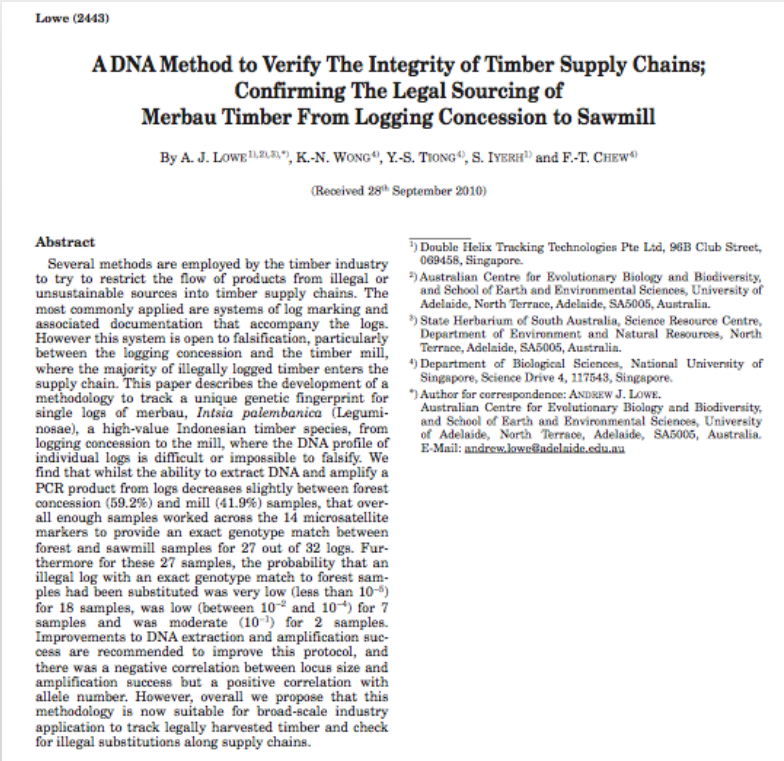
Of 32 samples, 27 exact match, 5 did not amplify
Probability of substitution very low



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Timber Tracking
Next steps

- Integrate with other certification systems
- Evaluate extent of cost savings through DNA timber tracking

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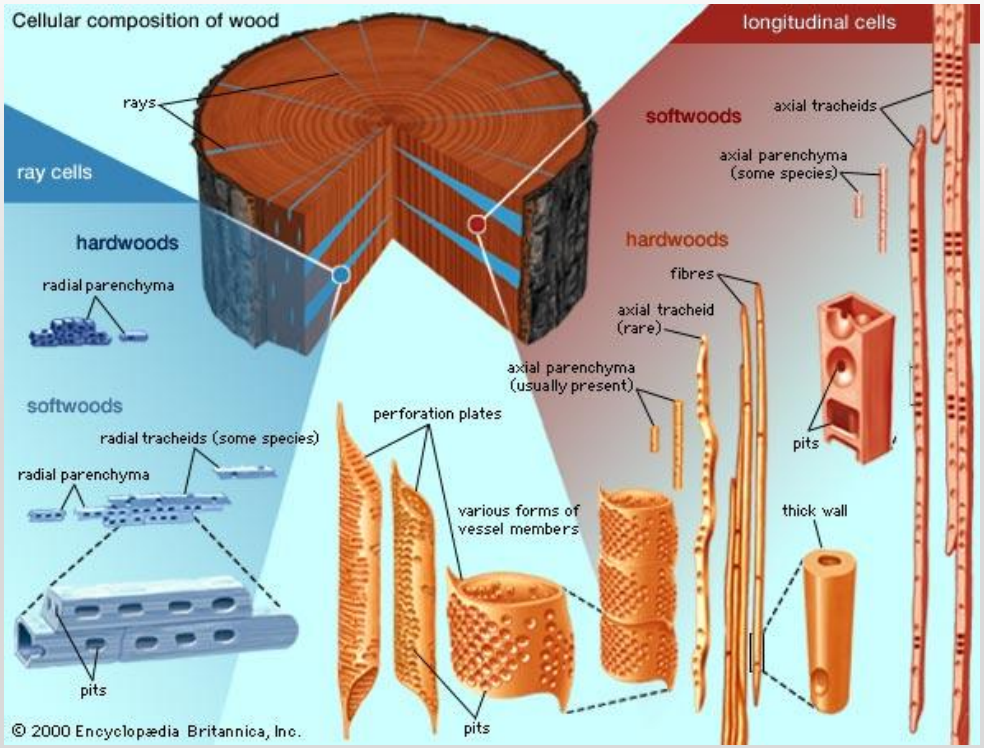
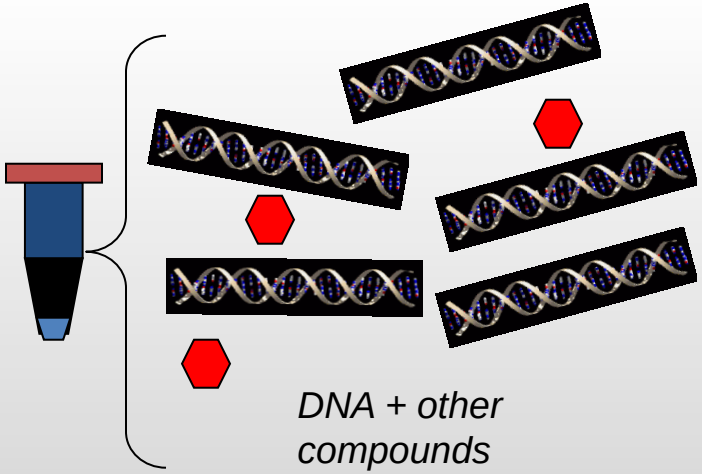
Timber Origin
A spot-check system

- DNA extraction from wood at different points of processing – extend tracking

Wood contains many secondary compounds that may affect success of DNA extraction





Including: cellulose, lignin, hemicellulose, resins, waxes, trace elements

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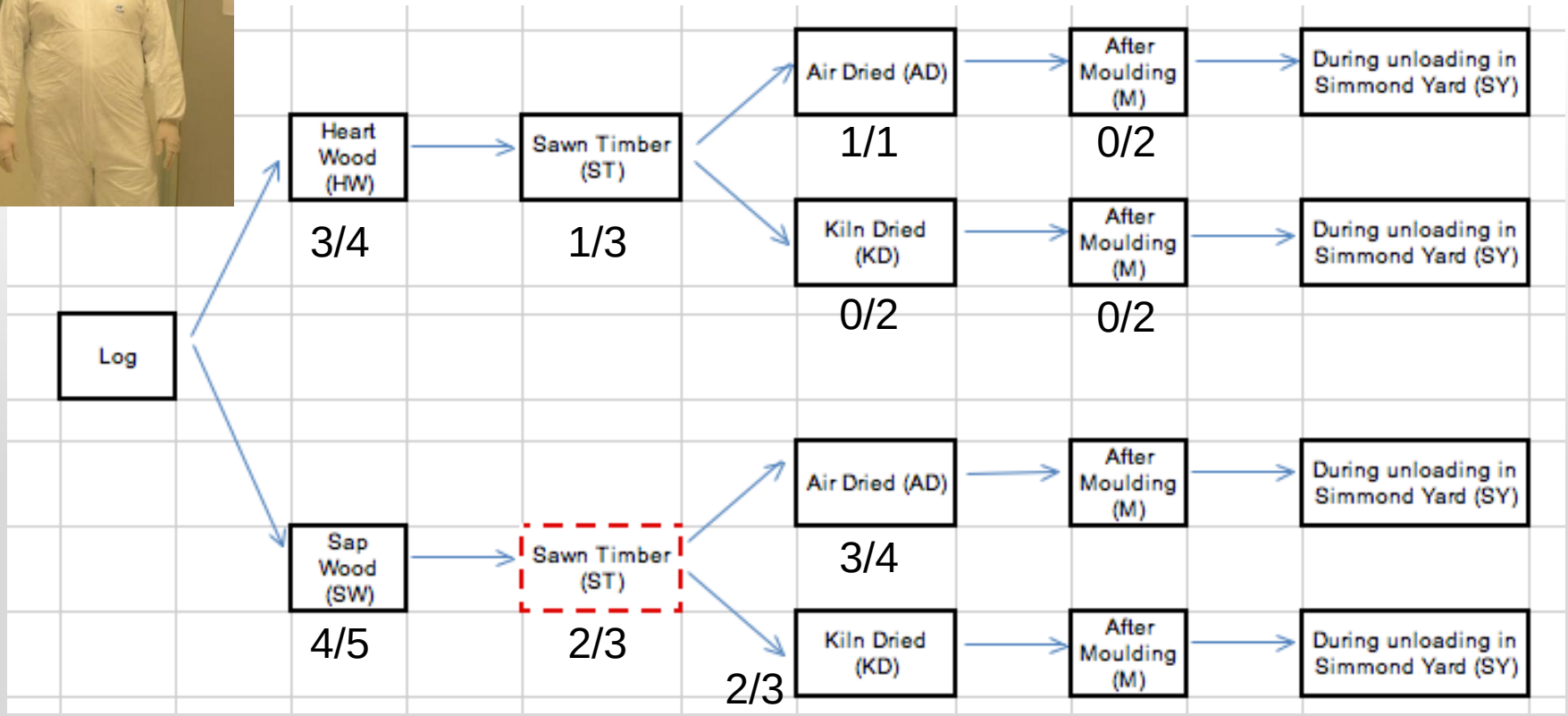
Success by treatment

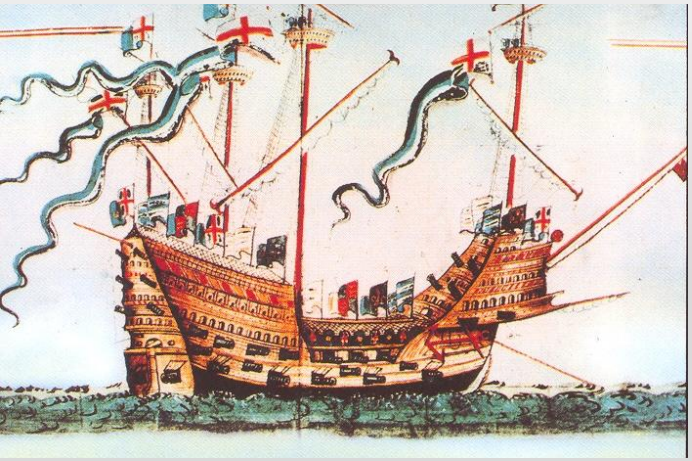


Number below treatment: # successful PCR / # extracted

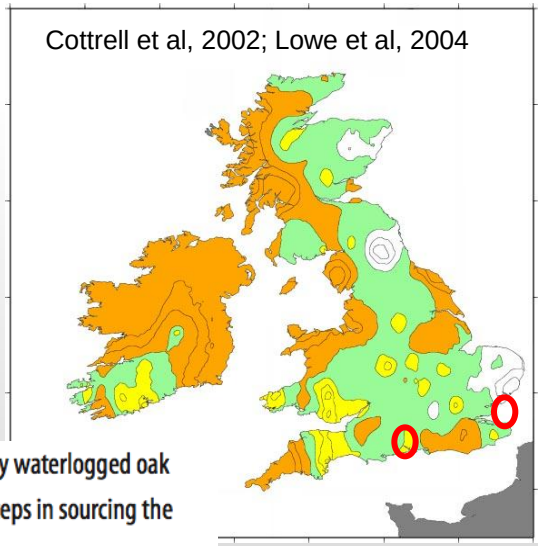
Several DNA extraction methods systematically tested (BoTAB)

Progressing source of origin -DNA extraction

- Extraction of DNA from wood
  - Temperate species – well developed
  - Tropical species – developing



Cottrell et al, 2002; Lowe et al, 2004



Chloroplast DNA from 16th century waterlogged oak in a marine environment: initial steps in sourcing the Mary Rose timbers

Alanna K. Speirs[1], Glenn McConnachie[2] and Andrew J. Lowe[3,4]

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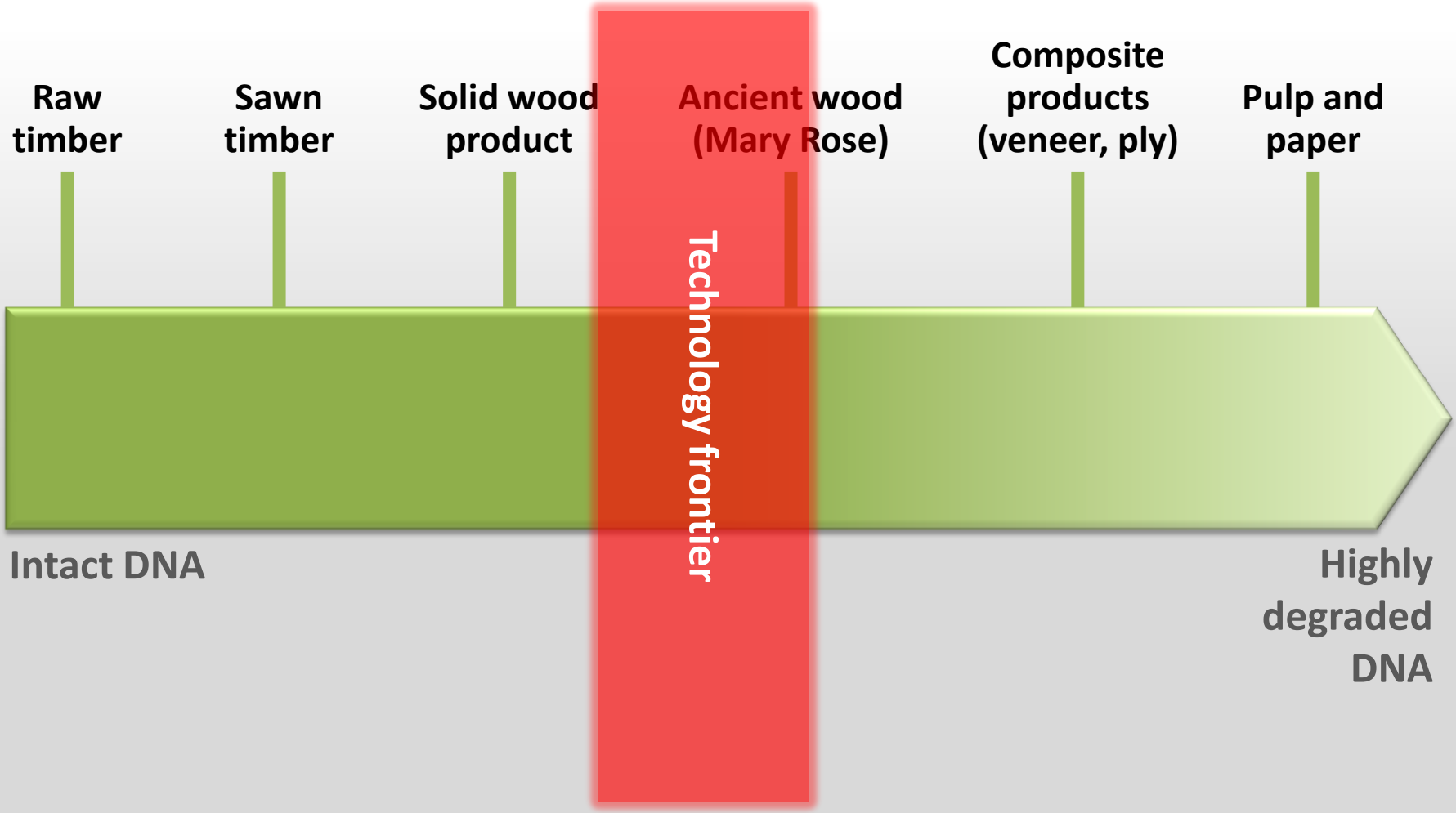
University of Adelaide
Adelaide SA 5000 Australia

ABSTRACT

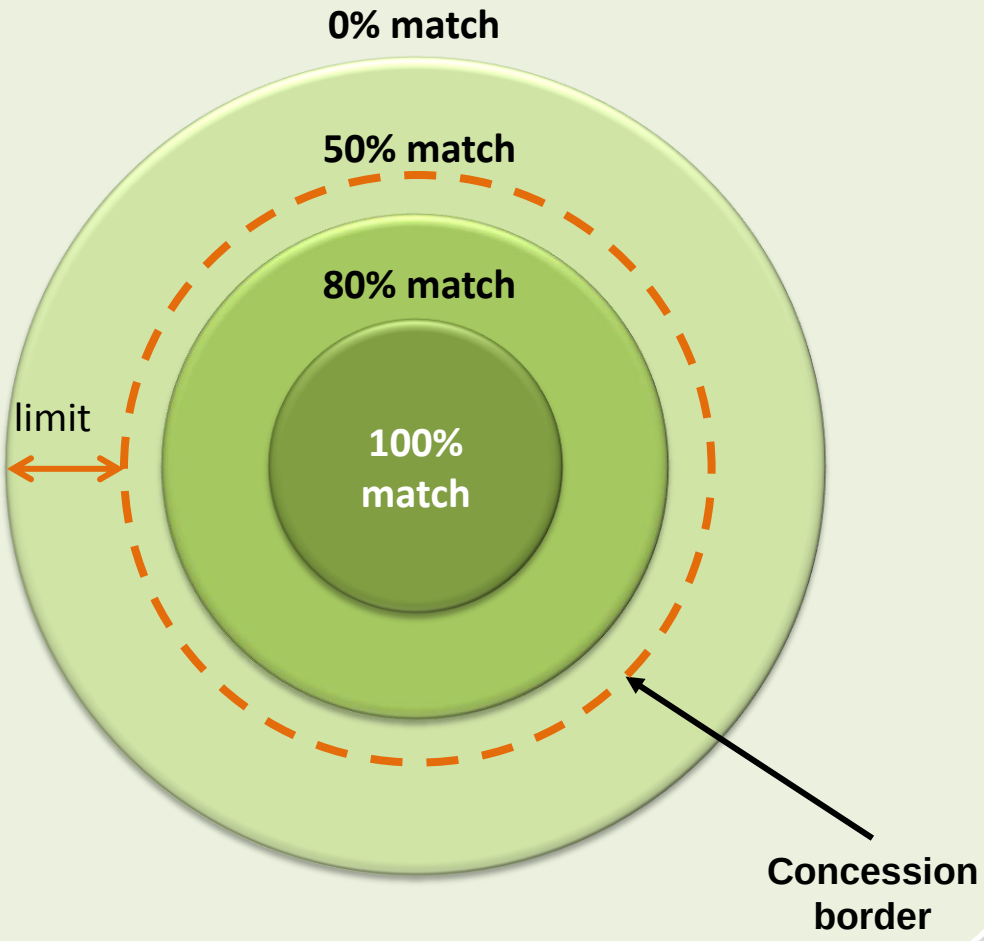
This paper reports initial results of a palaeogenetic analysis of timbers from the hull of the English Tudor flagship *Mary Rose*. The study is the first step in assessing the feasibility of extracting and amplifying chloroplast DNA (cpDNA) from these timbers, which were preserved in a marine environment for more than four centuries. The ultimate goal of this research is to determine the provenance of oak (*Quercus* spp.) used in the ship's manufacture, following previous work demonstrating that the chloroplast genome of modern European oak populations exhibits a strong phylogeographic structure. Experimental trials revealed that extraction methods developed for modern oak wood were inadequate owing to the presence of polymerase chain reaction (PCR) inhibitors in the *Mary Rose* timbers. A series of treatments were tested to develop a new extraction protocol, resulting in cpDNA recovery from one archaeological sample. These results represent the first successful extraction and amplification of cpDNA from waterlogged archaeological oak wood from a marine environment.

Current DNA Extraction Capabilities



Origin of Timber
Example: INDONESIA

- Area:
  250,000ha
- Species:
  Merbau



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Timber Origin
A spot-check system

- Discriminate between populations of merbau (*Intsia* spp.) in S.E. Asia
  - High value timber (flooring/decking)
  - Discriminate species
  - Discriminate Malaysian and Indonesian sources
  - Assign to concessions?
  - Funding from Singaporean government

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Timber Origin

A spot-check system

260 individuals screened

Intsia bijuga
Singapore and New Guinea

Intsia palembanica
Sabah and Papua

16 microsatellites
(14 nuclear, 2 chloroplast)

All populations significantly differentiated, structure higher with cpSSRs than nSSRs

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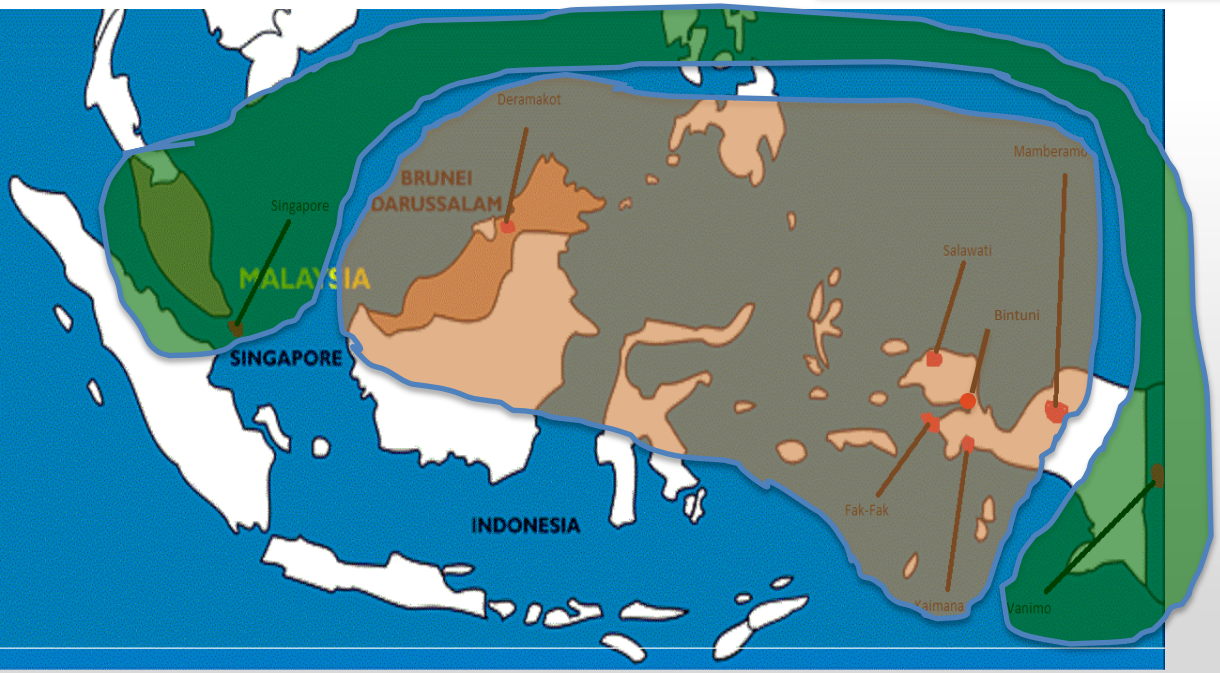
Timber Origin
A spot-check system



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Assignment test results

- *Intsia bijuga* *vs* *Intsia palembanica*
  - 98.1% confidently assigned, some taxonomic issues – reassign by clusters

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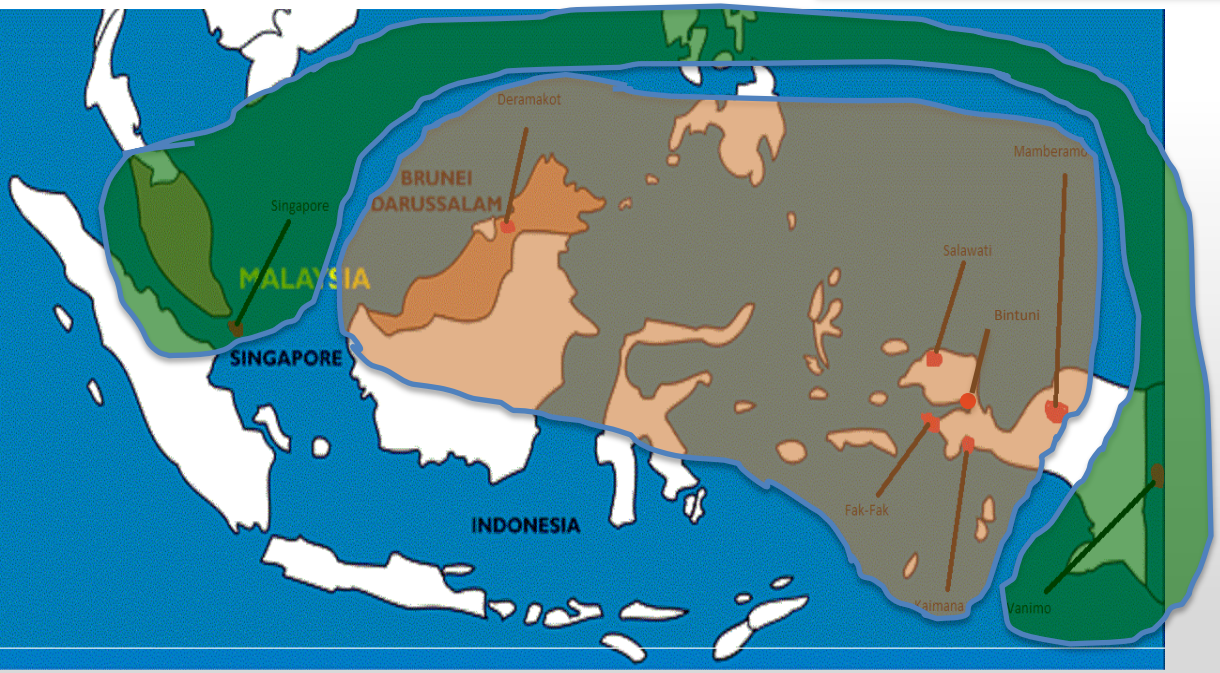
Timber Origin
A spot-check system



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Sabah and Papua

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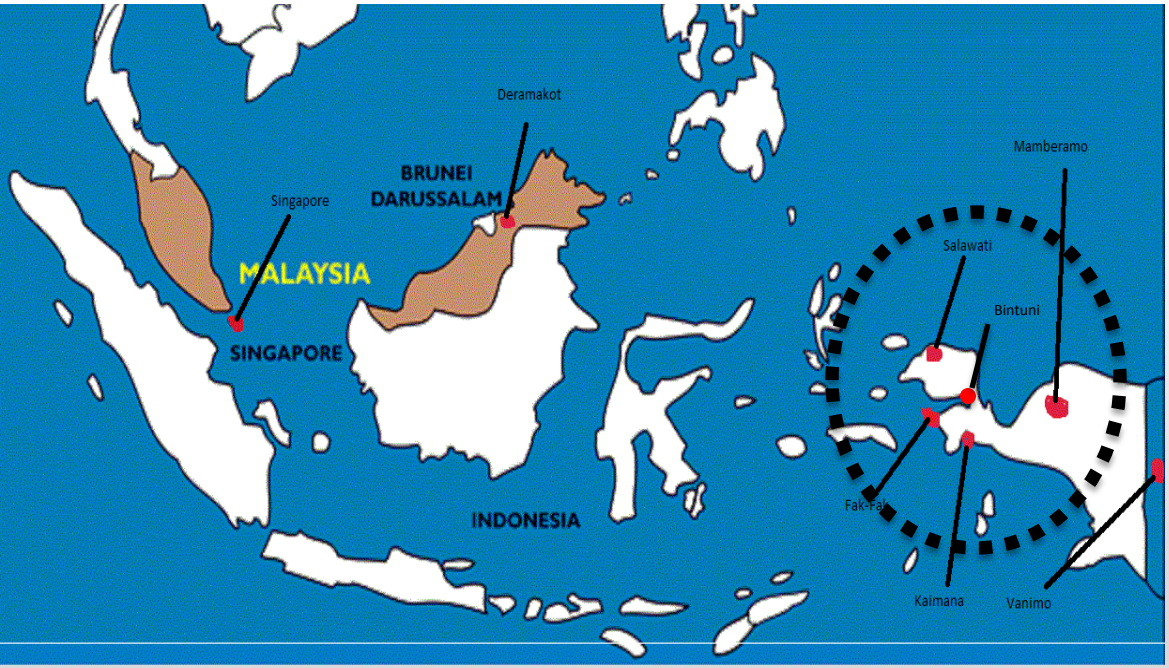
Assignment test results

- Differentiation between region
  - *Intsia bijuga* - Sinpore vs New Guinea: 100% correctly reassigned
  - *Intsia palembanica* – Sabah vs Papua : 95.6% correctly reassigned

Timber Origin
A spot-check system



260 individuals screened

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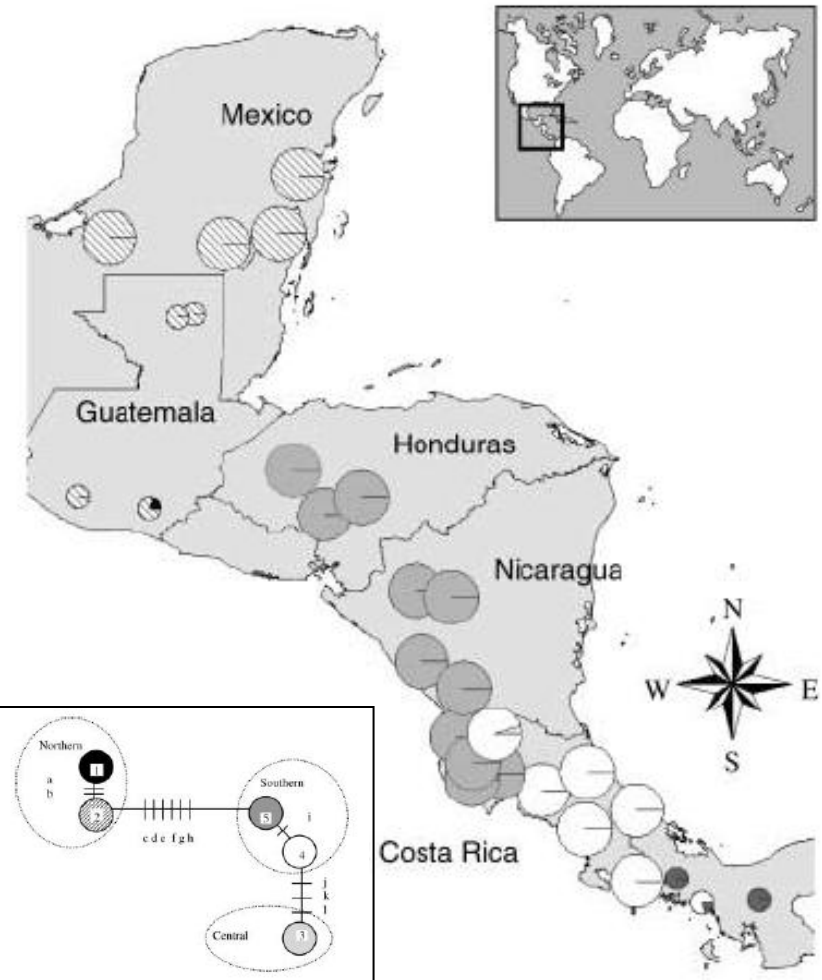
Assignment test results

- Differentiation between concessions (*Intsia palembanica* – Papua)
  - Proportion of reassignment 67.9% - some failures, need to repeat
- Ongoing work with Singaporean gov funding and vTI, Germany

Progressing region of origin testing using phylogeographic structure

Spanish cedar
Cavers et al 2006



Phylogeographic structure of Spanish cedar

Progressing region of origin testing using phylogeographic structure

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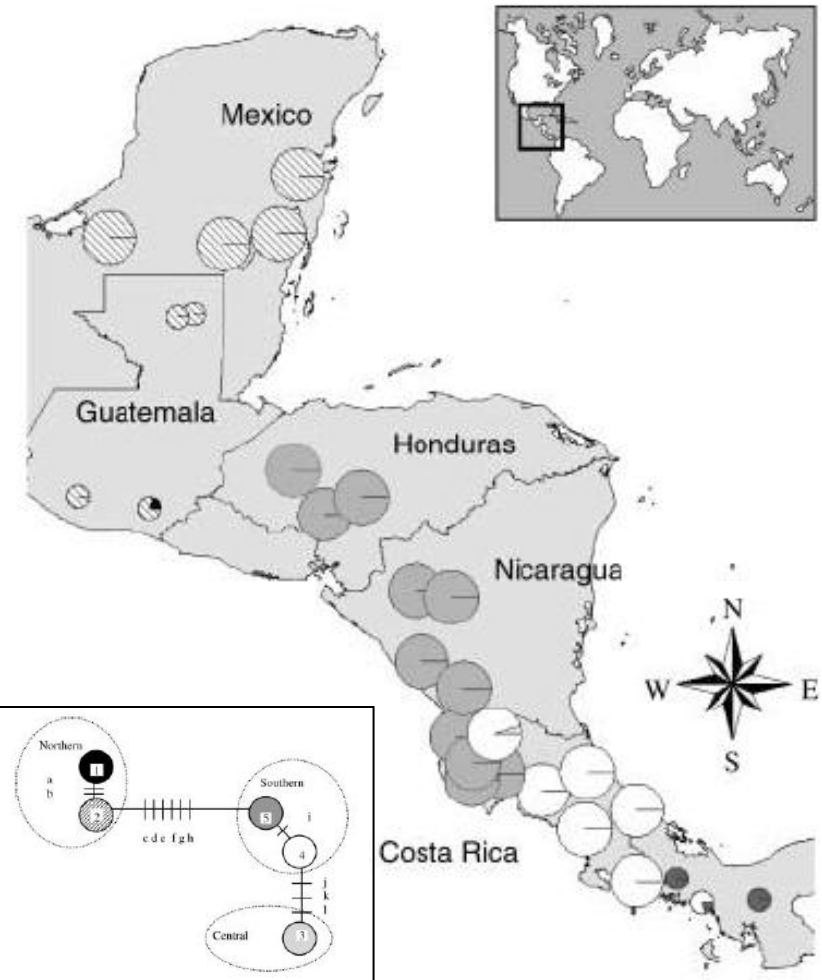


Phylogeographic structure of Spanish cedar

Used to test origin of unknown provenance used for breeding by Queensland Forestry

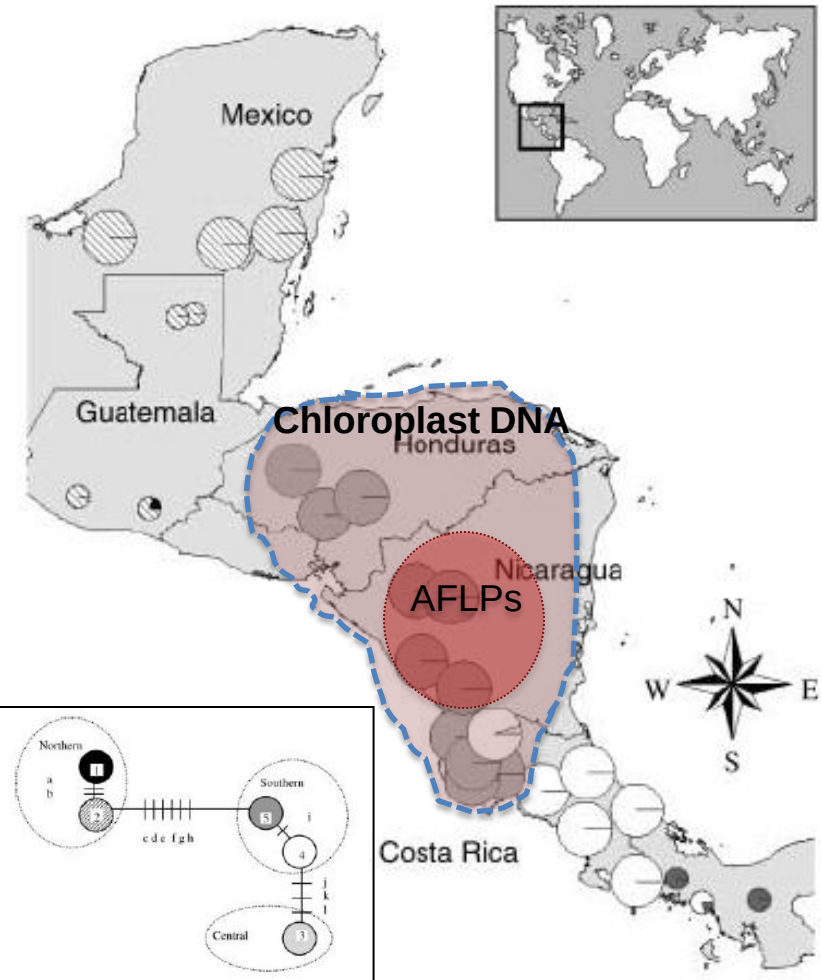
Is it pure *Cedrela odorata* or cross with *Toona*?

Where did it come from?

Used cpDNA and AFLPs

Progressing region of origin testing using phylogeographic structure

Spanish cedar
Cavers et al 2006



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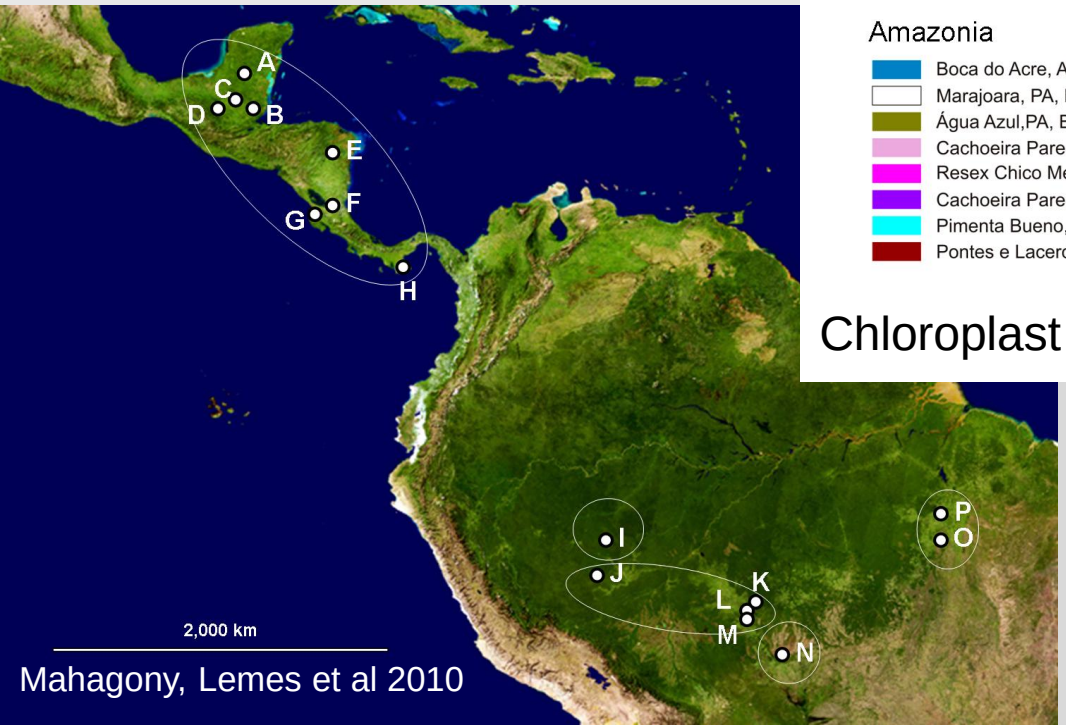
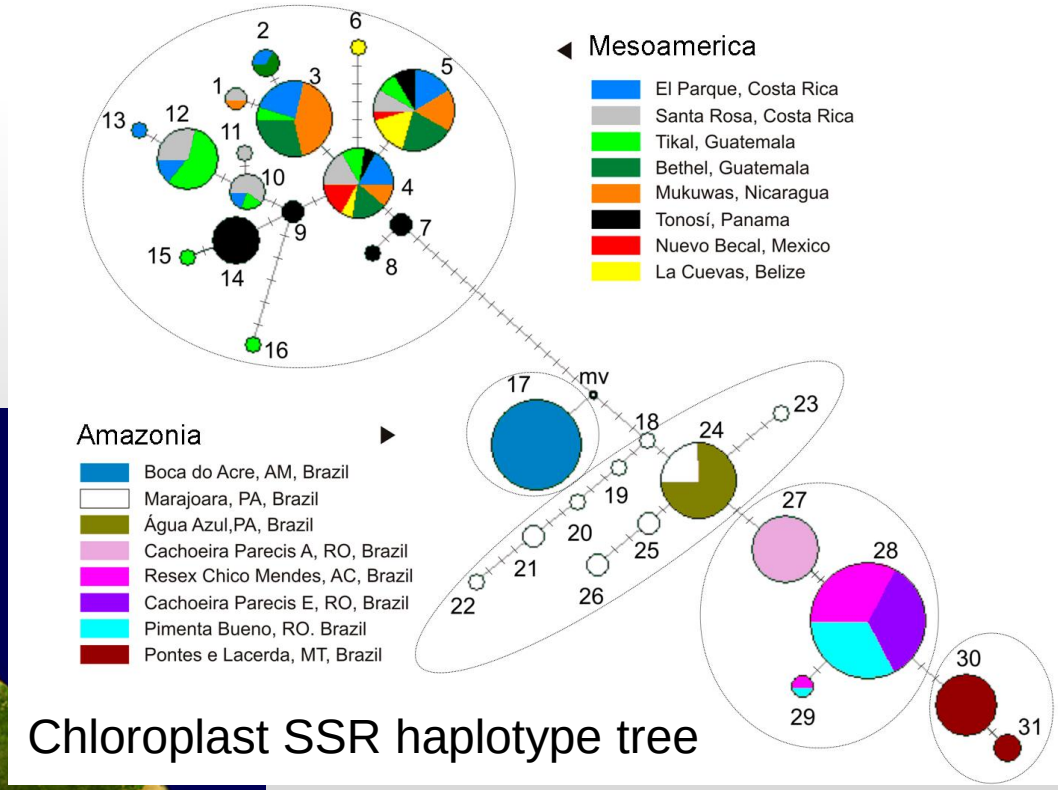


Mahagony, Lemes et al 2010



Chloroplast SSR haplotype tree

Species and regional database

- SEEDSOUCE - Rangewide genetic data 16 neotropical tree species, Including; mahogany, Spanish cedar, Brazil nut
- Partners: UK, Australia, Costa Rica, Italy, Germany, Brazil, France, Ecuador, USA
- Many other projects now also coming to fruition

- Call to build international database combining species, regional and concession data with tools for analysis

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Conservation

- Partnership with Global Conservation Standard
- Genetic profiling of conservation areas
  - Develop genetic identity for conservation stands for future random testing
- TreeBOL
  - International initiative to DNA barcode all trees

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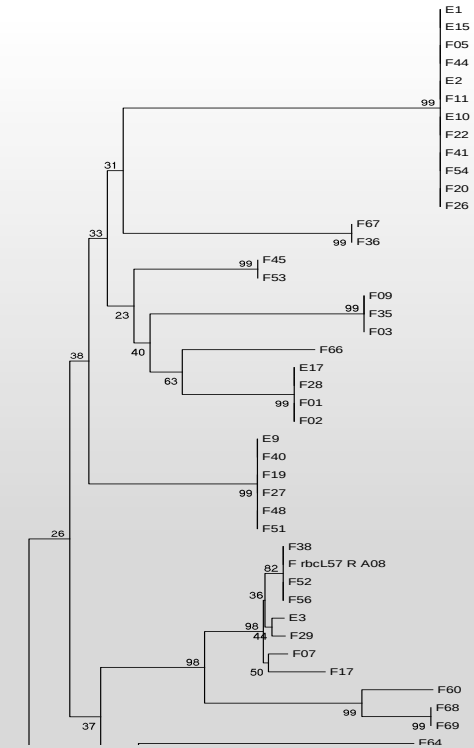
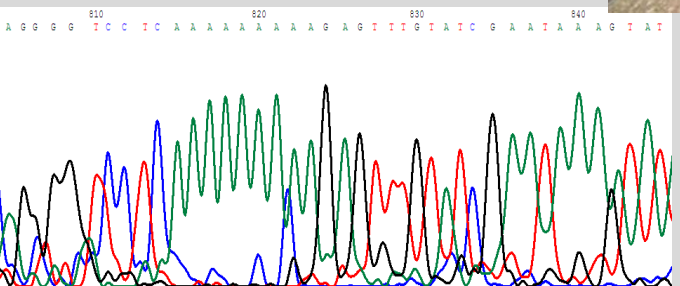
ATH
Australian Tropical Herbarium

International Barcode of Life (IBOL)

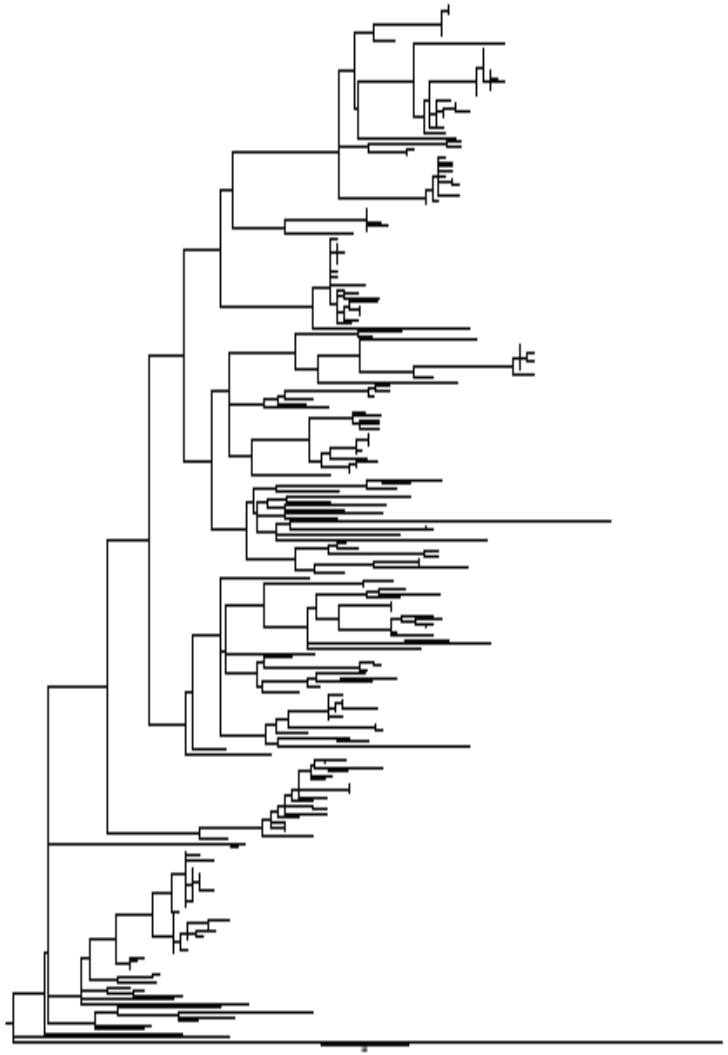


Over next 5 years aim to generate unique DNA barcode for 500,000 species incl. 100,000 plants

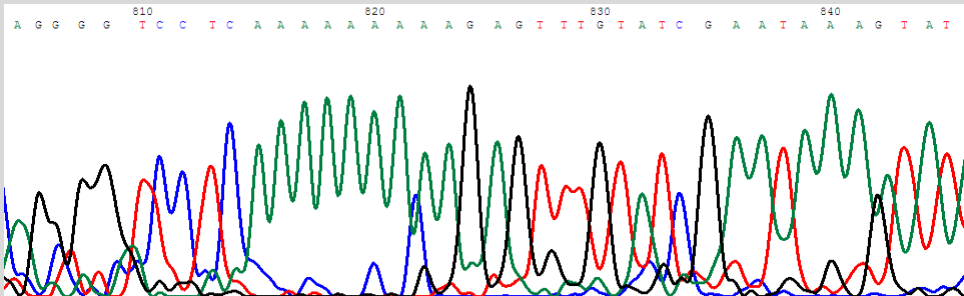
Range of international efforts – e.g. Australia



DNA Barcoding tropical trees



- TreeBoL
- International initiative to barcode trees
- Focus on tropical regions
  - Americas, Africa, SE Asia
- Data in Barcode of Life Database (IBOL)
- Open access to promote identification of CITES species
- DoubleHelix leading new funding initiative (CBOL/IBOL)



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Timber Origin
Next steps

- Optimise marker selection and extraction protocols
- Improve accuracy
- Accelerate through partnership
  - Develop benefits sharing model

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The potential of scientific methods

- DNA is **simple**
- DNA is **complementary** to existing initiatives
- DNA will **reduce** overall **cost of compliance**
- DNA **reduces the burden on supply chain members**
- DNA encourages **self-regulation → Quality Control**
- DNA **costs will** continue to **fall**