European commission DG RESEARCH
INCO-DEV, International role

Developing know-how for the improvement and sustainable management of teak genetic resources.

TEAKDIV

Final Scientific Report December 2007

Research programme	Major target	Additional target
	Tools for sustainable development	Systems research on natural capital and the human environment
Key action	Technologies for sustainable crop and animal production: building blocks for improvement. Cash crops and forestry (c.ii-1)	Strategies for rural productivity; ecosystem management for sustainability (b.i)
Region code	Asia / ALA	
Contract Number	Project number: ICA4-CT-2001-10094	
Project homepage	http://www.edinburgh.ceh.ac.uk/teakdiv	

Section 1: PROJECT IDENTIFICATION Information to be provided for project identification	NOT CONFIDENTIAL

Title of the project: Developing know-how for the improvement and sustainable management of teak genetic resources.		
Acronym of the project: TEAKDIV		
Type of contract: Shared cost RTD		Total project cost 1 083 821
Contract number ICA4-CT-2001-10094	Duration (in months) 36 + 12 Months	EU contribution 920 000
Commencement date 1st January 2002		Period covered by the final report 01.01.04 – 31.12.06

PROJECT COORDINATOR

Name: Stephen Cavers	Title: Dr.	Address: Centre for Ecology and Hydrology – Edinburgh Bush Estate, Penicuik, Midlothian, EH26 0QB
Telephone: +44 (0)131 445 4343	Telefax: +44 (0)131 445 3943	E-mail address: scav@ceh.ac.uk

Key words Teak, genetic-resources, management, molecular, modelling
World wide web address http://www.edinburgh.ceh.ac.uk/teakdiv/home_page/index.htm List of participants Following

List of participants:

Partner 1 (Project co-ordinator):
Dr. Stephen Cavers – Centre for Ecology and Hydrology, CEH-Edinburgh, Bush Estate, Penicuik, Midlothian EH26 0QB, UK. Tel: (+44) (0)131 445 4343. Fax: (+44) (0)131 445 3943. Email: scav@ceh.ac.uk

Partner 1 (Financial co-ordinator):
Mr. Neil Ramsay – Natural Resources Research Council. Email: nram@wpo.nerc.ac.uk

Partner 2 (Contractor):
Dr. Indira Edakkeppurath Puthanveettil – Kerala Forest Research Institute, Peechi, 680653, Thrissur, India. Tel: (+91) 487 2699 037. Fax: (+91) 487 2699 249. Email: indira@kfri.org

Partner 3 (Contractor):
Dr. Sudarsono – Institut Pertanian Bogor - Bogor Agricultural University, Department of Agronomy, Faculty of Agriculture, J1. Raya Pajajaran, 16143, Bogor, Indonesia. Tel: (+62) 251 326 429. Fax: (+62) 251 312 032. Email: agrspsipb@indo.net.id

Partner 4 (Contractor):
Dr. Apichart Vanavichit – Rice Gene Discovery Center, Kasetsart University, Kamphaengsaen Campus, 73140, Kamphaengsaen, NakornPathom, Thailand. Tel: (+66) 34 xxx xxx. Fax: (+66) 34 xxx xxx. Email: apichart@dna.kps.ku.ac.th

Partner 4 (Scientific coordinator):
Dr. Hugo Volkaert – BIOTEC, Centre for Agricultural Biotechnology, Kasetsart University, Kamphaengsaen Campus, 73140, Kamphaengsaen, NakornPathom, Thailand. Tel: (+66) 34 282 494 ext 305. Fax: (+66) 34 282 498. Email: hugo.v@ku.ac.th

Partner 5 (Contractor):
Dr. Dominique Van Der Straeten – Department of Molecular Genetics, Universiteit Gent, K.L. Ledeganckstraat 35, 9000, Gent, Belgium. Tel: (+32) 9 264 5185. Fax: (+32) 9 264 5333. Email: dominique.vanderstraeten@ugent.be

Partner 6 (Contractor):
Dr. Hubert Wellendorf - Royal Veterinary University, Sektion Arboretet, Institut for Oekonomi, Skov og Landskab, KVL, Kirkegaardsvej 3A, 2970, Hørsholm, Denmark. Tel: (45) 35 283 628. Fax: (45) 35 283 629. Email: hwe@kvl.dk

Note:
Former coordinator, Dr. Andrew Lowe is still cooperating with this progress from his new position at University of Adelaide. He can be reached at:
University of Adelaide, Australian Centre for Evolutionary Biology and Biodiversity, School of Earth & Environmental Sciences, The University of Adelaide South Australia 5005, Australia. Tel: (+61) 8 8303 5280; Fax: (+61) 8 8303 4364. Email: andrew.lowe@adelaide.edu.au

Section 2: PROJECT SUMMARY NOT CONFIDENTIAL

The project had the following objectives:

To trace and quantify genetic diversity of teak within its natural range, DNA markers were used to assay the current distribution of genetic diversity within and between populations, investigate its mating system and establish historical migration patterns.

To evaluate the amount of contemporary gene flow through pollen and seed, hypervariable microsatellite DNA markers have been developed for parentage analysis. The molecular work was complemented by field observations of teak flower insect pollinators.

To assess the influence of human disturbance, the genetic diversity in teak forests that have been undisturbed, lightly or heavily disturbed have been assessed and compared for both population genetic diversity and contemporary gene flow processes.

Results and Milestones:

WP1 Development of practical assays for studying genetic diversity in teak

Microsatellite clones have been isolated and sequenced and primers designed for use with teak. Levels of polymorphism of markers have been screened and microsatellite sequence data have been made publicly available through the EMBL database. Primer pairs for the analysis of nuclear and cytoplasmic DNA markers have been developed. Levels of polymorphism have been assessed and sequence data have been made publicly available through the EMBL database. The Project website (http://www.edinburgh.ceh.ac.uk/teakdiv/home_page/index.htm) contains several result files and a database facility has been incorporated to accommodate marker storage and assessment. A catalogue of alleles has been developed. A training workshop was held in Thailand in September 2002 to share DNA analysis methods to all partners and a data analysis workshop has been held at the Kerala Forest Research Institute, India, in December 2003. A third workshop was held at Bogor Agricultural University in November 2005 to discuss the results.

WP2 Assessment of genetic diversity in teak over its natural range

Molecular assessment of chloroplast DNA haplotypes and selected nuclear loci has been done to establish a molecular inventory of genetic diversity and allow analysis of historical migration patterns, i.e. phylogeography. Very little diversity was observed in the chloroplast loci. Three polymorphic chloroplast mononucleotides and one base substitution were found. In contrast, a very large diversity was observed at each of the studied nuclear genes. In spite of a comparatively more intensive sampling in Thailand compared to India, many more alleles were found in India. All alleles found in Thailand and Laos, and all except one of the alleles found in Indonesia, are confined to a small region in each of the phylogenetic networks. For almost all genes studied, the Thai/Lao/Indonesian alleles are shared with, or phylogenetically related to, alleles found in northeastern India. Southwestern India has the largest number of alleles, several of which are shared with eastern India. This would imply an origin for teak in southwestern India with a migration to northeastern India and then to Myanmar, Thailand, Laos and via Sumatra to Java in Indonesia.

WP3 Population genetic diversity in teak forests
Many teak populations were sampled for genetic analysis over a wide range of teak's natural distribution. The genotypes at selected nuclear gene loci were obtained to compile a molecular inventory to quantify genetic diversity in natural populations that reflects long-term gene flow and mating system differences.

WP4 Gene flow through pollen and seed dispersal
Microsatellite genotyping of DNA extracted from adults, seedlings and seeds in several populations has allowed the estimation of pollen dispersal curves for teak in natural forests (Kerala, Java and Thailand), one plantation (Kerala) and a seed orchard (Indonesia) *and an estimation of the variation in male mating success*.

WP5 Analysis of the impact of human disturbance on genetic diversity
An inventory of genotypes at selected nuclear gene loci and microsatellites has been established for disturbed and undisturbed forests to compare loss of heterozygosity, loss of alleles and change in allele frequencies due to human-mediated land-use changes. A population genetic simulation model has been developed that integrate population genetic and diversity measurements. The combination of actual population data and incorporation of the simulation model should allow quantification of genetic diversity in populations with different levels of human disturbance and an assessment of the loss of genetic diversity due to human disturbance.

WP6 Identification of effective insect pollinators in teak
Insect pollinators have been studied in selected populations of teak in India, Indonesia and Thailand. For several of the insects determination to the species level remains problematic. A list of insects visiting teak flowers in natural populations and plantations has been compiled for India, Indonesia and Thailand.

WP7 Analysis of the genetic diversity data
Data on microsatellite, nuclear gene and chloroplast DNA genetic markers variation has been compiled for analysis and publication. The analysis concentrated on comparing historical and contemporary gene flow and the level and partitioning of genetic diversity between undisturbed and disturbed populations. Computer packages to analyse the genetic diversity and gene flow data at several levels of organisation have been demonstrated and a handbook instructing genetic data analysis has nearly been completed. Final data assimilation will devise best practise genetic resource management strategies for teak.

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Partner 1 (Project co-ordinator):
Dr. Stephen Cavers – Centre for Ecology and Hydrology, CEH-Edinburgh, Bush Estate, Penicuik, Midlothian EH26 0QB, UK. Tel: (+44) (0)131 445 4343. Fax: (+44) (0)131 445 3943. Email: scav@ceh.ac.uk

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Dr. Indira Edakkeppurath Puthanveettil – Kerala Forest Research Institute, Peechi, 680653, Thrissur, India. Tel: (+91) 487 2699 037. Fax: (+91) 487 2699 249. Email: indira@kfri.org

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Dr. Sudarsono – Institut Pertanian Bogor - Bogor Agricultural University, Department of Agronomy, Faculty of Agriculture, J1. Raya Pajajaran, 16143, Bogor, Indonesia. Tel: (+62) 251 326 429. Fax: (+62) 251 312 032. Email: agrspsipb@indo.net.id, pertaipb@bogor.indo.net

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Dr. Apichart Vanavichit – Rice Gene Discovery Center, Kasetsart University, Kamphaengsaen Campus, 73140, Kamphaengsaen, NakornPathom, Thailand. Tel: (+66) 34 xxx xxx. Fax: (+66) 34 xxx xxx. Email: apichart@dna.kps.ku.ac.th

Partner 4 (Scientific coordinator):
Dr. Hugo Volkaert – BIOTEC, Centre for Agricultural Biotechnology, Kasetsart University, Kamphaengsaen Campus, 73140, Kamphaengsaen, NakornPathom, Thailand. Tel: (+66) 34 282 494 ext 305. Fax: (+66) 34 282 498. Email: hugo.v@ku.ac.th

Partner 5 (Contractor):
Dr. Dominique Van Der Straeten – Department of Molecular Genetics, Universiteit Gent, K.L. Ledeganckstraat 35, 9000, Gent, Belgium. Tel: (+32) 9 264 5185. Fax: (+32) 9 264 5333. Email: dominique.vanderstraeten@ugent.be

Partner 6 (Contractor):
Dr. Hubert Wellendorf - Royal Veterinary University, Sektion Arboretet, Institut for Oekonomi, Skov og Landskab, KVL, Kirkegaardsvej 3A, 2970, Hørsholm, Denmark. Tel: (45) 35 283 628. Fax: (45) 35 283 629. Email: hwe@kvl.dk

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University of Adelaide, Australian Centre for Evolutionary Biology and Biodiversity, School of Earth & Environmental Sciences, The University of Adelaide South Australia 5005, Australia. Tel: (+61) 8 8303 5280; Fax: (+61) 8 8303 4364. Email: andrew.lowe@adelaide.edu.au

FINAL REPORT

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1. OBJECTIVES AND EXPECTED ACHIEVEMENTS

The continued destruction of tropical forests calls for a greater effort to produce timber in plantations and to manage the remaining forests in a sustainable way. Genetic improvement of quality hardwood species is one avenue to alleviate some of the pressure to extract timber from natural forests. Teak (*Tectona grandis* L. f.) is an important tree species in the natural forests of India, Myanmar, northern Thailand and Indonesia. As a fast growing quality hardwood species, it is widely planted in its original area of distribution and in other countries in tropical Asia, Africa and Latin America. Teak genetic resources have been dramatically altered during the last 50 to 100 years through uncontrolled logging and indiscriminate movement of planting materials.

This project has developed specific DNA marker tools that can be used in tree breeding programmes and for the management of genetic resources. The developed markers have been applied to compile information on the geographical distribution of genetic diversity and gene flow, at different spatio-temporal scales and in forest stands with different levels of human impact. Together with the DNA analysis, studies have been undertaken to determine the pollen vector in natural populations. This information has been integrated to draft guidelines for the future conservation and management of teak genetic resources in nature, and for the efficient use of these resources in breeding and plantation programmes in Asia, Africa and Latin America.

This project had three scientific activities.

- To trace and quantify genetic diversity of teak within its natural range, DNA markers were used to assay the current distribution of genetic diversity within and between populations, investigate its mating system and establish historical migration patterns.

- To evaluate the amount of contemporary gene flow through pollen and seed, hypervariable microsatellite DNA markers were developed for parentage analysis. The molecular work has been complemented by field observations of teak flower insect pollinators.

- To assess the influence of human disturbance, the genetic diversity in teak forests that have been lightly or heavily disturbed has been assessed and compared for both population genetic diversity and contemporary gene flow processes.

2. PROJECT WORKPLAN

2.1 Introduction

During the first year of the project, specific protocols and assays to study genetic diversity in teak were developed. Microsatellites and markers for specific nuclear genes were developed. Markers for chloroplast and mitochondrial loci were screened for diversity. The different alleles have been sequenced for genealogical analysis and to establish allelic relationships.

As a result of this phase, practical protocols to detect genetic diversity and a catalogue of different alleles for each marker system are now available. A workshop was organised to train all the participants in the developed protocols and assays, and to agree on a system for scoring the alleles and communicate the results among the participants and to outside interested parties.

The developed protocols and information on different alleles were then be used to study the genetic diversity at different spatial and temporal scales. Each of the participants in India, Indonesia and Thailand collected tissue samples and analysed them locally. Sequencing of alleles from representative individuals was used to link the results from the different studies together.

Several populations, representing little disturbed or highly disturbed natural stands and plantations, were studied intensively for contemporary gene flow and identification of insect pollinators. Additional populations were analysed for population genetic parameters that yield information on the long-term effects of gene flow. All these populations and additional individual trees from germplasm collections and provenance tests covering the whole natural range of teak were analysed for diversity at slowly evolving genetic markers (chloroplast and nuclear genes) to study the phylogeography of teak.

The genetic diversity information obtained can be combined, analysed and interpreted to give recommendations for conservation of genetic diversity, guidelines for establishing and managing teak seed orchards and breeding programmes and indicate some issues that should be taken into account when moving teak germplasm for plantation. Practical software tools for undertaking such a diverse range of phylogeographic, population genetic and paternity analyses has been compiled. Furhter analysis will be geared specifically for use by those interested in assessing genetic diversity and structure for conservation and management purposes.

2.2 Project structure, planning and timetable

Overview:

According to the workplan, the first year of TEAKDIV activity focused on development of specific protocols to study genetic diversity in teak and on getting all Asian partners up to date concerning the methods to be used and their laboratories equipped so that the project can be efficiently implemented.

Microsatellite markers, some nuclear gene markers and chloroplast markers have been developed and are now being applied on plant materials collected by the Indian, and Indonesian partners.

Even though the project has started quite late in 2002, it did still achieve the goals for the first year. However, two major obstacles resulted in a significant slowing down of the project's progress during the second and third year.
Due to very late submission of accounting information by the Indonesian partner, the contractors did not receive any new funding until halfway through 2004. As a result, the Indian and Thai partners were forced to slow the pace of their research.

The Thai partner suffered an additional set-back. Due to reorganisation within the Thai Royal Forest Department, a permission to collect samples from the forest reserves and national parks in Thailand has not yet been granted. To circumvent this problem, students at the Center of Agricultural Biotechnology were used as proxies to apply for separate permissions to collect teak leaf samples. One permission to do the collection for workpackage 3 was granted end of August 2004. Another separate permission to collect seed, seedlings and leaf samples to complete workpackage 4 has been applied for and was granted early in 2005. The original permission to do the research, including vegetation and soil studies is still being evaluated by the Department of National Parks, Wildlife and Plant Protection. A request to make some changes to the proposal was received in December 2006!

Because of these delays, permission has been sought and was granted by the European Union to extend the project duration by one year, until December 31, 2005.

3. PROJECT RESULTS

Workpackage 1: Development of practical assays for studying genetic diversity in teak

Item	Date	Status
Milestones and expected results		
Primers of microsatellites for use in WP3, 4	Month 12	Complete
Primers and sequences for nuclear and cytoplasmic DNA markers for use in WP2, 3	Month 12	Complete
Set-up of a project webpage on which results will be published	Month 12	Complete
Training workshop to transfer DNA analysis methods	Month 12	Complete
Deliverables		
D1. Microsatellite sequences and primers with an assessment of their polymorphism	Month 12	Complete
D2. Nuclear and cytoplasmic gene markers for teak	Month 12	Complete
D3. Catalogue of alleles observed at	Preliminary month 16, final	Complete

each of the loci in teak). Made available through a web-based database.	month 32	

Notes on deliverables, milestones and expected results

D1. Microsatellite sequences and primers with an assessment of their polymorphism

The microsatellite sequence data have been made publicly available through the EMBL/GENBANK database. A publication detailing the development of these primers, together with an example of their operational use is being drafted. Ten of the microsatellite loci have been used for analysis in WP4. They displayed a fair level of polymorphism in the Kerala populations, but lower level of polymorphism in the Thai and Indonesian population.

D2. Nuclear and chloroplast gene markers for teak

Fourteen chloroplast fragments have been studied for diversity, but only two of them, the *pet*B and *psa*A fragments, contain informative polymorphic sites.

A set of markers for nuclear loci has been developed: CAT1, ADH2, G3PDHcy1, G3PDHcy2, G3PDHcp, AATcy, TFL1, FLS1, IDH1, IPI1, IPI2, AP3, MAPK1 and NDPK1.

D3. Catalogue of alleles observed at each of the loci in teak (month 16, elaborated month 32).

The chloroplast sequence data have been made publicly available through the EMBL database.

Nuclear gene sequences have been obtained and are available through the EMBL database.

Training workshop to transfer DNA analysis methods

Achieved in month 10

Technical details

Fourteen chloroplast fragments have been studied: *mat*K, *ndh*A, *ndh*F-*trn*L, *pet*A-*psb*J, *pet*B, *psa*A, *psb*A, *rbc*L-*trn*V, *rpl*16 intron, *rpo*A, *trn*T-*trn*L, *trn*Y-*psb*D, *trn*Y-*rpo*B and *psa*B-*psb*C. All fragments consist mainly of non protein coding sequences, mostly intergenic regions but also introns (*mat*K, *ndh*A, *rpl*16 and *pet*B).

Even though the total length of chloroplast fragments studied in this project reached 25,000 base pairs, none of the chloroplast markers revealed reproducible polymorphisms on SSCP gels. This was rather unexpected.

All fragments were sequenced in at least one tree from Thailand, one tree from southwestern India and one tree from eastern India, though for most fragments (*mat*K, *ndh*A, *ndh*F-*trn*L, *pet*A, *pet*B, *psa*A, *psb*D, *rpl*16, *trn*T-*trn*L, *trn*Y-*psb*D) covering a total length of almost 9000 bp, sequences from more than 10 trees were obtained. Sequencing of these fragments revealed 3 polymorphic sites: two mononucleotide repeat polymorphisms in the *pet*B fragment, and one nucleotide substitution polymorphism in the *psa*A fragment. In a second stage, more than 50 trees from a wide range of populations were sequenced for the *pet*B, *psa*A, and *ndh*A fragments, a total of approximately 2400 bp. This large scale sequencing revealed one additional mononucleotide repeat in the *psa*A fragment of teak trees from Orissa, but confirmed the absence of polymorphisms in the *ndh*A fragment. This indicates that the true number of polymorphisms is probably a bit higher than what has been found so far, but still very low.

Table 1: Overview of chloroplast fragments amplified and sequenced

locus	primer name	primer sequence	amplified length	*Tectona* sequenced length	sequence length >10 samples	Polymorphism
*mat*K	cp-matK-for	ggAACTAgTCggATggAgT	2553	2514	1700	0
	cp-matK-rev	ACTCAATggTAgAgTACTCg				
*ndh*A	cp-ndhA-for	gCTgCTCAATCTATTAgTTATgA	1069	1023	900	0
	cp-ndhA-rev	TgTgCTTCAACTATATCAACTgT				
*ndh*F-*trn*L	cp-ndhF-for	TCCCCTACACgATTAgTTAC	2250	1963	700	0
	cp-trnL-rev	TAAgAgCAgCgTgTCTACCA				
*pet*A-*psb*F	cp-petA-for	ATTgTATgTgCCAATTgCCA	2264	2211	735	0
	cp-petA-rev	TATCAgCAATgCAgTTCATC				
*pet*B	cp-petB-for	gAATATgAgTgTgTgACTTg	1611	1571	700	2
	cp-petB-rev	gTCACACACTCCCATAATCC				
*psa*A	cp-psaA-for	AAATCgTgAgCATCAgCATg	1078	1038	850	2
	cp-psaA-rev	CCgAggAgAACAggCCATTC				
*psb*A	cp-psbA-for	gCAATTTTAgAgAgACgCgA	995	955	0	0
	cp-psbA-rev	ACgTTCgTgCATAACTTCCAT				
*rbc*L-*trn*V	cp-trnV-for	CgAACCgTAgACCTTCTCgg	3820	2611	650	0
	cp-rbcL-rev	gCTTTAgTCTCTgTTTgTgg				
*rpl*16	cp-rpl16-for	gCTATgCTTAgTgTgTgACT	937	897	750	0
	cp-rpl16-rev	CATTCTTCCTCTATgTTgTTT				
*rpo*A	cp-rpoA-for	CCATTTCgTCgTCCAgTAgC	1279	1239	0	0
	cp-rpoA-rev	CAgTggAAgTgTgTTgAATC				
*trn*T-*trn*L	cp-trnT-for	CATTACAAATgCgATgCTCT	1302	1262	660	0
	cp-trnL-rev	ggggATAgAgggACTTgA				
*trn*Y-*psb*D	cp-trnY-for	gCTggATTTgAACCAgCgTAg	3300	3218	760	0
	cp-psbCD-rev	CCATTAAAgAgCgTTTCCACg				
*trn*Y-*rpoB*	cp-trnY-rpoB-for	ACgCTggTTCAAATCCAgCTCg	4300	3100	0	0
	cp-rpoB-rev	CTgATgATAgAACTAgAATAgA				
*psa*B-*psb*C	cp-psaB-for	gTTACTTTTTATTggCATTggAA	3600	2400	0	0
	cp-psbC-rev	TATgTCTCTCCTAgAAgTTggTT				
Total				26102	8405	4

The nuclear gene markers used in this project have been developed from two sources. Initially, consensus PCR amplification primers were developed based on alignments of homologous genes from a wide range of plant species. This way, one CAT gene, two ADH genes, three G3PDH genes, one cytoplasmic and one mitochondrial AAT gene, two IPI genes, two IDH genes, one cystein proteinase inhibitor, one chitinase, two TFL like genes and one AP3 gene were obtained. Based on the initial sequences, locus-specific PCR amplification primers were designed to amplify individual fragments. Some of the gene fragments contained only short introns (AATm, CPI and CHIT) and thus it was decided not to try to use them for SSCP analysis. However, the CPI and CHIT introns contained a SSR which was used for the geneflow analysis.

Later on, with additional funding from the Center for Agricultural Biotechnology, a cDNA library was developed and about 200 inserts were sequenced. For 20 of these protein coding regions, primers were developed to detect polymorphisms in the corresponding genomic regions. Nineteen of these amplified a single fragment and were used to detect polymorphisms using SSCP assays. All of them revealed polymorphism. One (FLS1) was then further used in the population genetic analysis, while for two other genes (MAPK and NDPK) enough information was obtained to show their potential usefulness, but they were not used in the population genetic study.

The CAT1, G3PDHcy1 and G3PDHcy2 loci have been used to genotype trees in all populations. The markers for G3PDHcp, TFL1 and FLS1 have been used for all populations in Thailand, Laos and Indonesia but only some populations from India. The ADH2 marker has been used to genotype trees in the Kerala populations and sequences have been obtained from a sample of Thai trees, but it was not used further. However, it could be used in future studies as well. The IDH1, IPI1, IPI2, AATcy, AP3, MAPK and NDPK markers have not been widely used yet.

Table 2: Overview of nuclear gene fragments amplified and sequenced

locus	primer names	primer sequences	amplified length	Total number of alleles	# alleles in southwestern India	# of alleles in eastern India	# of alleles in Thailand, Laos,
CAT1	Tg-CAT1-for Tg-CAT1-rev	ATTCTCCACTgTCATCCATg ATCAAAgTTTCCCTgCATCC	367-392	22	14	12	
G3PDHcy1	Tg-GPDcy1-for Tg-GPDcy1-rev	gTATTTTATTCACCTTCTgAgC ATgCTTgACCTACACAACgg	618-627	35?	21	16	
G3PDHcy2	Tg-G3PDHcy2-for Tg-G3PDHcy2-rev	gTACTTTATTCACCATCTgAgT ATgCTTgACCTACATgAACA	596-597	20	15	9	
G3PDHcp	Tg-G3PDHcp-for Tg-G3PDHcp-rev	gCTgCTAAggTATTAgATgg gACACAAgCTTCATgAAggA	800	24	13	12	
TFL1	Tg-TFL1-for Tg-TFL1-rev	CCACATCTAAgggAgCACTTA AATATCCgTgACTATCCTgCgAg	823-825	23	12	10	
FLS1	Tg-FLS1-for Tg-FLS1-rev	ggTTgggTTgATCATTTgTTCC TACTCTCCTTgTTTACTgTggT	838	35	14	16	
ADH2	Tg-ADH2-for Tg-ADH2-rev	TgggAAgCCAAggTAACTAAg TgCAACATTCAATgTTAgCA	1000	>16	12	8	
AATcy	Tg-AATcy-for Tg-AATcy-rev	gCgCCCTgAgCATTgTAAgC gCCTTCAgCTCAATTgTCCA	963-966	-	-	-	
MAPK1	Tg-MAPK1-for Tg-MAPK1-rev	TTggTCTTgCACgAACgAACTC AAAATggCTCTgAgCATACTgg	600	-	-	-	
AP3	Tg-AP3-for Tg-AP3-rev		600	-	-	-	
NDPK	Tg-NDPK-for Tg-NDPK-rev	CTgCAAAgCCCTTCTTCAATgg CAgCAATTCCTTCtgggAACCA					
IDH1	Tg-IDH1-for Tg-IDH1-rev	TgATggAgATgTgCAgAgTgA ACAgTCTTgCgTTgTCATCCA					
IPI1	Tg-IPI1-for Tg-IPI1-rev	TgAAgAgAATgCTCTTggTAg ATCTggATTTgggTgCACgC					
IPI2	Tg-IPI2-for Tg-IPI2-rev	AAgAAAAggCTCTTggTATTg ATCTggATTTgggTgCgCAT					

Workpackage 2: Assessment of genetic diversity in teak over its natural range

Item	Date	Status
Milestones and expected results		
Selection of accessions for genetic analysis	Month 12	Complete
Molecular assessment of cytoplasmic DNA haplotypes and selected nuclear loci	Month 20	Complete
Deliverables		
D4. Molecular inventory of genetic diversity reflecting historical migration patterns – phylogeography	Month 30	Complete

D4. Molecular inventory of genetic diversity reflecting historical migration patterns – phylogeography

A database with sequences of all alleles for all studied chloroplast and nuclear gene loci has been prepared. We are quite confident that this dataset covers all alleles of medium and high frequency in teak. Some low frequency alleles might have been missed in India (especially for the highly polymorphic loci such as G3PDHcp and FLS1). Most of the low frequency alleles from the Thai populations have probably been identified. The lowest frequency alleles in Thailand account for just 1 to 3 chromosomes out of nearly 1000 sampled.

Table3: populations and samples used for WP2

code	name	location	country	longitude	latitude	#	Remarks
Tg1-01	Varhani	Thrissur district, Kerala	India	10°63'N	76°32'E	2	
Tg1-02	Thamaravellachal	Thrissur district, Kerala	India	10°50'N	76°37'E	2	Protected area
Tg1-03	Kaduvappara	Konni district, Kerala	India	9°09'N	77°00'E	2	
Tg1-04	Kattathi	Konni district, Kerala	India	9°10'N	76°57'E	2	Protected area
Tg1-05	Poochappara	Nilambur district, Kerala	India	11°38'N	76°26'E	2	
Tg1-06	Padukka	Nilambur district, Kerala	India	11°20'N	76°21'E	2	Protected area
Tg1-07	Tholpetty	Wyanad district, Kerala	India	11°57'N	76°05'E	2	
Tg1-08	Bavelli	Wyanad district, Kerala	India	11°51'N	76°05'E	2	Protected area
Tg1-09	Varhachal	Kerala	India			2	
Tg1-11	Masale Valley	Karnataka	India	11°55'N	76°10'E	3	Provenance trial, Thailand
Tg2-01	Mahwas	Valsad district, Gujarat	India	20°46'N	73°24E	2	
Tg2-02	Sara	Valsad district, Gujarat	India	20°47'N	73°24E	2	Protected area
Tg2-03	Ghotgharakpur	Madhya Pradesh	India	22°51'N	79°53'E	2	Protected area
Tg2-04	Disharad	Madhya Pradesh	India	22°51'N	79°53'E	2	
Tg3-01		Rajastan	India			2	
Tg4-01	Muhamadabad	Andrapradesh	India			2	
Tg4-02	Bandaguda	Andrapradesh	India			2	
Tg4-03	Adilabad	Andrapradesh	India			2	
Tg4-04		Chhattisghargh	India			2	
Tg4-05	Pech Tiger Reserve	Maharashthra	India			2	
Tg4-06	Allapally Plains	Maharashthra	India	19°23'N	80°07'E	3	Provenance trial, Thailand
Tg5-01	Purunakote	Orissa	India	20°--'N	84°--'E	3	Provenance trial, Thailand
Tg5-02	Bakbahal	Orissa	India	15°04'N	105°53'E	3	Provenance trial, Thailand
Tg5-03	Balunda	Orissa	India	19°52'N	85°05'E	2	Protected area

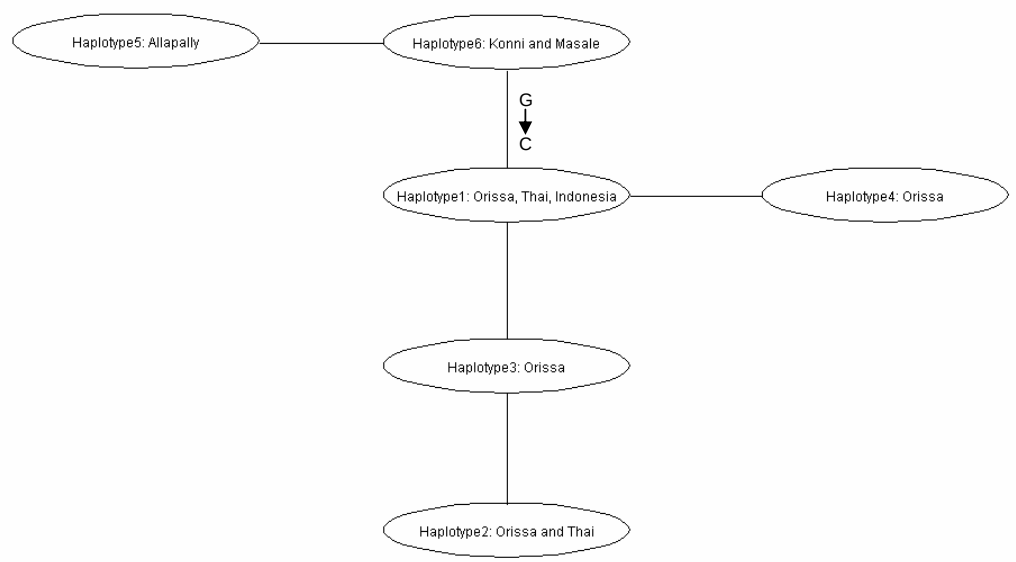
Tg5-04	Ranjin	Orissa	India	19°52'N	85°05'E	2	
Tg6-01	Northern Myanmar		Myanmar			5	Provenance trial, Kerala
Tg7-01	Southern Myanmar		Myanmar			5	Provenance trial, Kerala
Tg8-01	Potharam	Potharam district, Rachaburi	Thailand	13°43'N	99°52'E	3	Highly degraded
Tg8-02	SaiYoke	SaiYoke NP, Kanchanaburi	Thailand	14°23'N	98°52'E	3	Highly degraded
Tg8-03	MaeMoei	MaeMoei NP, Tak	Thailand	17°25'N	98°03'E	3	Low density teak
Tg8-04	ChiangDao	ChiangDao NP, ChiangMai	Thailand	19°40'N	98°54'E	3	Low density teak
Tg8-05	PratuuPhaa	Ngao district, Lampang	Thailand	18°30'N	99°49'E	3	Logged, but still high density teak
Tg8-06	TonSakYai Forest Park	TonSakYai Park, Uttaradit	Thailand	17°39'N	100°34'E	3	Small protected area
Tg8-07	SriSatchanalai	SriSatchanalai NP, SukhoThai	Thailand	17°31'N	99°23'E	3	Heavily logged
Tg8-09	MaeRewa,	MaeWong NP, Kamphaengphet	Thailand	16°--'N	99°--'E	3	Small area, logged
Tg8-11	BanTak-MaeRamat	Mae Wildlife Sanctuary, Tak	Thailand	17°--'N	99°--'E	3	Low density, logged
Tg8-12	ThaTaFang	Salaween NP, MaeHongson	Thailand	18°05'N	97°43'E	3	Heavily logged
Tg8-13	Salaween high altitude	Salaween NP, MaeHongson	Thailand	18°08'N	97°50'E	3	Low density teak
Tg8-14	MaeSalaab	Salaween NP, MaeHongson	Thailand	18°11'N	97°52'E	3	Heavily logged
Tg8-15	Northern Thailand	Northern Thailand	Thailand	17-20°N	98-99°E	34	Individual trees or small clusters
Tg8-16	DongSakNgam	MaeYom NP, Phrae	Thailand	18°45'N	100°11'E	3	Logged, but still high density teak
Tg8-17	LomDong	MaeYom NP, Phrae	Thailand	18°41'N	100°09'E	3	Heavily logged
Tg8-19	BanMaeChua	Denchai district, Phrae	Thailand	17°59'N	100°05'E	3	Very small area protected
Tg8-20	Pongsalee	ChiangRai district, ChiangRai	Thailand	19°50'N	99°47'E	3	Small area
Tg8-21	Savannakhet	Savannakhet Province	Laos	15°04'N	105°53'E	3	Provenance trial, Thailand
Tg8-22	Pakse South	Pakse Province	Laos	16°33'N	104°45'E	3	Provenance trial, Thailand
Tg9-			Indonesia				
Tg9-			Indonesia				
Tg9-			Indonesia				

Scientific results

Chloroplast markers
More than 20,000 base pairs of the chloroplast genome have been sequenced from at least 3 different trees, which accounts for about 12% of a typical plant chloroplast. About a third of this (7,755 bp) has been sequenced in at least 10 trees. In spite of this, very few polymorphisms were observed. The 4 polymorphic sites that were found make up a total of 6 haplotypes that contain phylogenetic useful information. An overview of the chloroplast loci sequenced and the areas studied in detail is given in table1 and figure1.

The phylogenetic network obtained from the chloroplast sites is shown in Figure 2. Though there is little structure in the network, it shows the division between the populations from Southern and Central India from the rest. This division is due to a nucleotide substitution in the psaA fragment. The other polymorphisms are due to changes in number of nucleotides in three mononucleotide repeats.

Figure 2: Phylogenetic network of chloroplast haplotypes



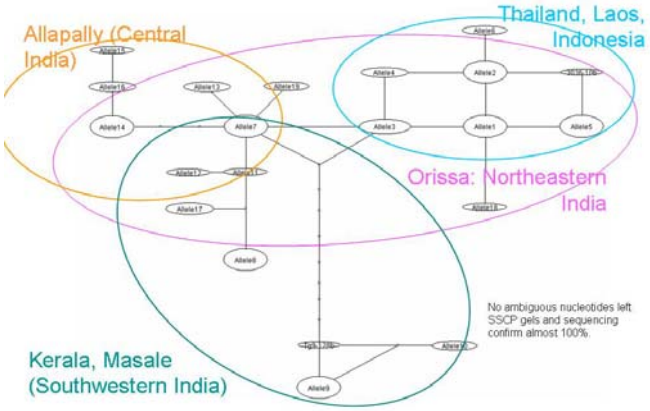
The data on nuclear loci are summarized in table2.

The nuclear haplotype networks are very informative for a phylogeographic interpretation of the diversity in teak. Teak in India is generally much more diverse than in Thailand. Teak in Indonesia contains a subset of the alleles found in Thailand. Some teak trees in southwestern India have haplotypes that are quite distant from the others. Teak in eastern India shares several alleles with teak in southwestern India, and another set of alleles are shared with Thailand and Indonesia. This leads us to conclude that teak originated probably in southwestern India, at some time migrated to eastern India, losing some alleles on the way. During the time though, new alleles were acquired through mutation. In the more recent past, teak spread out even further east into northern Myanmar, and then south and east through Myanmar, Thailand and some parts of Laos. The teak populations arriving in Thailand though had lost a substantial part of its diversity on the way

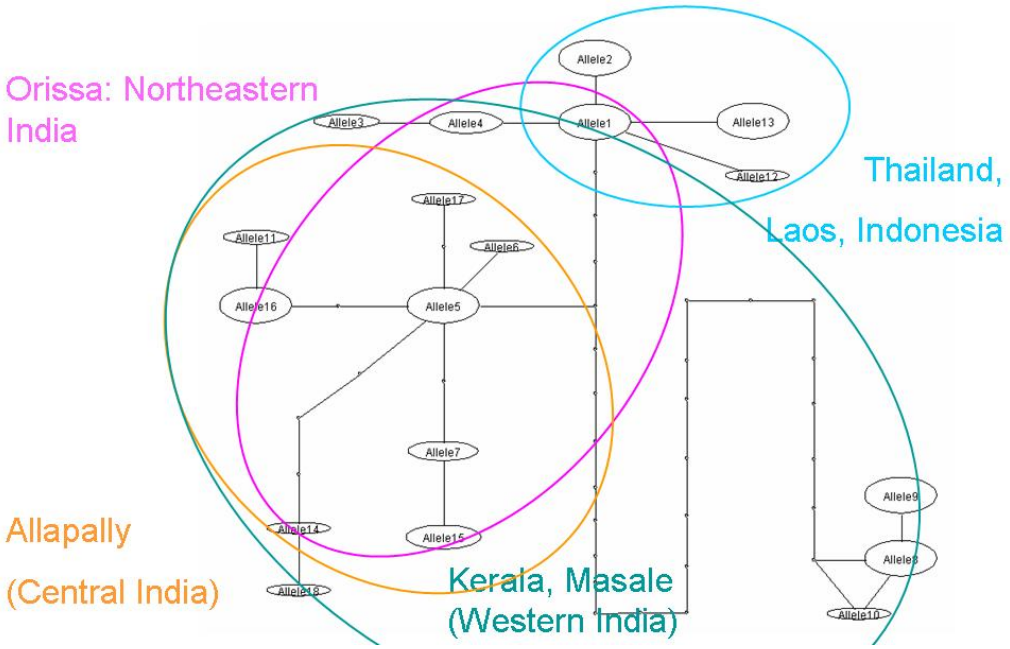
(bottleneck effect). However, teak has been long enough in the region to have acquired a few new mutations. At some time in the past it was even possible for teak populations to move from southern Myanmar to Sumatra and Java in Indonesia. Probably the populations arriving on Java had gone through yet another severe bottleneck, because only a small number of alleles, all except one shared with Thai (and probably Myanmar) populations can be found. No new alleles could be confirmed, thought they might exist. For some trees from Java, an origin from southwestern India resulting from a more recent introduction could be assumed. Only two trees carrying alleles typically found in southwestern India (2 loci) could be confirmed.

Figure 3: Phylogenetic network of nuclear alleles. a: CAT1 haplotype network; b: G3PDHcy2 haplotype network

a



b


Orissa: Northeastern India
Thailand, Laos, Indonesia
Allapally (Central India)
Kerala, Masale (Western India)
Allele2
Allele3
Allele4
Allele1
Allele13
Allele12
Allele17
Allele11
Allele6
Allele16
Allele5
Allele7
Allele9
Allele14
Allele15
Allele8
Allele18
Allele10

Workpackage 3: Population genetic diversity in teak forests

Item	Date	Status
Milestones and expected results		
Selection of accessions for genetic analysis	Month 12	Complete
Collection of tissue samples and DNA extraction	Month 24	Complete
Molecular inventory of genotypes at selected nuclear gene loci and microsatellites	Month 30	Complete
Deliverables		
D5. Quantification of genetic diversity in natural populations, reflecting long-term gene flow and mating system	Month 32	Complete

Notes on deliverables, milestones and expected results

Identification of populations and accessions for analysis

All partners have chosen sites for collection and analysis. Collection of samples has been completed. A list of populations and sampling information is given in table 4.

D5. Quantification of genetic diversity in natural populations, reflecting long-term gene flow and mating system

All populations were assayed for a minimum of three nuclear gene loci. Additionally, all the Indian populations were assayed for three microsatellite loci.

Table 4: populations and samples used for WP3

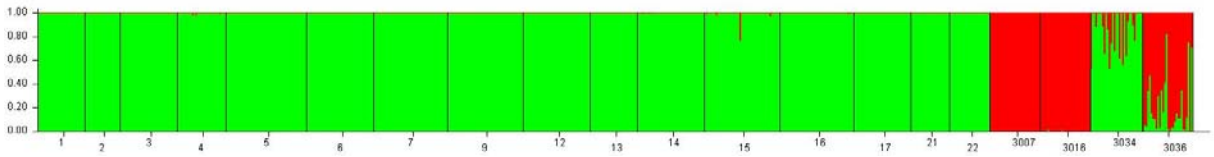
code	name	location	country	longitude	latitude	#	Remarks
Tg1-01	Varhani	Thrissur district, Kerala	India	10°63'N	76°32'E	40	
Tg1-02	Thamaravellachal	Thrissur district, Kerala	India	10°50'N	76°37'E	40	Protected area
Tg1-03	Kaduvappara	Konni district, Kerala	India	9°09'N	77°00'E	40	
Tg1-04	Kattathi	Konni district, Kerala	India	9°10'N	76°57'E	40	Protected area
Tg1-05	Poochappara	Nilambur district, Kerala	India	11°38'N	76°26'E	32	
Tg1-06	Padukka	Nilambur district, Kerala	India	11°20'N	76°21'E	40	Protected area
Tg1-07	Tholpetty	Wyanad district, Kerala	India	11°57'N	76°05'E	40	
Tg1-08	Bavelli	Wyanad district, Kerala	India	11°51'N	76°05'E	40	Protected area
Tg1-09	Varhachal	Kerala	India			40	
Tg1-11	Masale Valley	Karnataka	India	11°55'N	76°10'E	30	Provenance trial, Thailand
Tg2-01	Mahwas	Valsad district, Gujarat	India	20°46'N	73°24E	40	
Tg2-02	Sara	Valsad district, Gujarat	India	20°47'N	73°24E	40	Protected area
Tg2-03	Ghotgharakpur	Madhya Pradesh	India	22°51'N	79°53'E	40	Protected area
Tg2-04	Disharad	Madhya Pradesh	India	22°51'N	79°53'E	40	
Tg3-01		Rajastan	India			40	
Tg4-01	Muhamadabad	Andrapradesh	India			40	
Tg4-02	Bandaguda	Andrapradesh	India			40	
Tg4-03	Adilabad	Andrapradesh	India			40	
Tg4-04		Chhattisghargh	India			40	
Tg4-05	Pech Tiger Reserve	Maharashthra	India			40	
Tg4-06	Allapally Plains	Maharashthra	India	19°23'N	80°07'E	30	Provenance trial, Thailand
Tg5-01	Purunakote	Orissa	India	20°--'N	84°--'E	31	Provenance trial, Thailand

Tg5-02	Bakbahal	Orissa	India	15°04'N	105°53'E	30	Provenance trial, Thailand
Tg5-03	Balunda	Orissa	India	19°52'N	85°05'E	40	Protected area
Tg5-04	Ranjin	Orissa	India	19°52'N	85°05'E	40	
Tg8-01	Potharam	Potharam district, Rachaburi	Thailand	13°43'N	99°52'E	28	Highly degraded
Tg8-02	SaiYoke	SaiYoke NP, Kanchanaburi	Thailand	14°23'N	98°52'E	23	Highly degraded
Tg8-03	MaeMoei	MaeMoei NP, Tak	Thailand	17°25'N	98°03'E	34	Low density teak
Tg8-04	ChiangDao	ChiangDao NP, ChiangMai	Thailand	19°40'N	98°54'E	29	Low density teak
Tg8-05	PratuuPhaa	Ngao district, Lampang	Thailand	18°30'N	99°49'E	48	Logged, but still high density teak
Tg8-06	TonSakYai Forest Park	TonSakYai Park, Uttaradit	Thailand	17°39'N	100°34'E	44	Small protected area
Tg8-07	SriSatchanalai	SriSatchanalai NP, SukhoThai	Thailand	17°31'N	99°23'E	45	Heavily logged
Tg8-09	MaeRewa,	MaeWong NP, Kamphaengphet	Thailand	16°--'N	99°--'E	35	Small area, logged
Tg8-12	ThaTaFang	Salaween NP, MaeHongson	Thailand	18°05'N	97°43'E	35	Heavily logged
Tg8-13	Salaween high altitude	Salaween NP, MaeHongson	Thailand	18°08'N	97°50'E	28	Low density teak
Tg8-14	MaeSalaab	Salaween NP, MaeHongson	Thailand	18°11'N	97°52'E	35	Heavily logged
Tg8-16	DongSakNgam	MaeYom NP, Phrae	Thailand	18°45'N	100°11'E	35	Logged, but still high density teak
Tg8-17	LomDong	MaeYom NP, Phrae	Thailand	18°41'N	100°09'E	35	Heavily logged
Tg8-21	Savannakhet	Savannakhet Province	Laos	15°04'N	105°53'E	30	Provenance trial, Thailand
Tg8-22	Pakse South	Pakse Province	Laos	16°33'N	104°45'E	26	Provenance trial, Thailand
Tg9-			Indonesia				
Tg9-			Indonesia				
Tg9-			Indonesia				

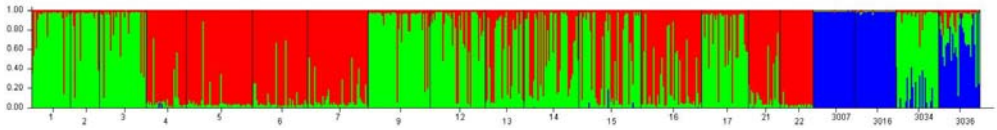
Scientific results

Using 6 nuclear gene loci, analysed by SSCP and sequencing, STRUCTURE readily differentiates the populations from southwestern India (Allapally and Masale) from the rest. Assuming three origin populations, the program divides the Thai populations in two. With 4 or more original populations, no new insights are gained. Likelihood values for more than 3 populations also do not increase as rapidly any more.

K = 2



K = 3



K = 4

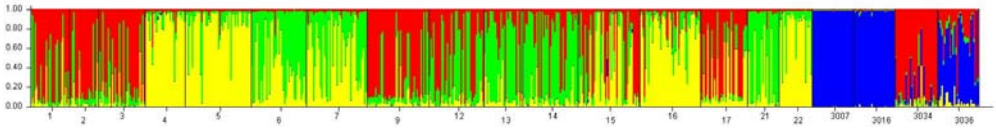
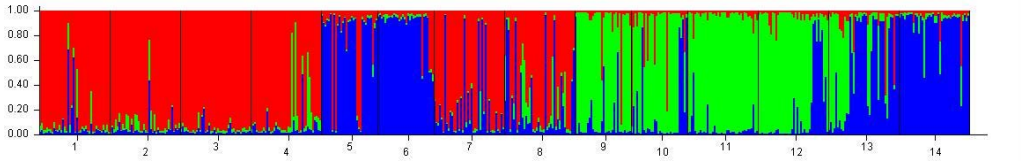


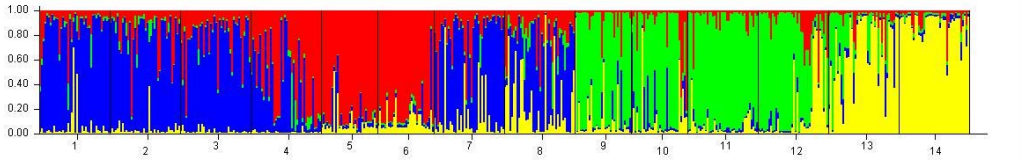
Figure 4 population assignment based on 6 nuclear gene loci
1 = Tg8-01, 2 = Tg8-02, 3 = Tg8-03, 4 = Tg8-04, 5 = Tg8-05, 6 = Tg8-06, 7 = Tg8-07, 9 = Tg8-09, 12 = Tg8-12, 13 = Tg8-13, 14 = Tg8-14, 15 = Tg8-15, 16 = Tg8-16, 17 = Tg8-17, (all Thailand), 21 = Tg8-21, 22 = Tg8-22 (Laos), 3007 = Tg4-06 Allapally (Maharashthra), 3016 = Tg1-11 Masale (Karnataka), 3034 = Tg5-01 Purunakote, 3036 = Tg5-02 Bakbahal, (both Orissa)

Using the STRUCTURE analysis for 4 microsatellite loci, the 8 Kerala populations and 6 populations from other parts of India are clearly divided in two separate groups. Assuming 3 original populations, also here a further split occurs within the Kerala populations. With a set of four assumed original populations, the Orissa populatins separate from the other populations. With 5 or more assumed origins, the likelihood improves gradually, but there essentially remains a separation in 4 geographic regions.
K = 2

K = 3



K = 4



K = 5

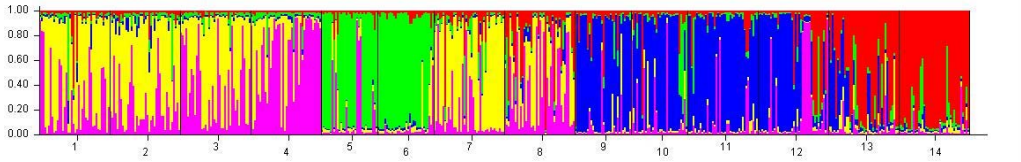


Figure 5: Population assignment based on 4 microsatellite loci
1 = Tg1-01, 2 = Tg1-02, 3 = Tg1-07, 4 = Tg1-08, 5 = Tg1-05(Poochappara, Nilambur district), 6 = Tg1-06 (Padukka, Nilambur district), 7 = Tg1-03, 8 = Tg1-04, 9 = Tg2-02, 10 = Tg2-01, 11 = Tg2-03, 12 = Tg2-04, 13 = Tg5-03 (Balunda, Orissa), 14 = Tg5-04 (Ranjin, Orissa).

Analysis of the two datasets clearly indicate a split between Kerala and Central India populations (Tg1, Tg2 and Tg4) on the one hand and the Orissa populations grouping together with the Thai and Laotian populations, which can be combined with the Indonesian samples. A separation of the Kerala populations from the rest of Central India is quite likely. More detailed analysis with Thai and Laotian populations only indicates that two teak provenances can be distinguished (south and west vs. east) with a transition zone in between. The exact area of each of the Thai zones still need to be established more in detail. A separate analysis of the populations from Kerala also lends support for two subregions, with the Nilambur area slightly different from the rest of Kerala.

The largest proportion of the genetic diversity is within populations, with only a small fraction of the diversity that can be attributed to differences between populations

Workpackage 4: Gene flow through pollen and seed dispersal

Item	Date	Status
Milestones and expected results		
Selection, inventory and mapping of populations	Month 12	Completed
Collection of seed and tissue samples and DNA extraction	Month 18	Completed
Microsatellite fingerprints of seedlings and mature trees	Month 26	Completed
Deliverables		
D6. A pollen dispersal curve, for teak in natural forests and plantations	Month 32	Completed
D7. Estimation of the variation in male mating success	Month 32	Completed

Notes on deliverables, milestones and expected results

Identification of populations and accessions for analysis

All partners have chosen sites for collection and analysis. Collection has been completed. Maps of the plots are available.

D6. A pollen dispersal curve, for teak in natural forests and plantations

D7. Estimation of the variation in male mating success

Pollination was studied in a seed orchard in Indonesia, a plantation in Kerala, India and in natural stands in Indonesia, Kerala and Thailand.

Table 5: populations and samples used for WP4

code	name	location	country	longitude	latitude	adults	Embryos/seed	regeneration
Tg1-01	Varhani	Thrissur district, Kerala	India	10°63'N	76°32'E	109	102	0
Tg1-02	Thamaravellachal	Thrissur district, Kerala	India	10°50'N	76°37'E	178	180	100
Tg1-03	Kuthiran	Konni district, Kerala	India	10°34'N	76°22'E	200	135	0
Tg8-09	MaeRewa,	MaeWong NP, Kamphaengphet	Thailand	16°--'N	99°--'E	182	>200	15
Tg8-14	MaeSalaab	Salaween NP, MaeHongson	Thailand	18°11'N	97°52'E	89	>200	12
Tg8-16	DongSakNgam	MaeYom NP, Phrae	Thailand	18°45'N	100°11'E	184	>200	20
Tg8-17	LomDong	MaeYom NP, Phrae	Thailand	18°41'N	100°09'E	86	>200	10
Tg9-	Java	Cepu, Seed orchard	Indonesia					
Tg9-			Indonesia					
Tg9-	Muna	Muna Island	Indonesia					

Scientific results

Gene flow in Indonesia seed orchard

Kerala

Seven microsatellite markers were amplified and used in the analysis. The number of observed alleles for 7 the microsatellite markers varied between 2 and 8 in the disturbed population, between 3 and 9 in the undisturbed population as well as between 3 and 8 in the plantation, with overall mean number of alleles per locus of 5.86 in the disturbed population, 6.71 in the undisturbed population and 6.29 in the plantation. A total of 49 distinct alleles were obtained from the parent generation and

the allelic richness in parental population was found to retain in the progeny population.

Table . Number of Alleles in selected parental populations

Population type		Locus name						
		AC01	AC28	AG04	AG14	AG16	AC44	CPI-MS
Disturbed	parents	8	5	5	7	6	7	2
	progeny	7	5	5	6	6	6	3
Undisturbed	parents	7	5	8	8	8	6	3
	progenies	8	5	8	8	9	6	3
Plantation	parents	8	7	5	7	7	7	3
	progenies	8	7	5	7	6	6	3

The overall observed mean number of alleles is slightly lower in disturbed population than in undisturbed and plantation populations.

Table . Allelic richness in populations

Population type		Locus name						
		AC01	AC28	AG04	AG14	AG16	AC44	CPI-MS
Disturbed	Parents	6.987	5.000	4.999	6.000	6.000	6.000	2.000
	Progenies	7.464	5.000	5.000	6.851	6.000	6.873	2.458
Undisturbed	Parents	8.000	4.906	6.999	7.000	8.887	5.000	2.999
	Progenies	7.928	4.846	7.444	7.710	8.305	5.989	2.978
Plantation	Parents	8.000	5.998	4.992	6.901	6.000	6.000	2.887
	Progenies	7.997	6.470	5.000	6.999	6.962	6.353	3.000
Locus allelic richness		7.926	6.470	5.000	6.933	6.991	6.485	3.000

The allelic richness was similar between parental population and progenies and also shows not much difference between plantation as well as disturbed and undisturbed natural teak populations.

Polymorphic Information Content

Polymorphic Information Content (PIC) is a measure of informativeness related to expected heterozygosity and is calculated from allele frequencies (Botstein *et al.,* 1980; Hearne *et al.,* 1992). Genetic markers showing PIC value higher than 0.5 are normally considered as informative in population-genetic analyses (Botstein *et al.* 1980). The average PIC for the samples are 0.603 for plantation, 0.586 for disturbed natural teak forest and 0.501 for undisturbed natural teak forest (Table 3 and Fig. …).

Table . Polymorphic Information Content

Locus Name	Kuthiran (plantation)	Thamaravellachal (disturbed forest)	Varhani (undisturbed forest)
AC01	0.609	0.455	0.605
AC28	0.440	0.263	0.280
AG04	0.609	0.409	0.690
AG14	0.790	0.756	0.782
AG16	0.772	0.773	0.775
AC44	0.642	0.264	0.321
CPI-MS	0.361	0.588	0.650
Mean PIC	0.603	0.501	0.586

Hence, the allelic richness and the average PIC value of the selected populations shows that the resolving power of the loci is sufficient and the output is suitable for unbiased estimation of individual reproductive success and its parentage, as well as for pedigree reconstruction. The information on allelic richness is also necessary for the scientific management of seed orchards.

Maximum Number of Alleles

Figure: 1 AC01 Thamaravellachal

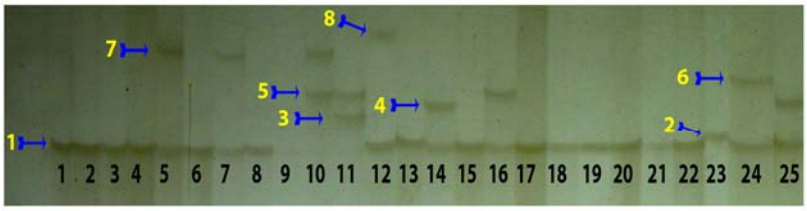


Figure: 2 AC28 Kuthiran

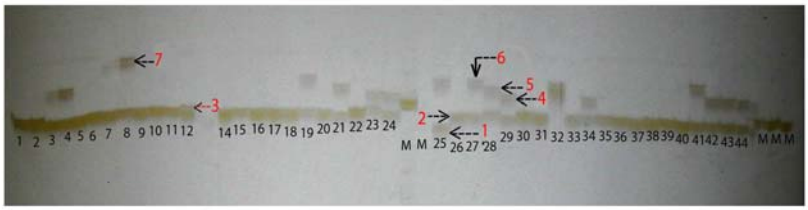


Figure: 3 AG04 Vazhani

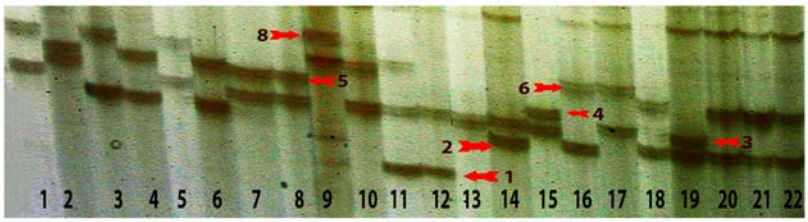
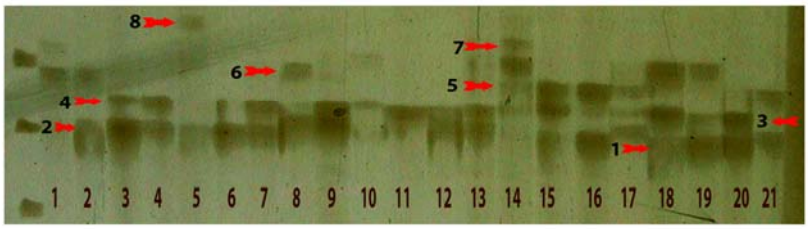


Figure: 4 AG14 Vazhani



Observed and expected heterozygosity estimates were from allele frequencies assuming Hardy-Weinberg equilibrium (Nei, 1987) as calculated using an unbiased formula executed in Cervus software. Expected heterozygosity is a useful measure

of informativeness of a locus. Loci with expected heterozygosity of 0.5 or less are in general not very useful for large-scale parentage analysis.

The mean observed heterozygosity in disturbed and undisturbed forests are 0.4764 and 0.5843, which were lower than the expected heterozygosity 0.5293 and 0.6215 respectively. In teak plantation, observed heterozygosity is slightly larger (0.6496) than the expected heterozygosity (0.6389) (Table).

Gene diversity calculated through Fstat software shows values higher in plantation (0.651) than undisturbed population (0.614) and disturbed population (0.563) (Table).The gene diversity difference between parental population and its progenies revealed that there is diversity loss in plantation (0.038) and disturbed forest (0.079), but the higher value of 0.010 was found in undisturbed population.

Table … Gene diversity and Heterozygosity

Disturbed forest				
Locus name	H_o	H_e	Gene diversity	
			Parents	Progeny
AC01	0.430	0.474	0.518	0.421
AC28	0.251	0.273	0.340	0.209
AG04	0.431	0.435	0.516	0.348
AG14	0.663	0.787	0.823	0.729
AG16	0.613	0.804	0.789	0.774
CPI-MS	0.274	0.312	0.307	0.317
AC44	0.673	0.620	0.645	0.590
Mean	0.4764	0.5293	0.563	0.484
Undisturbed forest				
AC01	0.551	0.636	0.566	0.688
AC28	0.314	0.296	0.294	0.298
AG04	0.683	0.727	0.742	0.702
AG14	0.721	0.811	0.816	0.800
AG16	0.567	0.805	0.806	0.800
CPI-MS	0.361	0.385	0.401	0.372
AC44	0.893	0.691	0.670	0.708
Mean	0.5843	0.6215	0.614	0.624
Plantation				

AC01	0.660	0.637	0.649	0.613
AC28	0.446	0.463	0.487	0.427
AG04	0.633	0.655	0.673	0.624
AG14	0.766	0.818	0.813	0.811
AG16	0.751	0.803	0.794	0.791
CPI-MS	0.414	0.423	0.470	0.344
AC44	0.877	0.674	0.671	0.676
Mean	0.6496	0.6389	0.651	0.613

Inbreeding Coefficient (*F*is)

The heterozygote deficiencies or the inbreeding coefficient with in population were estimated as *F*is is calculated through Fstat. The average inbreeding estimates in disturbed population is 0.070, in undisturbed population is 0.018 and in teak plantation is -0.033 (Table).

Table … Inbreeding Coefficient (F*is*)

Disturbed forest		
Locus name	Inbreeding coefficient (*F*is)	P-value for *F*is (5% nominal level adjusted is 0.00238)
AC01	0.069	0.0833
AC28	0.083	0.0976
AG04	-0.034	0.7619
AG14	0.129	0.0024
AG16	0.303	0.0024
CPI-MS	0.014	0.5000
AC44	-0.185	1.0000
Mean	0.070	0.0024 *
Undisturbed forest		
AC01	0.038	0.2690
AC28	-0.122	1.0000
AG04	0.036	0.2571

AG14	0.139	0.0071
AG16	0.233	0.0024
CPI-MS	-0.136	0.9643
AC44	-0.270	1.0000
Mean	0.018	0.2048 NS
Plantation		
AC01	-0.040	0.8643
AC28	0.014	0.3857
AG04	0.056	0.1071
AG14	0.025	0.2595
AG16	-0.018	0.6857
CPI-MS	0.063	0.1667
AC44	-0.305	1.0000
Mean	-0.033	0.9881 NS

Gene flow through *F*st and Nm value

*F*st is the fixation index, which measures variation in allele frequencies among populations. By using Fstat software, the *F*st value was calculated based on 7 microsatellite markers. The *F*st value evaluated in the studied population with its progenies was 0.024 in disturbed plot. The value (0.010) obtained is similar in both undisturbed and plantation of teak with its next generation progenies. In disturbed plot the *F*st value has calculated with its forest floor seedlings was found to be 0.015. The *F*st value was used to evaluate gene flow parameter *N*m, the product of the effective size of individual populations (*N*) and the rate of migration among them (m) .The estimates of *N*m value among three selected teak populations and individual population with next generation was also calculated using the formula, *N*m= 1/4(1/ *F* st-1). The *N*m value obtained from the excecution of whole parental population with its next generated teak population in disturbed plot was found to be 10.17. The Nm value obtained from both undisturbed and teak plantation was remains the same as 24.75. The gene flow in the disturbed population with its forest floor seedlings was found to be 16.42.

Population type	*F*st	Nm
Disturbed(Parents	0.024	10.17

with Progenies)		
Disturbed(Parents with Forest floor seedlings)	0.015	16.42
Undisturbed(Parents with Progenies)	0.010	24.75
Plantation((Parents with Progenies	0.010	24.75

Parameter	**Parents**	**Seeds**	**Saplings on forest floor**
Undisturbed natural teak			
Gene diversity	**0.613**	**0.624**	**-**
Allelic richness	**6.290**	**6.094**	**-**
Inbreeding (Fis)	**0.022**	**0.072**	**-**
(Fst/Gst')Genetic differentiation	between parents **0.016**		**-**
Disturbed natural teak			
Gene diversity	**0.570**	**0.494**	**0.584**
Allelic richness	**5.748**	**4.856**	**6.255**
Inbreeding (Fis)	**0.108**	**0.072**	**0.071**
(Fst/Gst') Genetic differentiation	between parents and seeds **0.222**		between parents and seedlings **0.022**
Plantation			
Gene diversity	**0.663**	**0.608**	**-**
Allelic richness	**6.651**	**5.405**	**-**
Inbreeding (Fis)	**-0.006**	**-0.011**	**-**
(Fst/Gst') Genetic differentiation	between parents **0.025**		**-**

Cervus Analysis

The data collected with respect to all the progenies and adult trees in each population was used for analysis by Cervus version 3. Cervus analyses genetic data from co-dominant genetic markers such as microsatellites (STRs), SNPs and allozymes. It assumes that markers are autosomal and that the species is diploid. It also assumes that markers are inherited independently of each other, in other words that they are unlinked.

The heterozygous and homozygous individuals were identified from all the selected populations, as the microsatellite markers are codominant. The allele identification

was done for all the markers. The data collected with respect to all adult trees and progenies were analysed through the software Cervus version 3. The genotypic fingerprints of each of the seedlings/embryos were compared with all the adult trees in respective plots find out the potential pollen parents or pollen donors.

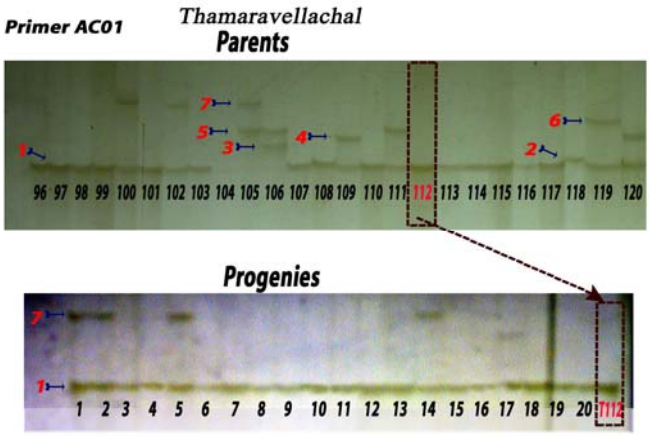


Figure Comparison of alleles from progenies with mother parent

Through maximum likelihood method, by comparing each and every band, the male parent could be identified. From this the distance between seed parent and the pollen parent was measured, which gave the structure of pollen dispersal. Through the analysis of the fingerprints, the parents of the seedlings in the forest floor were identified. Once the parents are identified the distance of seeds moved from the mother parent could be measured, which gave the structure of seed dispersal. The

closest parent was taken as the mother parent, as was done usually. The percentage of natural crossing and selfing was estimated from the data generated through parentage analysis. Parentage testing allowed for selfing and other forms of inbreeding.

A simple approach to parentage analysis relies on a process of exclusion. The genotypes of candidate parents are compared against the offspring's genotype (taking account of the other parent's genotype, if available), and are excluded as parents if a mismatch occurs at one or more loci. With few candidate parents and highly polymorphic loci, this process should usually leave just a single non-excluded candidate parent. However in less favourable circumstances it is common that multiple candidate parents remain non-excluded. In this case the exclusionary approach is inadequate because there is no way to identify which non-excluded candidate parent is the true parent.

Likelihood, on the other hand, can be used to statistically distinguish non-excluded candidate parents. For each locus likelihood captures two sources of information about the candidate parent that exclusion does not:

1. The frequency of the offspring allele or alleles that could have come from candidate parent.
2. Whether or not the candidate parent is heterozygous or homozygous.

The purpose of Cervus is to use this information to identify the candidate parent that is most likely to be the true parent.

Seed setting in selected teak populations is presented in Table … In disturbed population seed setting was found to be maximum with 47.39 per cent. Around 38.89 seeds produced only single embryo while, 6.21 per cent seeds produced two embryos, 1.63 per cent produced three embryos, 0.65 per cent produced four embryos and rest of the seeds produced any embryos. In plantation population, the percentage of seed setting was around 32.31 per cent. Here four embryo presented seeds amounts 0.28 per cent, 0.58 per cent seeds presented three embryos, two embryos were presented by 3.34 per cent seeds and 28.13 per cent of the seeds presented single embryo. Compared to other populations, seed setting in the undisturbed population was found to be the least (19.3 per cent). In this population

seeds producing three or four embryos were absent and the percentage of seeds producing single embryo was found to be 18.2 per cent followed by two embryo producing seeds (1.1 per cent).

Table … Seed setting in selected teak populations

Population type	No. of seeds	1 embryo	2 embryo	3 embryo	4 embryo	0 embryo	% Seed setting
Disturbed	306	119	19	5	2	151	47.39
Undisturbed	456	83	5	0	0	368	19.3
Plantation	359	101	12	2	1	243	32.31

Contemporary gene flow and mating system

Pollen dispersal was traced in natural teak forest at Thamaravellachal and Varhani representing disturbed and undisturbed populations based on human impact. For comparison, a plantation at Kuthiran was also included in the study. Around 180 progenies from disturbed, 109 from undisturbed and 135 from plantation were collected. Seed setting was poor in teak and hence embryos were used to obtain the progeny requirement in all the three plots.

Pollen Dispersal in the disturbed population

After fingerprinting the 178 potential parents and the 180 progenies with seven microsatellite markers, the genotypic fingerprints of each of the seedlings/embryos were compared with all the adult trees to find out the potential pollen parents or pollen donors. Through maximum likelihood method, by comparing each and every band, the male parent could be identified. From this, the distance between seed parent and the pollen parent was measured, which gave the structure of pollen dispersal.

The result of the analysis showed that the distance of pollen flow was maximum in disturbed population (414 m). Main distance range of pollen flow was found to be 151- 200 m (Fig. ..). Here, out of the total 180 progenies, 42 have their male parents within the main distance range (151-200), which contributes to 23.33 per cent of the total progenies. This was followed by the male parents within a range of 101-150 m and constitutes 17.78 per cent of the progenies. Only one of the progeny has got the pollen parent in the maximum distance i.e., 414 m which represent only 0.56 per cent of the total.

Table 3.Percentage of progenies receiving pollen from different distance classes

Distance of pollen flow (m)	Number of progenies	Percentage of progenies
1—50	21	11.67
51—100	31	17.22
101—150	32	17.78
151—200	42	23.33
201—250	17	9.44
251—300	13	7.22
301—350	11	6.11
351—400	5	2.78
401—450	1	0.56

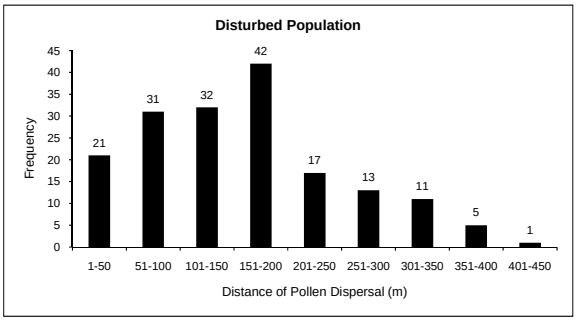


Fig. .. Frequency of Pollen Dispersal in different distance classes

Crossing and Selfing

It is understood from the studies of mating system that, cross pollination explicitly dominated in the sample progenies (Table ..). Out of the total 180 progenies, 173 were cross fertilized (96.11 per cent) and rest was self fertilized. The increased percentage of cross pollination helps the teak to be highly diverse genetically.

Table 3. Pattern of pollination in the sampled progenies

Pollination pattern	No. of progenies	Percentage of progenies
Cross pollinated	173	96.11
Self pollinated	7	3.89
Grand total	180	100.00

Fertility pattern

Female fertility pattern of teak population in this plot showed that female parents receive pollen from almost all sides (Fig. .). It is also revealed that most of the individual mother trees are pollinated by many pollen donor trees. The number of pollen donors ranged from 13 to 19. A total of 91 teak trees (52.3 per cent) contributed pollen to produce the 180 progenies analysed. Out of the total 91 male parents a large proportion i.e., 69 per cent, crossed with only one female parent while 23 per cent of male donors crossed with 2 female trees. A single male parent (tree number 165) contributed pollen to a maximum of 7 female parents and producing 11 offspring. Two of the trees (tree number 101 and 106) has produced 5 and 6 progenies by donating pollen to 5 different females and another two trees (tree 98 and 79) produced 7 progenies by crossing with 6 and 3 different female parents. One of the teak tree (number 2), has produced 6 progenies through crossing with 2 different females.

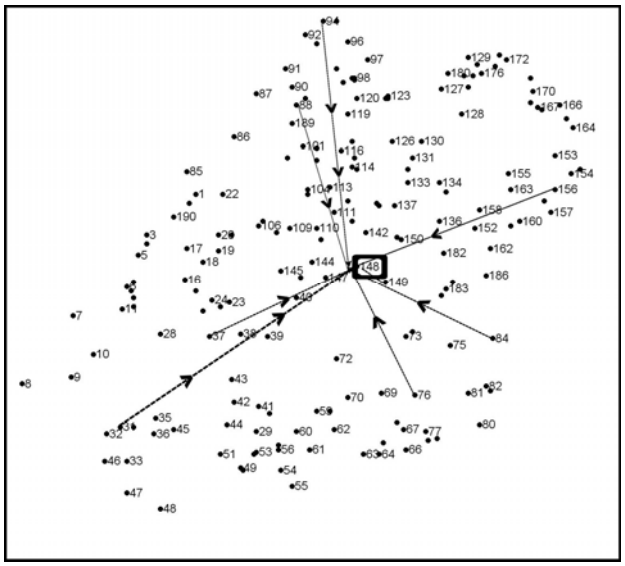


Fig . Pollen flow pattern from different pollen donors to mother tree

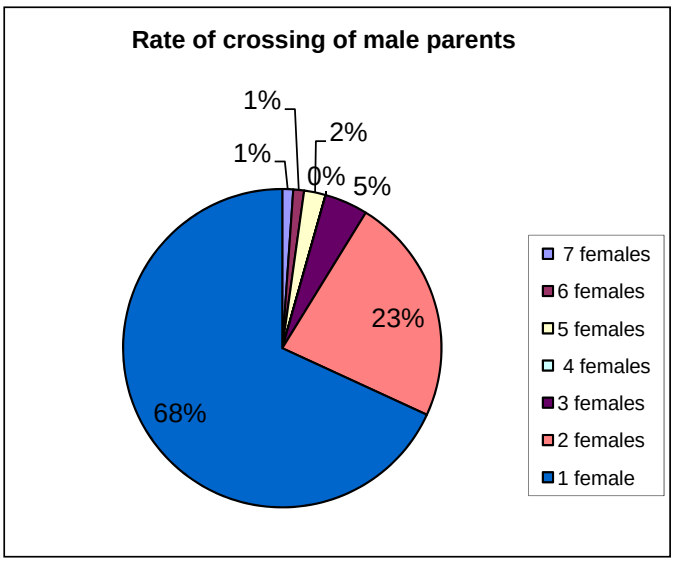


Fig 3. Percentage of male parents crossed with one or more females

Pollen Dispersal in the undisturbed population

The analysis of pollen dispersal with 109 adult teak trees and 102 progenies from undisturbed population shows that, the pollen transfer is mainly in the range of 101-150 m. That is 27 progenies (26.47 per cent), have their male parents within this

distance range. Here only two of the progenies (1.96 per cent) have their male parents in the maximum distance of 300 m. 2.94 per cent of the progenies have their male parents in the distance range of 201-250 m (Table and Fig.).

Table 3.Percentage of progenies receiving pollen from different distance classes

Distance of pollen flow (m)	Number of progenies	Percentage (%)
1-50	18	17.65
51-100	14	13.73
101-150	27	26.47
151-200	22	21.57
201-250	3	2.94
251-300	2	1.96

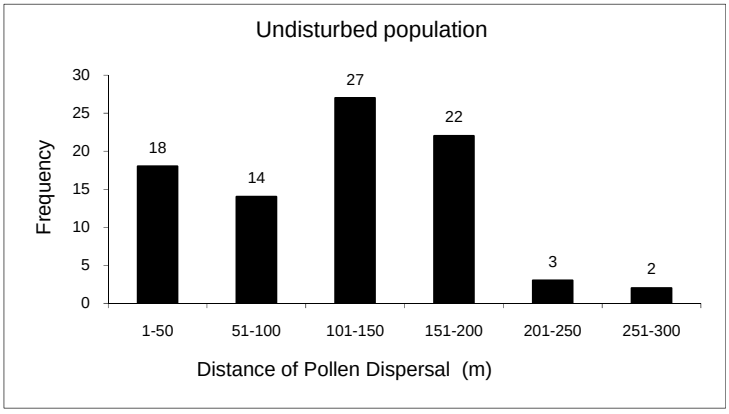


Fig. . Frequency of pollen dispersal in different distance classes

Crossing and Selfing

From the studies on mating system revealed that, out of 109 progenies analysed, 11 were found to be self-pollinated and the percentage was 10.1. Out of these11 progenies, 7 were produced by the self-pollination of a single tree and 4 of them were produced by the self-pollination of another tree. The remaining 98 progenies, 86 (78.9 per cent) were produced by cross-pollination with the male parents within the plot and 12 progenies (11.01 per cent) were produced by the pollination of male parents outside the plot. So the total cross-pollinated progenies were 88.91 per cent (Table). Here the increased percentage of self pollination with a single tree may be due to the reason of flowering early than the other trees.

Table . Pattern of pollination in the sampled progenies

Pollination pattern	No. of progenies	Percentage of progenies
Cross pollinated	98	88.91
Self pollinated	11	10.1
Pollinated out side the plot	12	11.01
Grand total	109	100.00

Fertility pattern

It is also understood from the undisturbed natural teak forest that an individual mother trees are pollinated by many pollen donor trees from almost all sides of the plot (Fig.) and it ranged from 4-13. Male fertility pattern shows that individual pollen parents are donating pollen to many mother parent trees. The maximum pollination capacity of a single tree (tree number 51) is found to be 8, out of which 7 were self pollinated ones. Here out of 51, two of the trees (tree number 50 and 57) donated pollen to 3 different female parents and produced 5 progenies as well as one of the tree (Tree number 12) has donated pollen to 2 different female parents and produced 5 progenies. (Appendix 2).

In this plot, out of 109 trees, 50 per cent (51 trees) contributed pollen to 9 female parents to produce 86 crossed progenies and 11 self-pollinated progenies.

Out of these 51 trees, 33 male parents (65 per cent) which, donated pollen to only one female parent, 15 male parents (29 per cent) donated to two female parents and 3 male parents (6 per cent) donated pollen to 3 different female parents. Only a maximum of 3 different female parents were pollinated by a single male parent (Fig.)

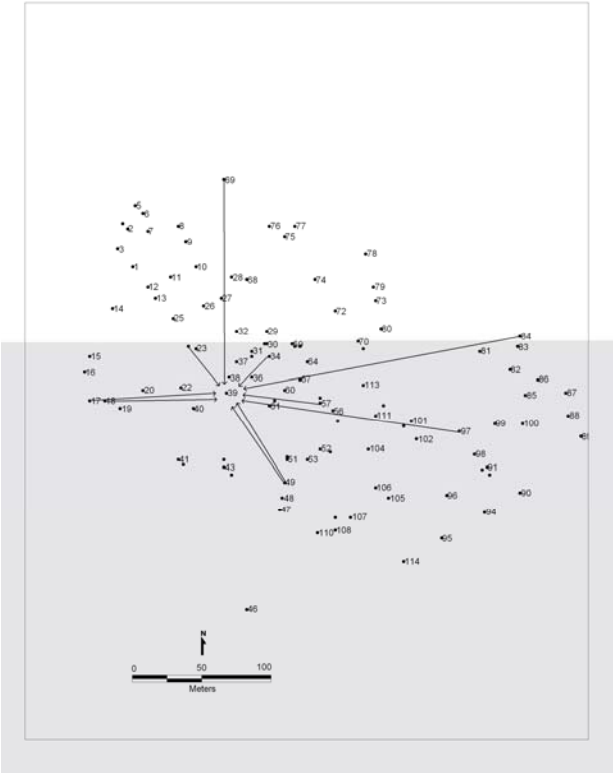


Fig . Pollen flow pattern from different pollen donors to mother tree

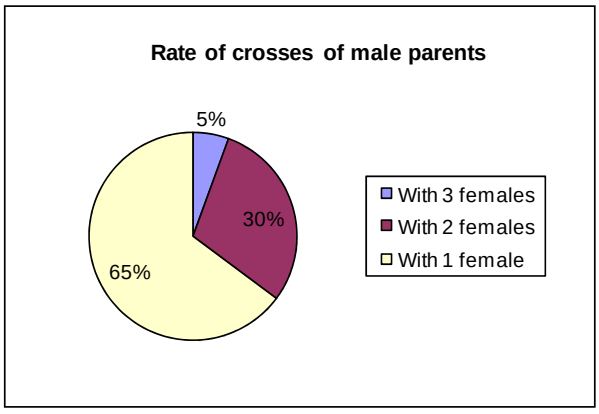


Fig 3. Percentage of male parents crossed with one or more females

Pollen dispersal in the plantation

With regard to the teak plantation, this result shows that the pollen dispersal is mainly in the range of 51-100 m. Out of the total 135 progenies tested, 42 progenies (31.11 per cent) have their male parents within this main distance range followed by 38 progenies (28.15 per cent) having their male parents in 1-50 m distance range. Five of the progenies (2.963 per cent) have their male parents in a maximum distance range of 201-250 m (Fig.).

Table 3.Percentage of progenies receiving pollen from different distance classes

Distance of Pollen flow (m)	Number of progenies	Percentage of progenies
1-50	38	28.15
50-100	42	31.11
101-150	27	20
151-200	14	10.37
201-250	4	2.963

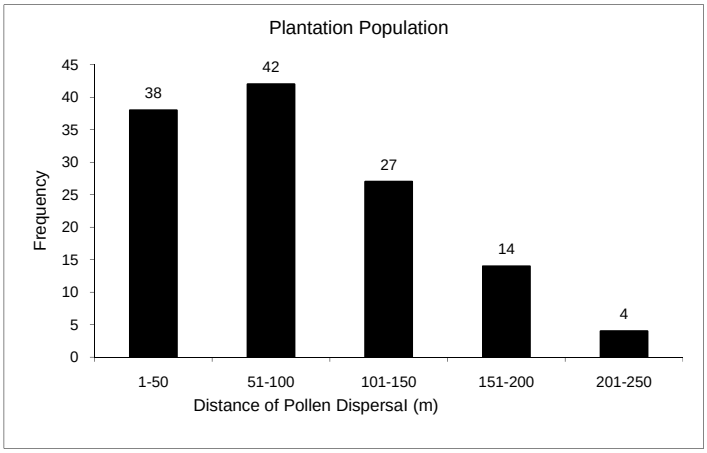


Fig. . Frequency of pollen dispersal in different distance classes

Crossing and Selfing

It is revealed from the studies of mating system that, cross pollination explicitly dominated in the sample progenies (Table ..). Out of the total 135 progenies, five (3.70 per cent) were self-pollinated. The remaining 130 progenies, 125 (92.59 per cent) were produced by cross pollination with male parents within the plot and 5 progenies (3.7 per cent) were produced by pollination of male parents out side the plot. So, the total cross pollinated progenies were 96.3 per cent. The increased percentage of cross pollination helps the teak to be highly diverse genetically.

Table . Pattern of pollination in the sampled progenies

Pollination pattern	No. of progenies	Percentage of progenies
Total cross pollinated	130	96.3
Self pollinated	5	3.7
Pollination from outside the plot	5	3.7
Grand total	135	100.00

Fertility pattern

Fertility pattern of teak plantation showed, the pollen donor range of 7-18 had donated pollen to 9 female parents. Here also female parents were found to be receiving pollen from all the sides of the plot. The maximum crossing ability of an efficient pollen donor (tree number 194) in this plot was found to be 5 by donating pollen to 3 different female parents. Another single tree (tree number 169) has produced 4 progenies by donating pollen to 3 different female parents, one of the other (tree number 110) also donated pollen to 2 different female parents and produced 4 progenies (Appendix 3).

Out of the 200 total teak trees in the plot, 92 (46 per cent) contributed pollen to produce 130 progenies. Three trees produced 5 progenies through self fertilization. Out of the total 92 male parents 81 per cent trees crossed with only one female parent. Each of the 17 per cent of male donors crossed with 2 female trees and only 2 per cent were in the group of donating pollen to 3 female parents (Fig.)

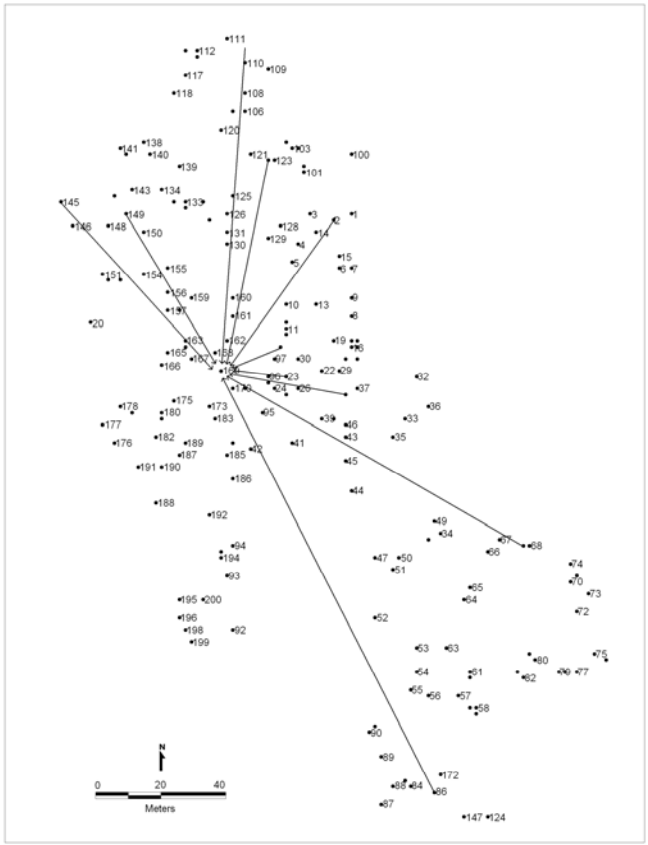


Fig . Pollen flow pattern from different pollen donors to mother tree

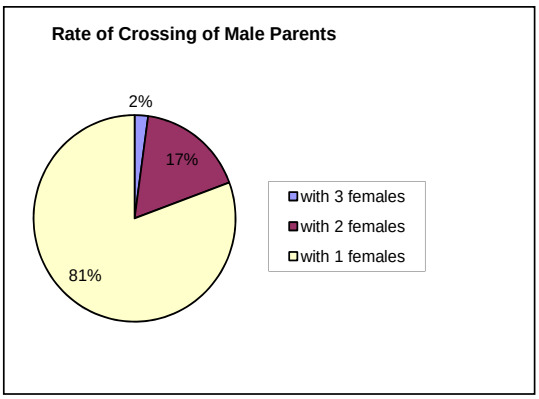


Fig 3. Percentage of male parents crossed with one or more females

Seed Dispersal

From the disturbed population at Thamaravellachal, 100 seedlings grown on the forest floor were selected for seed dispersal studies. The parents of these seedlings have been identified through maximum likelihood method through the software program cervus version 3.

Here, we can see that the main dispersal of seeds from its mother parent is within a range of 51 to 100 m. Maximum number seedlings (33 seedlings/ 33 per cent) were dispersed mainly in a distance range of 51-100 m. Four seedlings were dispersed to a maximum distance range of 251-300 m. Seed dispersal of main range was found to be followed by 101 to 150 m (23 per cent) (Table and Fig. 4) .This result also found that the seed dispersal was also took place in all sides of the plot.

Table. Percentage of progenies receiving pollen from different distance classes

Distance of pollen flow (m)	Number of progenies	Percentage of progenies
1—50	8	8
51—100	33	33

101—150	23	22
151—200	16	16
201—250	10	10
251—300	4	4

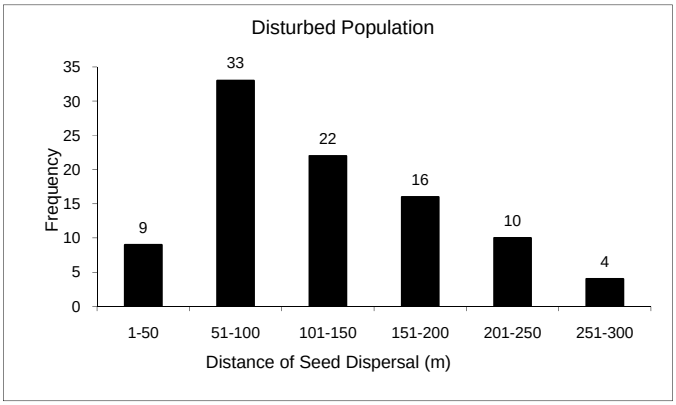


Fig. . Frequency of seed dispersal in different distance classes

Workpackage 5: Analysis of the impact of human disturbance on genetic diversity

Item	Date	Status
Milestones and expected results		
Molecular inventory of genotypes at selected nuclear gene loci and microsatellites from WP3 / 4	Month 18	Completed
Computer programmes that integrates population genetic and diversity measurements	Month 24	Completed
Comparison of the data with data from undisturbed populations: loss of heterozygosity, loss of alleles, changes in allele frequencies	Month 32	Completed
Deliverables		
D8 Quantification of genetic diversity in populations with different levels of human disturbance	Month 32	Completed
D9 Estimation of the loss of genetic diversity due to human disturbance	Month 34	Completed

Notes on deliverables, milestones and expected results

It is very difficult, if not impossible to identify undisturbed populations that are large enough for population genetic analysis. In Thailand, several of the populations are clearly more disturbed than others with the most southern and western populations having the highest level of disturbance. The Potharam population is currently a village, with some individual trees remaining here and there and a few small plots with trees. The SaiYoke population consists of individual trees left at the edges of agricultural fields, and a small strip of forest between the fields and a limestone outcrop. The ThaTaFang (Salaween NP) population has a long history of exploitation for export of teak logs. Its proximity to the Salween river, along which teak logs were floated to the Moulmein harbour in then British controlled Burma.

To avoid collecting samples from planted teak trees, only large trees, or sprouts originating from old stumps were collected.

In India, paired sets of protected and unprotected populations were sampled, but truly undisturbed populations are very hard to find. The stand structure and tree sizes clearly indicate that also in the protected populations, human disturbance must have occurred. There is a complete lack of large teak trees. The populations have been analysed for 4 microsatellite loci and 3 nuclear gene loci, contrasting the protected (undisturbed) and unprotected (disturbed) populations. The results of the analysis indicate that there are very few statistically significant differences. For some microsatellite loci, the unprotected forests had slightly higher number of alleles and levels of diversity.

The geographical pattern observed in India, in Thailand and within Kerala probably results in clearer genetic differences between the populations than any differences in disturbance.

Comparison of protected and unprotected populations in India

The polymorphic information content (PIC) of four loci AC01, AC28, AG04, AG14, was calculated for disturbed and undisturbed populations. From this (Table: 3) it was observed that PIC is slightly higher in undisturbed population than in disturbed populations. (Fig 4**)**

Table: 3 Polymorphic information content data

Locus	Undisturbed	Disturbed	P-value
AC01	0.627	0.583	
AC28	0.507	0.458	
AG04	0.5	0.395	
AG14	0.705	0.649	

The mean number of alleles per population in the case of disturbed population is in a range of 5.00 to 6.75 and expected heterozygosity ranges from 0.477 to 0.645. But in case of undisturbed population, mean number of alleles per locus per population is in a range of 4.75 – 6.25 and expected heterozygosity ranges from 0.477 to 0.645. The range seen in expected heterozygosity values results from both the variation in number of alleles per locus, and allele frequency distribution within populations. The data were analyzed using Cervus 3.0.3 and Fstat 2.9.3.2 (2000) softwares.

Gene diversity per locus and sample was estimated using an unbiased estimator (Nei, 1987)

$$H_{sk} = \frac{n_k}{n_k - 1}\left(1 - \sum p_{ik}^2 - H_{ok} / 2n_k\right)$$

where n_k is the size of sample k, P_{ik} is the frequency of allele A_i in sample k and H_{ok} is the observed proportion of heterozygotes in sample k. The results showed that the estimated gene diversity is higher in undisturbed population than disturbed populations in all the locations (Table 3 & Fig.3) but the differences are not significant (t-test). With respect to the undisturbed populations the highest gene diversity (0.721) was observed at Orissa and lowest value (0.538) at Wayanad. In the case of disturbed populations the highest value (0.64) was observed in Konni and the lowest (0.48) in Madhya Pradesh.

Table.4 Genetic diversity within each population

Area	Undisturbed	Disturbed	t-value
Trichur (Kerala)	0.608	0.596	0.076^{ns}

Konni (Kerala)	0.649	0.639	0.124 ^{ns}
Wayanad (Kerala)	0.538	0.526	0.090 ^{ns}
Nilambur (Kerala)	0.594	0.532	0.370 ^{ns}
Orissa	0.721	0.629	0.837 ^{ns}
MadhyaPradesh	0.645	0.480	0.903 ^{ns}
Gujarat	0.657	0.575	0.449 ^{ns}

Relatively good levels of multi allelism were observed at all four microsatellite loci studied. Mean number of alleles per locus in case of disturbed populations is in a range of 5.00 to 6.75, while for undisturbed populations it ranges from 4.75 to 6.25. The result shows that in every location, the disturbed populations have a slightly higher number of alleles than undisturbed populations except the populations in Gujarat where disturbed and undisturbed populations have the same average number of alleles.

Table : 6 Number of alleles per locus in each of the populations

Population	Orissa Undi	**Orissa Dis**	MP Undis	**MP Dist**	Gujar Und	**Guja Dist**	Thrissu Undist	**Thriss Dist**	Konni Undis	**Konni Dist**	Nilam Undis	**Nilamb Dist**	**Wynad Undist**	**Wynad Dist**
AC01	6	**8**	9	**7**	7	**8**	7	**6**	5	**8**	5	**5**	**4**	**6**
AC28	6	**4**	4	**7**	5	**7**	5	**6**	4	**4**	4	**3**	**3**	**3**
AG04	5	**5**	2	**2**	4	**2**	6	**7**	4	**4**	4	**4**	**4**	**5**
AG14	6	**7**	4	**6**	5	**4**	7	**8**	6	**6**	6	**8**	**6**	**8**
Mean	**5.75**	**6**	**4.75**	**5.5**	**5.25**	**5.25**	**6.25**	**6.75**	**4.75**	**5.5**	**4.75**	**5**	**4.25**	**5.5**

The microsatellite data showed that the current differences in the level of disturbance is not a threat to allele frequency in the fragmented populations. In addition, there was no evidence for a loss of alleles in disturbed populations, which is a more sensitive indicator of a population bottleneck than is the loss of heterozygosity (Allendorf, 1986).

Table 7. Allele frequency in different loci

Locus	Undisturbed	Disturbed
AC01	6.14	6.86
AC28	4.43	4.86
AG04	4.14	4.14
AG14	5.71	6.71

Leberg, (2000) conducted a study and by Spearmans rank correlation analysis, he identified two significant relationships, the correlations between population size and number of alleles, and between fragment isolation and inbreeding coefficient. The correlation between population size and allelic richness was non-significant. (Leberg, 2002).

Allelic richness is defined as the number of alleles in a sample/population, standardized for sample/population size.

Table 12 Allelic Richness

Populations	Undisturbed	Disturbed	**t-value**
Trichur	5.74	5.9	0.249 ns
Konni	5.06	4.31	0.917 ns
Wayanad	5.07	4.09	1.027 ns
Nilambur	4.53	4.52	0.002 ns
Orissa	5.48	5.46	0.018 ns
Madhya Pradesh	4.95	4.62	0.185 ns
Gujarat	5.09	4.97	0.078 ns

The allelic richness was compared between disturbed and undisturbed population and tested by using t-test. In all cases, t-value was found to be non-significant indicating that there is no difference in the allelic richness between disturbed and undisturbed population.

Expected heterozygosity (H_e) was estimated using an unbiased formula from allele frequencies assuming Hardy-Weinberg equilibrium (Nei 1987). Overall expected heterozygosity across all loci was estimated using the Cervus software. Results of He comparisons?

The observed heterozygosity (H_O) per locus and populations was estimated (Table.13). With regard to undisturbed populations, the highest heterozygosity value (0.677) was observed in the population of Wayanad. The lowest value (0.326) was observed in the undisturbed population of Madhya Pradesh. In the case of disturbed

populations, the maximum value (0.559) was observed in Konni and the minimum in Madhya Pradesh (0.254).

Table.13: Expected and Observed Heterozygosity of Disturbed and Undisturbed Populations

Populations	H_E Undisturbed	H_O Undisturbed	H_E Disturbed	H_O Disturbed	Significance
Trichur	0.604	0.592	0.591	0.552	
Konni	0.637	0.672	0.645	0.559	
Wayanad	0.536	0.677	0.521	0.521	
Nilambur	0.547	0.584	0.593	0.484	
Orissa	0.718	0.532	0.625	0.519	
Madhya Pradesh	0.643	0.326	0.477	0.254	
Gujarat	0.654	0.428	0.570	0.338	

For *T. grandis* the genetic consequences of forest disturbances have been measured as a decrease in genetic diversity, loss of heterozygosity, loss of alleles and change in allele frequencies. The result obtained form this study clearly shows that logging activities and the frequent fires resulting in loss of nature trees adds the severity of gene loss. In the 7 disturbed populations of *T. grandis* compared with 7 undisturbed populations, the rate of genetic diversity is lower in disturbed populations. The effect of logging can also be observed in heterozygosity because it is observed that heterozygosity is decreased in disturbed populations.

Table 12 Inbreeding Coefficient

Populations	Undisturbed	Disturbed
Trichur	0.068	0.074
Konni	0.105	0.134
Wayanad	0.005	0.09
Nilambur	0.185	-0.105
Orissa	0.267	0.175
Madhya Pradesh	0.504	0.472
Gujarat	0.464	0.416

Workpackage 6: Identification of effective insect pollinators in teak

Item	Date	Status
Milestones and expected results		
Selection, inventory and mapping of populations (same as in WP4)	Month 12	Completed
Construction of observation towers	before flowering season 1	Completed
Collection of insects visiting insect flowers	end of flowering season 1 + end of flowering season 2	completed
Study of pollen grains on insect body	end of flowering season 1 + end of flowering season 2	completed
Study of behaviour of a few selected insect species in relation to teak pollination	end of flowering season 2	Completed
Deliverables		
D10. A list of insects visiting teak flowers in natural populations and plantations with information on the behaviour and ecology of effective insect pollinators	Month 34	Completed

Notes on deliverables, milestones and expected results

Identification of populations and accessions for analysis

All partners have chosen sites for collection and analysis

Scientific results

Several species of insect have been observed, summarized in table3

Species	Family	Country	Visitor?	Pollinator?

Workpackage 7: Analysis of the genetic diversity data

Item	Date	Status
Milestones and expected results		
Comparison of data on microsatellite, nuclear gene and chloroplast DNA genetic markers	for publication	Ongoing
Comparison of data on historical gene flow (WP2 and 3) and contemporary gene flow (WP5)	for publication	Ongoing
Comparison of data between undisturbed and disturbed stand for population differentiation parameters (WP4) and contemporary gene flow (WP5)	for publication	Ongoing
Deliverables		
D11. Computer package to analyse the genetic diversity and gene flow data at several levels of organisation	Month 28	Ongoing
D12. Recommendations concerning number, size and location of *in situ* conservation areas and sustainable management in natural populations and seed orchards	Month 36	Ongoing

Notes on deliverables, milestones and expected results

Several computer software packages have been used to analyze the information. Most of the results have been detailed in WP2, WP3, WP4 and WP5, and are summarized here.

The phylogeographic analysis indicates a southwestern Indian origin for teak, based on the number of alleles and the diversity of gene sequences found in Kerala. Population composition analysis with the STRUCTURE software clearly separates the southwestern Indian populations from eastern India and Thailand, Laos and Indonesia. Covering very large areas, from Orissa through Myanmar, Thailand to Indonesia, very little genetic differentiation can be detected, indicating high levels of gene flow. The high levels of gene flow are confirmed by the pollination analysis, where pollen dispersal appears to happen over long distances, with several trees between the pollen donor and seed parent. The numbers of males contributing pollen to the fertilization is high, and the number of trees pollen is dispersed to is also high. Correspondingly, selfing rates are very low.

2.3 Description of the Workpackages

Workpackage 1: Development of practical assays for studying genetic diversity in teak

Workpackage number:	1 Development of practical assays for studying genetic diversity in teak					
Phase:	Initiation					
Start date:	Jan 2002					
Completion date:	Dec 2002					
Current status:	Finished					
Partners responsible:	P5					
Participants	P1	P2	P3	P4	P5	P6
Person months	1	2	2	24	15	1
Already devoted persons months	1	2	2	24	15	1

The objective is to develop adequate DNA assays to detect and characterise genetic diversity in teak for use by the participants in developing countries. Microsatellites have been isolated, sequenced and characterised to be used as genetic markers. Other markers for cytoplasmic and nuclear gene loci have been developed. The different alleles found at several cytoplasmic and nuclear gene loci have been cloned and sequenced. The result of this development of assays is a catalogue of alleles that have been detected and an allelic clone library. This information will be publicized in a web-based database and the cloned alleles will be distributed to each participant to be used as standards in the further study of diversity in teak.

It is the purpose of the laboratories responsible for the molecular genetic analysis that each performs the same procedures with comparable equipment. To achieve this, a training workshop was organised in October 2002 when each of the participants learnt about procedures for marker detection, scoring, and analysis in teak. At the coordination meeting in India in December 2003, training was provided in using different computer programmes for population genetic analysis.

Workpackage 2 Assessment of genetic diversity in teak over its natural range

Workpackage number:	2 Assessment of genetic diversity in teak over its natural range					
Phase:	Core Activities					
Start date:	Jan 2003					
Completion date:	Dec 2004					
Current status:	Finished					
Partners responsible:	P4					
Participants	P1	P2	P3	P4	P5	P6
Person months	4	34	24	30	0	4
Already devoted persons months	0	32	22	32	0	4

Teak occurs naturally in three regions. The largest contiguous area is found in India, south of 23^{o}30' N. Another area is Myanmar, northern Thailand, western Laos and the bordering area in Southwestern China. There is no natural teak forest that links the two areas. Teak is believed also to occur naturally on a few western islands of Indonesia. On these and other islands teak has been in cultivation for a long time.

Teak accessions are being collected from natural stands and germplasm collections over its entire range and assayed for cytoplasmic and nuclear DNA markers. The collection will be considered as samples of geo-referenced individuals across the natural range. This analysis will evaluate the overall patterning and partitioning of the genetic variation across the entire range.

2.1 Genetic diversity in accessions collected in India.
2.2 Genetic diversity in accessions collected in Indonesia.
2.3 Genetic diversity in accessions collected in Thailand.

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Workpackage 3: Population genetic diversity in teak forests

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	3 Population genetic diversity in teak forests Core Activities April 2003 Aug 2005 Ongoing P4					
Participants	P1	P2	P3	P4	P5	P6
Person months	6	44	27	32	10	6
Already devoted persons months	2	12	12	12	3	3

Molecular markers have indicated that tree populations generally contain a wealth of diversity, which is unequally distributed within and between populations. Both the type of allele and the frequency distribution of each allele can differ substantially among populations from different areas. This distribution of diversity reflects the historical pattern of gene flow and the breeding system of the species.

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To obtain information about the long-term dynamics of population genetic parameters in teak, populations from different regions of the natural range of teak will be assayed for genetic diversity at nuclear genome loci. In several locations, paired populations with different histories of human disturbance are being selected, and in each population, 30 to 50 mature trees are being sampled.

Existing software packages will be used to calculate standard population genetic parameters, including F_{is}, F_{st} and F_{it}. The proportion of population differentiation will be examined using AMOVA and gene flow between alleles estimated from F_{st} calculations and examination of the distribution and phylogenetic relationship of private nuclear alleles, to assess:

3.1 Nuclear gene markers to assess diversity in Indian teak populations (P1, 2).
3.2 Nuclear gene markers to assess diversity in Indonesian populations (P1, 3).
3.3 Nuclear gene markers to assess diversity in Thai teak populations (P1, 4).

Workpackage 4 Contemporary gene flow through pollen and seed dispersal

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	4 Contemporary gene flow through pollen and seed dispersal Core Activities April 2003 Dec 2005 Ongoing P4					
Participants	P1	P2	P3	P4	P5	P6
Person months	7	36	20	30	13	0
Already devoted persons months	2	12	6	6	4	4

The objective is to trace and quantify pollen and seed dispersal in terms of distances and rates (mean, median and maximum). Paternity analysis based on microsatellite markers or private alleles will be used to monitor effective natural pollination events in undisturbed forests, disturbed forests and plantations. The seed and pollen dispersal curves are important tools in estimating the minimum area that is required for the conservation of a viable population of trees. Pollen dispersal curves are also useful in management of seed orchards.

Comparison of contemporary gene flow in undisturbed and disturbed forests will be very useful for drafting guidelines concerning the changes in contemporary gene flow due to human disturbance. By comparing the contemporary gene flow in natural forests and single species plantations, guidelines will be drafted for plantation management to conserve genetic diversity. Study populations have been selected and tree, seed and seedling samples are being collected for:
4.1 Parentage analysis in Indian natural teak stands and plantations (P1, 2, 5, 6).
4.2 Parentage analysis in Indonesian natural stands and plantations (P1, 3, 5, 6).
4.3 Parentage analysis in Thai natural teak stands and plantations (P1, 4, 5, 6).

Workpackage 5: Analysis of the impact of human disturbance on genetic diversity

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	5 Analysis of the impact of human disturbance on genetic diversity Core Activities Aug 2003 Dec 2005 Ongoing P1					
Participants	P1	P2	P3	P4	P5	P6
Person months	4	22	12	16	0	5
Already devoted persons months	2	6	6	6	0	3

Teak has been selectively logged over the past century and may already have lost some of its genetic diversity in certain areas. By comparing the genetic diversity within populations that have been moderately or heavily degraded with the genetic diversity in undisturbed or little disturbed populations (studied in WP3 and WP4), an estimate could be made about the diversity that has already been lost.

Using several measures that indicate the level of disturbance (number of stumps observed, presence of old logging roads, frequency of forest fires, presence or absence of natural regeneration, distance to inhabited areas, records of recent

disturbance etc), selected populations are being classified as heavily disturbed, intermediately disturbed or little disturbed. Whenever possible paired populations of WP 3 and WP4 have been selected so that genetic diversity measures for these populations can be contrasted to each other. For all populations, the set of parameters of human disturbance that has been measured will be used as covariates in the genetic analysis.

For the analysis existing software will be used to calculate estimates of population genetic diversity (e.g. Shannon's and Nei's) and estimates of standard population genetic parameters (particularly examination of Fis which can be used to show a deficit of heterozygotes in a population and is one of the signs of inbreeding). Multiple regression analysis will be used for correlating variables of:
5.1 Genetic marker diversity in disturbed teak forests in India.
5.2 Genetic marker diversity in disturbed teak forests in Indonesia.
5.3 Genetic marker diversity in disturbed teak forests in Thailand.

Workpackage 6: Identification of effective insect pollinators for teak

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	6 Identification of effective insect pollinators for teak Core Activities June 2002 Sep 2005 Ongoing P4					
Participants	P1	P2	P3	P4	P5	P6
Person months	0	9	9	9	0	2
Already devoted persons months	0	6	8	2	0	1

This research aims to identify insect species that visit teak flowers in natural habitats and quantify the teak flower visits. Observations are being conducted and descriptions being be made of the foraging behaviour of the important teak flower visiting insects to evaluate the pollination effectiveness. The observations are being compared between the natural forest, degraded forests and plantations. The observations are being done within the same stands and at the same time when also the molecular genetic data are collected (WP5). The data from the molecular study will be compared to the observations made on the insects.
6.1 Observations on insects in natural forests and plantation in India.
6.2 Observations on insects in natural forests and plantation in Indonesia.
6.3 Observations on insects in natural forests and plantation in Thailand.

Workpackage 7: Analysis of the genetic diversity data

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	7 Analysis of the genetic diversity data Final Jan 2004 Dec 2005 Preparing P1					
Participants	P1	P2	P3	P4	P5	P6
Person months	10	12	12	12	6	4
Already devoted persons months	5	0	0	0	0	0

Data that have been gathered in the previous workpackages will be analysed for several aspects of population genetics and gene flow. Through discussion with end-users, reports will be prepared dealing with several aspects of the use of teak genetic resources and its conservation. Appropriate computer software will be made available to analyse the data, and a database of results constructed. Wherever possible, the phylogenetic information contained in the assayed markers will be exploited to gain insight into processes of genetic diversity and gene flow.

Several topics will be analysed, grouped in four themes
7.1 Teak phylogeography, identification of genetic markers specific for certain origins.
7.2 Teak genetic diversity conservation: identification of areas of high genetic diversity and patterns in genetic diversity and recommendations for establishment of conservation areas.
7.3 Teak pollen flow and seed dispersal and implications for seed orchard management.
7.4 Manual for management of teak genetic diversity in its natural area and implications for plantation in other regions (P1, 2, 3, 4, 5, 6).

4. ROLE OF PARTICIPANTS

Partner 1 (Financial coordinator) CEH

Stephen Cavers – Centre for Ecology and Hydrology, CEH-Edinburgh, Bush Estate, Penicuik, Midlothian EH26 0QB, UK. Tel: (+44) (0)131 445 4343. Fax: (+44) (0)131 445 3943. Email: scav@ceh.ac.uk

Scientific team
Samantha Davies (PhD student), Andy White (modelling, Heriot-Watt University), Stephen Cavers (data analysis), Andrew Lowe

Contractual links to other participants
None

Sub-contracted work during the first reporting period
Heriot Watt University has been subcontracted to help with computer simulation modelling of gene flow for workpackage 7. The results of this work are presented in the relevant section following.

Research activities
(S. Davies, A. White, A. Lowe, S. Cavers)

Workpackage 1 Development of practical assays for studying genetic diversity in teak

Web-based database (D3, C. Bacles)

The development of a web-based database cataloguing alleles observed at each of the molecular markers screened in the sampled teak populations is in progress. The structure of the database has been finalized and preliminary information on sample collection in each country is being put together by partners. The DNA database will be geo-referenced by allowing a link with a geographic information system based map (Figure 1) so that input DNA information for new samples can be compared to that of referenced samples of known origin. The database will be built during the third year of the project, awaiting for collection of molecular data for the sampled populations.

Workpackage 2 Assessment of teak genetic diversity – Phylogeography
Workpackage 3 Population genetic diversity in teak forests
Workpackage 4 Contemporary gene flow
Workpackage 5 Analysis of the impact of human disturbance
Workpackage 6 Identification of effective insect pollinators for teak

Whilst not directly involved in the fieldwork and laboratory experiments for these workpackages, CEH has helped partners in resolving significant problems and in developing optimum methodology to achieve the deliverables and milestones of the

workpackages. Many of those issues were discussed during the second project meeting at KFRI, India (8^{th}-12^{th} December 2003). Practical training in the use of several software packages was given during that meeting.

Workpackage 7 Analysis of the genetic diversity data

Simulation modelling (D11)

A spatial genetic simulation model is being developed to assist in estimating the impact of gene flow and habitat changes on the genetic resources of teak. This model operates at a population level scale using molecular data from workpackages 2 to 6 and includes a pollen dispersal component as well as a seed dispersal component as in the previous model (see annual report year 2). It is intended that when available realistic gene dispersal distances can be entered into the model and this can then be used to evaluate management strategies that maximise genetic diversity. A population scale model is currently being tested using model scenarios that are relevant to utilising timber from secondary regeneration of a tropical pioneer tree, *Vochysia ferruginea*, which has very similar population dynamics to teak and for which population data are available. This model can be used for teak when empirical data become available.

Other : Exploitation and dissemination of activities

Books

Lowe AJ, Harris SA, Ashton P (2004). *Ecological Genetics: Design, Analysis and Application*. Blackwells, Oxford. 326 pp.

Papers in refereed scientific journals

Davies S, White A, Lowe AJ (2004) An investigation into effects of long-distance seed dispersal on organelle population genetic structure and colonization rate: a model analysis. *Heredity*. **93**:566-576

Unsworth CP, Gerber SD, Lowe AJ (submitted, Jan 2005) Estimating short and long distance seed dispersal in plants using a bimodal probability mixture model. *Heredity*.

Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C (submitted, Dec 2004) Genetic resource loss following habitat fragmentation and degradation; reconciling predicted theory with empirical evidence. *Heredity*.

Partner 2 (Contractor) KFRI

Indira Edakkeppurath Puthanveettil – Kerala Forest Research Institute, Peechi, 680653, Thrissur, India. Tel: (+91) 487 2699 037. Fax: (+91) 487 2699 249. Email: indira@kfri.org

Scientific team
Dr. Indira E. P., Dr. M. Balasundaran, Dr. K. Mohanadas
Rajalakshmi, R. (postdoc)
Pramod N. Nair, Sabna Prabha S. (Ph. D. students)
Kannan, T. R. (technician)

Contractual links to other participants
None

Research activities
(Dr. Indira E. P., Dr. M. Balasundaran, Dr. K. Mohanadas)

Workpackage 1: Development of practical assays for studying genetic diversity in teak

Workpackage 2: Assessment of genetic diversity in teak over its natural range – Phylogeography
Collected samples from Kerala and other parts of India
Analyzed the samples using chloroplast and nuclear loci
Sequenced alleles

Workpackage 3 Population genetic diversity in teak forests
Collected samples from Kerala and other parts of India
Analyzed the samples using microsatellite and nuclear gene loci
Sequenced alleles

Workpackage 4 Contemporary gene flow through pollen and seed dispersal.
Collected samples from a protected and an unprotected natural stand and from a plantation in Kerala.
Analyzed the samples using microsatellite and nuclear gene loci

Workpackage 5 Analysis of the impact of human disturbance on genetic diversity
The collected data (WP2 and 3) were analyzed contrasting protected areas agains unprotected areas.

Workpackage 6 Identification of effective insect pollinators for teak

Selection, inventory and mapping of populations (same as in WP4

Construction of observation towers

Collection of insects visiting flowers

Study of pollen grains on insect

Study of behaviour of a few selected insect species in relation to teak pollination

List of identified insects recorded from teak inflorescence

Order/ Family /species	Frequency of visit	Remarks
HYMENOPTERA		
1. APIDAE		
Anthophora niveocincta Smith	High	Pollinator
Anthophora zonata Lin.	"	"
Apis cerana indica Fabricius	"	"
Apis florea Lin.	"	"
2. Apinae: Meliponini		
Lisotrigona mohandasi Jobiraj and Narendran	"	"
Melipona iridipennis Dall.	"	"
Trigonisca sp.	"	"
3. Anthophoridae		
Ceratina hieroglyphica Smith	Low	Pollinator
Nomada sp	"	'
4. COLLETIDAE		
Heriades sp.	"	"
Hylaeus sp	"	"
5. HALICTIDAE		
Halictus tectonae Narendran & Joberaj	High	"
Nomia curvipes Fabricius	"	"
Nomia ellioti Smith	"	
Nomia chalybeata Smith	"	"
Nomia basalis Smith	"	"
Lasioglossum sp. 1	"	"
Lasioglossum sp. 2	High	Pollinator
6. MEGACHILIDAE		
Megachile sp.	"	"
Megachile carbnria Smith	"	"
7. VESPIDAE		
Paraleptomenes sp.	"	"
Eumenes flavopicta Blanch	"	"
E. punctata saussure	"	"
Antepepona sp.	"	"
Anterhynchium sp.	"	"

Order/ Family /species	Frequency of visit	Remarks
Antodynerus ornatus Smith	"	"
Delta arcuata (Fb.)	"	"
Delta conoidus (Gemlin)	"	"
Delta petiolata (Fb.)	High	Pollinator
Rhynchium brunneum (Smith)	"	"
Ropalidia spatulata Van der Vecht	"	"
Sphecodex sp	"	"
Xenorhynchium abdomine (Illiger)	"	"
8. SPHECIDAE		
Chalybion bengalense Dalbhom	"	"
Sphex sericeus Fb.	"	"
Sphex sp.	"	"
LEPIDOPTERA RHOPALOCERA **ACRAEIDAE**		
Acraea violae Fb.	Low	Visitor
Danaidae		
Euploea core Cramer	"	"
Tirumala limniace leopardus (Butler)	"	"
ERYCINIDAE		
Abisara echerius (Stoll)	L	Visitor
Udaspes sp.	"	"
LYCAENIDAE		
Caleta caleta Hewitson	"	"
HESPERIDAE		
Celaenorrhinus leucocera Kollar	"	"
Potanthus sp	"	"
Tagiades litigiosa Moschler	"	"
NYMPHALIDAE		
Cupha erymanthis Drury	"	"
Hypolimnas bolina Lin.	Low	Visitor
Junonia almana Lin.	"	"
Junonia iphila Fruh slovfer	"	"

Order/ Family /species	Frequency of visit	Remarks
Junonia stygia	"	"
Neptis hylas Moore	"	"
PAPILIONIDAE		
Graphium agamemnon Lin.	Low	Visitor
Graphium doson Felder	Low	Visitor
Papilio polytes Lin.	"	"
PIERIDAE		
Catopsilia pomona Fb.	"	"
C. pyranthe Lin.	"	"
Delias eucharis Drury	"	"
SATYRIDAE		
Ythima huebneri Kirby	"	"
HETEROCERA **HYBLAEIDAE**		
Hyblaea puera Cramer	"	
Pyralidae		
Syngamia floridalis Zell.	"	Visitor
SYNTOMIDAE		
Eucromia polymena Lin.	"	"
HEMIPTERA **PENTATOMIDAE**		
Tessaratoma sp.	Low	Visitor

Diptera: unidentified 12 spp. **Coleoptera**: Unidentified 9 spp

Workpackage 7 Analysis of the genetic diversity data

Comparison of data on microsatellite, nuclear gene and chloroplast DNA genetic markers. (for publication)
Comparison of data on historical gene flow (WP2 and 3) and contemporary gene flow (WP5). (for publication)
Comparison of data between undisturbed and disturbed stand for population differentiation parameters (WP4) and contemporary gene flow (WP5). (for publication)

Significant difficulties or delays experienced during the third reporting period

Indicate any significant bottlenecks, delays or difficulties, which have affected your contribution against the workplan. Report on remedial actions taken or to be taken.

Due to shortage of funds in the first half of the year we were forced to restrict ourselves from travel to locate new areas and to map them and also procuring chemicals and other lab supplies.

Sub-contracted work during the third reporting period

Identify the sub-contractor. Report on the actual sub-contracted work for the reporting period and, where appropriate, present interim or final results and conclusions.

Not applicable

Partner 3 (Contractor) IPBogor

Dr. Sudarsono – Institut Pertanian Bogor - Bogor Agricultural University, Department of Agronomy, Faculty of Agriculture, J1. Raya Pajajaran, 16143, Bogor, Indonesia. Tel: (+62) 251 326 429. Fax: (+62) 251 312 032. Email: sudarsono@biotrop.org

Scientific team

Dr. Sudarsono, Dr. Asep, Dr. S. Iliyas
Mrs. Endah R. Palupi and Mr. Dirvamena Boer (PhD students)

Contractual links to other participants

None

Significant difficulties or delays experienced during the second reporting period

None

General remarks

Starting date of the TEAKDIV project on January 1, 2002

Third year responsibilities of IPB Project Team were to analyze the diversity of teak in Indonesia. DNA isolation has been conducted from dried leaf samples. DNA were obtained from most of the teak samples from various places in Indonesia. The quantity and quality of isolated DNA were adequate for conducting PCR using the selected primers for chloroplast genome-specific SSR, nuclear genome SSR, and gene specific markers. Using the isolated DNA from leaf samples and primer pairs originated from Thailands' counterpart, PCR amplification of specific DNA fragments have conducted in order to study the diversity of teak in Indonesia.

In accordance to the Insect Pollinator Studies, the first collection of insects associated with teak flower was conducted during the flowering season of 2001/2002 (December 2001 - March 2002) at Cepu Research Site. The second insect collection was conducted in 2003 at Dolok Research Site, Muna islands, South East Sulawesi. The collected insects were analyzed for the presence of teak pollen in their body, location of deposited pollen and the pollen load. Identification of the selected insect positively carry teak pollen was subsequently conducted. Insect studies have been completed in the third year of the TEAKDIV project. The final outcome of this activity include a list of insects visiting teak flowers, a list of potential polinators for teak, and the

identity of the insects that has the potential as pollinator from natural forest in Dolok Research Site and from managed forest in Cepu Research Site.

Two PhD students will continue to be associated with TEAKDIV Project. One of the project team, Ir Endah Retno Palupi is one PhD student who has conducted her PhD research with the support of TEAKDIV Project. Part of her PhD research is identification of potential insect pollinators in natural and managed teak forest. The other part of her PhD disertation will be gene flow studies in managed teak forest. The second PhD student, Ir Dirvamena Boer, MS also undertakes his PhD in association with TEAKDIV Project. He has been conducting diversity analysis for teak originated from Indonesia for his PhD research dissertation. He has also been conducted the gene flow studies in natural teak forest. One MSc student has joined the TEAKDIV project and conducted her MSc thesis with the support of TEAKDIV Project. For her MSc research, she has been working to specifically analyze the teak diversity originated from South Sulawesi. Therefore, there are two PhD and one MSc students who are working for their dissertation or master thesis researches under the support of TEAKDIV Project.

Coordination:
Other than internal coordination, coordination meetings with other TEAKDIV partners were not held. All consultation was through e-mail exchanges with the partners. The final coordination meeting was postponed until the end of 2005.

Research activities during the second reporting period
Workpackage 1: Development of practical protocols and assays for studying genetic diversity in teak.

In the third year of the project, there was not any workshop activity conducted in Indonesia. The planned third year meeting in Indonesia was postponed until the end of 2005. Personnels from Indonesian Team continues the necessary activities to implement the TEAKDIV projects, and to achieve the targeted milestones.

Workpackage 2 Assessment of genetic diversity in teak over its natural range – Phylogeography

General Objectives: To assess genetic diversity in teak accessions in its natural ranges in Indonesia.

Specific Objectives:

1. Select accession of teak from Indonesia for genetic analysis
2. Analyze selected teak accessions from Indonesia using cpSSR marker
3. Analyze selected teak accessions from Indonesia using nuclear loci markers

Progress of the Activities:

1. Various sites in Indonesia where teak forests or stand exist have been investigated (Table 1; Fig. 1) during the first year of the project. Dried samples from young leaf have been collected from most of these areas during the TEAKDIV Project implementation. Leaf samples from Teak Collection that belong to PERUM PERHUTANI and from TEAK museum, Cepu, Central Java have also been obtained in 2003.

Due to some problem in DNA isolation, some of the samples DNA were degraded and need to be reisolated in the year 2004. Moreover, some samples were collected from old leaf tissues and DNA isolation from such tissues was proven to be difficults. Whenever possible, young leaf materials were collected again from the field. Otherwise, the particular samples were not used in the genetic analysis.

2. During the year 2004, the isolated DNA has been used as template for PCR amplification activities. PCR amplification using specific primer pairs for chloroplast DNA and for gene specific markers have been initiated in the year 2004. Condition for PCR amplification developed by Thailand partners has been tested and worked very well on high quality DNA templates. Best procedures for PCR amplification under the Indonesian laboratory conditions has been worked out and established.
3. Most of the PCR amplified products have been analysed in agarose gel and not all gave positive amplification product. For certain DNA templates such as ones prepared from slighly older leaf, the estableshed PCR conditions gave negative results. For the positive PCR amplification products, no SSCP analysis was conducted until the end of the year 2004.
4. Since no SSCP activities has been conducted in the year 2004, allele assignment for the teak accessions from Indonesia based on cpSSR and gene spesific marker has not been obtained.
5. In the year 2005, molecular genetic analysis will be continued for all teak samples collected using chloroplast marker and nuclear DNA marker. SSCP analysis will be completed during the year 2005. In addition, identification of specific alleles among individual trees of the collected teak population will also be completed in the year 2005.

Table 1. Selected research sites, number of collected teak samples, and progress of research activities for the Work Package 2 (WP 2).

Site No.	Location	No. of teak samples	Remarks
1	Aceh Province (Pr) - Bukit Raya, Lhokseumawe - Blang Cot Baroh, Beureun - Blang Cot Tunong, Beureun	 6 10 14	 DNA Stock
2	Medan, N. Sumatra Pr.	9	Low DNA quality (degraded)
3	Bengkulu, Bengkulu Pr.	10	Low DNA quality (degraded)
4	South Sumatra Pr. - Simpang Oku - Lengikiki - Way Heling	 6 9 3	 DNA Stock, PCR on going DNA Stock, PCR on going DNA Stock, PCR on going
5	Lampung Pr.	20	Low DNA quality (degraded)
6	Bogor, West Java Pr.	10	Low DNA quality (degraded)
8	Cirebon, West Java Pr	20	Low DNA quality (degraded)
9	Teak Conservation Area, Tegal, Cental Java Pr.	25	Low DNA quality (degraded)
10	Teak Museum, Cepu, Central Java	32	DNA Stock, PCR on going
11	CSO, Cepu Central Java Pr	20	DNA Stock, PCR on going
12	Central Java Pr. - Boyolali - Wonogiri	 10 17	 Low DNA quality (degraded) DNA Stock
16	West Nusa Tenggara Pr. - Sekotong, Lombok - Wera, Sumbawa	 5 10	 DNA Stock, PCR on going DNA Stock, PCR on going
17	South East Sulawesi Pr. - Dolok, Muna Island - Wadila, Buton Island - Warangga, Muna Island - Wakuru, Buton Island - Wakonti, Buton Island - Napabalano, Muna Island - Jompi, Muna Island	 99 99 99 27 29 26 23	 PCR products with all SSR primer and gene specific primer (except TgG3PDHcp and Tg G3PDHcy)
18	South Sulawesi Pr. - Pare-Pare - Soppeng	 40 40	 PCR products with all SSR primer and gene specific

	- Biloka - Massepe	40 40	primer (except TgG3PDHcy)
19	Maluku Pr. - Soli Atas, Ternate - Taba Hijrah, Halmahera - Sidongali, Halmahera - Guraping, Halmahera - Guraring, Halmahera	 13 10 4 2 8	 DNA Stock, PCR on going Low DNA quality (degraded) DNA Stock, PCR on going Low DNA quality (degraded) Low DNA quality (degraded)

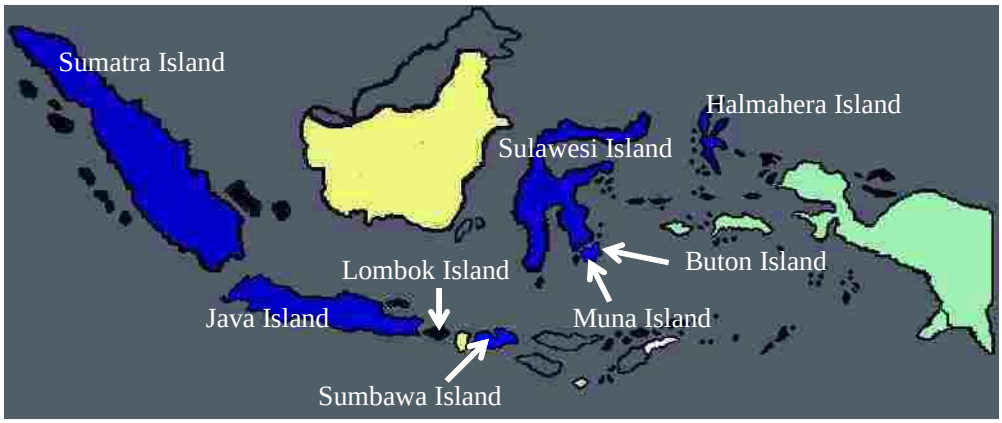

Figure 1. Map of the distribution of teak forest in Indonesia. **Blue color** indicated the presence of 'natural' or planted teak forest. **Yellow** – indicated the presence of recently introduced teak population in the respective islands.

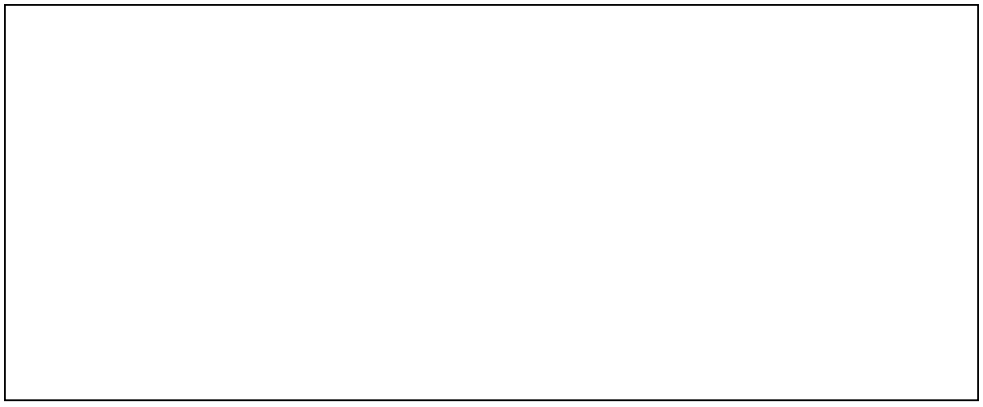

Figure 2. Results of the PCR amplification using Indonesian teak DNA as template and 15 teak specific primer pairs for chloroplast DNA, gene specific marker, and nuclear DNA.

Workpackage 3 Population genetic diversity in teak forests

General Objectives: To assess population genetic diversity in teak forest in Indonesia.

Specific Objectives:

1. Select accession of teak from Indonesia for genetic analysis
2. Analyze selected teak accessions from Indonesia using nuclear loci markers
3. Analyze selected teak accessions from Indonesia using microsatellite markers

Progress of the Activities:

1. Various sites in Indonesia where teak forests or stand exist have been investigated (Table 2) during the first year of the project. Dried samples from young leaf have been collected from most of these areas during the TEAKDIV Project implementation.
2. Similar to WP 2, some problem in DNA isolation have made some of the samples DNA were degraded and need to be reisolated in the year 2004. Moreover, some samples were collected from old leaf tissues and DNA isolation from such tissues was proven to be difficults. Whenever possible, young leaf materials were collected again from the field. Otherwise, similar to WP2 the particular samples were not used in the genetic analysis.
3. During the year 2004, the isolated DNA has been used as template for PCR amplification activities. PCR amplification using specific primer pairs for gene specific markers and microsatelite markers have been initiated in the year 2004. Condition for PCR amplification of macrosatelite markers developed by Thailand partners has been tested and worked very well on high quality DNA templates. Best procedures for PCR amplification of microsatelites markers under the Indonesian laboratory conditions has been worked out and established.
4. Most of the PCR amplified products have been analysed in agarose gel and not all gave positive amplification product. For certain DNA templates such as ones prepared from slighly older leaf, the estableshed PCR conditions gave negative results. For the positive PCR amplification products using nuclear specific markers, no SSCP analysis was conducted until the end of the year 2004.
5. As for the microsatelites marker, standard procedures for denaturing polyacrylamide gel electrophoresis that has been developed by partner in Thailand has been tested and worked well under Indonesian lab condition. The samples that have been tested positive for PCR products using agarose gels, have been analysed using denaturing polyacrylamide gel. Allele asignmenment for each SSR markers have been obtained from some of the teak samples analysed.
6. Since no SSCP activities has been conducted in the year 2004, allele assignment for the teak accessions from Indonesia used in WP3, based on gene spesific marker has not been obtained.

7. In the year 2005, molecular genetic analysis will be continued for all teak samples collected using nuclear DNA/gene specific markers and microsatelite markers. SSCP for gene specific marker analysis and denaturing acryllamide gel elecetrophoresis will be completed during the year 2005. In addition, identification of specific alleles of gene specific and microsatelites marker among individual trees of the collected teak population will also be completed in the year 2005.

Table 2. Selected research sites, number of collected teak samples, and progress of research activities for the Work Package 3 (WP 3).

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Site	Location	samples	Status of molecular analysis	Remarks
Comparison among old populations of teak stands in Indonesia				
1	Bengkulu, Bengkulu Pr.	10	Low DNA quality (degraded). Reisolation of DNA is neccessary	Teak stands, remnant of old population
2	Teak Conservation Area, Tegal, Cental Java	25	Low DNA quality (degraded). Reisolation of DNA is neccessary	Teak forest, Protected teak stand, Close to 100 yrs old
3	Teak Museum, Cepu, Central Java Province	32	DNA Stock, PCR on going	Teak stands, Protected teak stand, more than 100 yrs old
4	Napabalano Conservati-on Forest, Muna, SE Sulawesi Province	26	DNA Stock, PCR on going	Mixed forest, Protected teak stand, more than 100 yrs old
5	Donoloyo Conservation Forest, Wonogiri, Central Java Province	17	DNA Stock, PCR on going	Teak forest, Protected teak stand, more than 100 yrs old
'Natural Teak Forest' with different degree of human disturbances				
1	Dolok, Muna Island, SE Sulawesi Pr.	99	PCR with 11 primers	Undisturbed teak forest
2	Wadila, Buton Island, SE Sulawesi Pr.	99	DNA Stock, PCR on going	Trees were cut down completely and recover naturally to become teak forest
3	Warangga, Muna Island, SE Sulawesi Pr.	99	DNA Stock, PCR on going	Teak forest were selectively thinned and converted into seed orchard

Workpackage 4 Gene flow through pollen and seed dispersal.

General Objectives: To trace and quantify pollen and seed dispersal in term of distaces and rates (mean, median and maximum).

Specific Objectives:

1. Select accession of teak from Indonesia for pollen and seed dispersal (natural teak forest and teak plantation)
2. Analyze pollen flow in natural teak forest and teak plantation based on microsatelites markers
3. Analyze seed dispersal in natural teak forest based on microsatelites markers

Progress of the Activities:

1. Responsibility of IPB Project Team in the Work Package 4 (WP 4) is to conduct parentage analysis in natural teak stands and plantations in Indonesia. Various research sites in Indonesia have been selected (Table 3) during the year 2002. Dried leaf samples from young leaf have been collected from a number of potential male parents in each of the research sites.
2. For the natural teak stand, maps of potential male parents in the teak forest at three research sites were presented in the following Figure 3, 4, and 5, respectively. In the second and third year (2003 and 2004) of project implementation, additional seed samples have been collected from selected female parents from every research sites (Fig 3, Fig 4 and Fig 5). DNA isolation from collected leaf samples of potential male parents have also been conducted in 2003 and 2004. The quality of some isolated DNA has been evaluated using electrophoresis analysis and PCR.
3. In the year 2004, all collected seeds from research sites have successfully been germinated and grown in the plastic house for sources of leaf samples. A total of 24 seedlings from each of 20 female parents were obtained from Dolok research site, 24 seedlings from each of 15 female perents were from Warangga research site, while 24 seedlings from each of 20 female parents were from Sampolawa research site respectively. DNA isolation from seedling leaf samples have been conducted in 2004. The quality of some isolated DNA has been evaluated using electrophoresis analysis and PCR.
4. Dried leaf samples from randomly selected seedlings in the forest ground have also been collected from Dolok, Muna Island, southeastern Sulawesi research site. From this forest, a total of 57 seedling samples were collected for seed dispersal analysis. In addition, leaf samples for 20 individual parent trees and 20 seedlings have randomly been collected from 4 locations in southern Sulawesi (Pare-pare, Soppeng, Bilokka, and Massepe regency) for seed dispersal analysis.
5. Progress of molecular genetic analysis using micro-satellite marker for teak samples from plantation (Cepu Teak Plantation) and natural forest in Dolok, Warangga, and Sampolawa were summarized in Table 3.

6. Molecular genetic analysis using micro-satellite markers for teak samples from southern Sulawesi has been completed. All the parent trees and the seedlings have been subjected to genetic analysis using 9 microsatelite markers. Results of the analysis for five microsatelite markers were presented in the appendix of this report.
7. In the year 2005, molecular genetic analysis will be continued for all teak samples collected using microsatelite markers. In addition, analysis of pollen dispersal and seed dispersal in the plantation and natural teak forest will also be completed in the year 2005.

Table 3. Selected research sites, number of teak samples collected, andprogress of research activities for Work Package 4 (WP 4).

Site	Location	Samples	Status of molecular analysis	Remarks
Managed Teak Forest				
1	Clonal Seed Orchard, Cepu Central Java Pr (*)	100+ potential male parents, ca. 10 female parents (fmp), ca. 20 seedlings per fmp	On going analysis	Perum Perhutani Clonal seed orchard teak forest
'Natural Teak Forest'				
1	Dolok, Muna Island, SE Sulawesi Pr.	99 potential male parents, 20 fmp, 24 seedlings per fmp. 57 seedlings from forest ground	DNA Stock, PCR on going	Undisturbed teak forest, leaf samples from seedlings were collected
2	Wadila, Buton Island, SE Sulawesi Pr.	99 potential male parents, 20 fmp, 24 seedlings per fmp.	DNA Stock, PCR on going	Trees were cut down completely and recover naturally to become teak forest
3	Warangga, Muna Island, SE Sulawesi Pr.	99 potential male parents, 15 fmp, 24 seedlings per fmp.	DNA Stock, PCR on going	Teak forest were selectively thinned and converted into seed orchard

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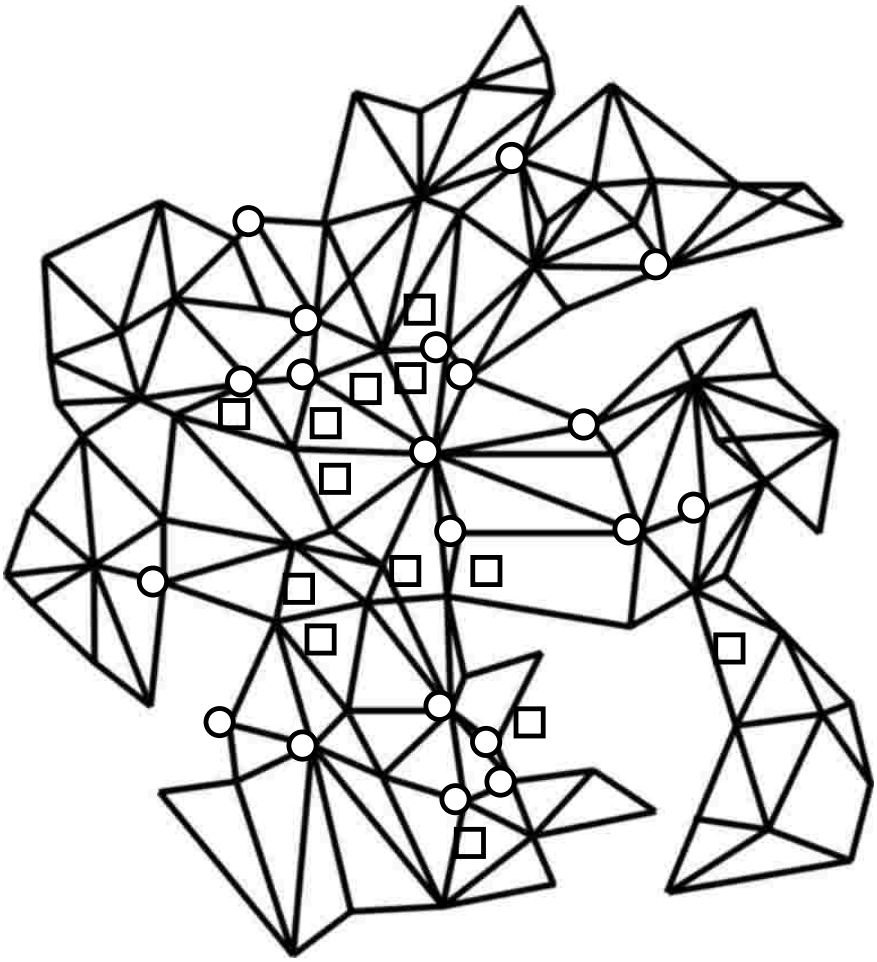

Figure 3. Map of position of potential male parents of teak trees at Dolok Research Site for the gene-flow study at natural teak forest. The circles indicated the position of selected female teak trees where the teak seeds have been harvested. From Dolok Research Site, collection of samples of teak seedling from the forest ground (boxes) and potential insect pollinators have also been conducted.

Figure 4. Map of position of potential male parents of teak trees at Wadila Research Site for the gene-flow study at natural teak forest. The circles indicated the position of selected female teak trees where the teak seeds have been harvested.

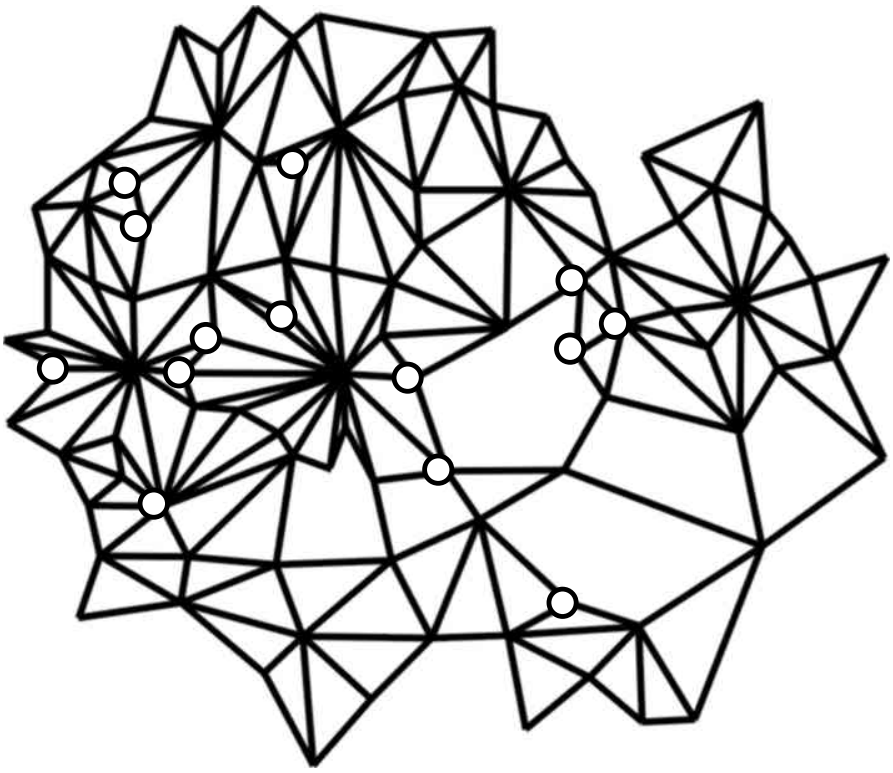

Figure 5. Map of position of potential male parents of teak trees at Warangga Research Site for the gene-flow study at natural teak forest. The circles indicated the position of selected female teak trees where the teak seeds have been harvested.

Workpackage 5 Analysis of the impact of human disturbance on genetic diversity

General Objectives: To analyse the impact of human disturbance on genetic diversity of teak in Indonesia.

Specific Objectives:

1. Select location of teak forest in Indonesia that are close to natural (undistuberd forest), slightly disturbed, and heavily disturbed teak forest in Indonesia
2. Analyze teak population from selected forest gene specific markers and microsatelite markers

Progress of the Activities:

1. Research site selected for this studies were the same as that used in the WP3 and WP 4 studies (Table 2). Threrefore, all information regarding research sites, sample collection and DNA isolation were similar to ones described in WP3 and WP4.
2. Molecular analysis data collected from WP3 and WP4 will also be used for WP 5. Progress of this research activity was similar to that described in WP3 and WP4.
3. In the year 2005, molecular genetic analysis will be continued for all teak samples collected using gene specific and microsatelite markers. In addition, analysis of impact of human disturbance on genetic diversity will be completed in the year 2005.

Workpackage 6 Identification of effective insect pollinators in teak

General Objectives: To identify effective insect pollinators in teak.

Specific Objectives:

1. Collect and identify potential polinators for teak in teak plantation
2. Collect and identify potential polinators for natural teak forest in Sulawesi

Progress of the Activities:

1. For the plantation forest, clonal seed orchard belonging to Perum Perhutani at Cepu, Central Java, has been selected for the teak polinator studies. Insect collection has been done in first year of research activity (2002). Second year collection has been conducted in 2003.
2. For the natural teak forest, potential insect pollinator from Dolok Research Site at Muna Island has been collected in the year 2003.
3. Identification of potential pollinators from both plantation and natural teak forest, respectively, has been conducted. The results indicated the existence of potential insect pollinators commonly found in both forests. In

addition, there are also unique potential insect pollinators existed only at Dolok Research Site but not at Cepu Research Site.

4. No further activities regarding the insect studies were conducted since 2003, except for identification of the collected insect speciments. Listing of insect visiting teak flower, characteristic of the insect such as whether they carry teak pollen, the position of the insect body where the teak pollen deposited, and pollen load have been described for each insect visiting teak flower. Possible teak polinators were predicted based on these characters.

Appendix:

Results of Parentage Analysis for Data from 4 sites in southern Sulawesi

1. Teak forest from four regency in southern Sulawesi (Bilokka, Ceddie, Massepe, and Welongge) were selected for one of the study site for seed dispersal (Work Package 4).
2. From each teak forest, 20 adults trees and 20 seedlings (close to each of the adults trees) were randomly selected.
3. The teak samples were subjected to microsatelite marker analysis and the genotypes of the individual sample were resolved using denaturing PAGE.
4. Data collected were subjected for analysis using CERVUS Program for parentage analysis using either both parents unknown or the identified parent from the first analysis was regarded as known parents and the data were reanalysed. Results of the analysis were presented in Table 1 and 2 (Bilokka), Table 3 and 4 (Ceddie), Table 5 and 6 (Massepe), and Table 7 and 8 (Welongge).
5. Map of the parent tree and seedling positions in the forest will be combined with the results of parentage analysis and used to determine teak seed dispersal in the four teak forest.

Table 1. Parent-Offspring analysis among samples of randomly selected adult trees and seedlings in the Bilokka, southern Sulawesi teak forest (Model: Both parent unknown)

Offspring ID	Known parent ID	Prob. non-exclusion	Candidate parent ID	LOD	Delta	Confidence
OffspringBS1	TreeBS6	0.11	TreeBS16	-0.03	0.00	
OffspringBS2	TreeBS20	0.20	TreeBS6	0.99	0.81	+
OffspringBS3	TreeBS12	0.13	TreeBS7	-0.07	0.00	
OffspringBS4	TreeBS16	0.02	TreeBS7	0.38	0.38	-
OffspringBS5	TreeBS17	0.03	TreeBS6	0.64	0.64	+
OffspringBS6	TreeBS20	0.27	TreeBS1	0.74	0.39	-
OffspringBS7	TreeBS7	0.38	TreeBS10	1.36	0.94	+
OffspringBS8	TreeBS15	0.30	TreeBS1	0.42	0.42	-
OffspringBS9	TreeBS4	0.15	TreeBS7	1.32	1.32	+
OffspringBS10	TreeBS4	0.05	TreeBS4	0.14	0.14	-
OffspringBS11	TreeBS10	0.39	TreeBS17	0.79	0.31	-
OffspringBS12	TreeBS6	0.17	TreeBS6	0.38	0.38	-
OffspringBS13	TreeBS6	0.14	TreeBS17	1.40	0.64	+
OffspringBS14	TreeBS10	0.39	TreeBS17	0.79	0.31	-
OffspringBS15	TreeBS7	0.02	TreeBS6	0.59	0.59	-
OffspringBS16	TreeBS7	0.17	TreeBS4	1.02	1.02	+
OffspringBS17	TreeBS4	0.25	TreeBS17	1.43	1.35	+
OffspringBS18	TreeBS10	0.10	TreeBS4	-0.41	0.00	
OffspringBS19	TreeBS10	0.10	TreeBS4	-0.41	0.00	
OffspringBS20	TreeBS2	0.01	TreeBS17	0.00	0.00	-

Table 2. Parent-Offspring analysis among samples of randomly selected adult trees and seedlings in the Bilokka, southern Sulawesi teak forest (Model: parent identified in Table 1 as known parent)

Offspring ID	Known parent ID	Prob. Non-exclusion	Candidate parent ID	LOD	Delta
OffspringBS1	TreeBS6	0.11	TreeBS16	-0.03	0.00
OffspringBS2	TreeBS20	0.20	TreeBS6	0.99	0.81
OffspringBS3	TreeBS12	0.13	TreeBS7	-0.07	0.00
OffspringBS4	TreeBS16	0.02	TreeBS7	0.38	0.38
OffspringBS5	TreeBS17	0.03	TreeBS6	0.64	0.64
OffspringBS6	TreeBS20	0.27	TreeBS1	0.74	0.39
OffspringBS7	TreeBS7	0.38	TreeBS10	1.36	0.94
OffspringBS8	TreeBS15	0.30	TreeBS1	0.42	0.42
OffspringBS9	TreeBS4	0.15	TreeBS7	1.32	1.32
OffspringBS10	TreeBS4	0.05	TreeBS4	0.14	0.14
OffspringBS11	TreeBS10	0.39	TreeBS17	0.79	0.31
OffspringBS12	TreeBS6	0.17	TreeBS6	0.38	0.38
OffspringBS13	TreeBS6	0.14	TreeBS17	1.40	0.64
OffspringBS14	TreeBS10	0.39	TreeBS17	0.79	0.31
OffspringBS15	TreeBS7	0.02	TreeBS6	0.59	0.59
OffspringBS16	TreeBS7	0.17	TreeBS4	1.02	1.02
OffspringBS17	TreeBS4	0.25	TreeBS17	1.43	1.35
OffspringBS18	TreeBS10	0.10	TreeBS4	-0.41	0.00
OffspringBS19	TreeBS10	0.10	TreeBS4	-0.41	0.00
OffspringBS20	TreeBS2	0.01	TreeBS17	0.00	0.00

Table 3. Parent-Offspring analysis among samples of randomly selected adult trees and seedlings in the Ceddie, southern Sulawesi teak forest (Model: Both parent unknown)

Offspring ID	Prob. non-exclusion	Candidate parent ID	LOD	Delta	Confidence
OffspringCP1	0.29	TreeCP12	1.20	0.02	-
OffspringCP2	0.52	TreeCP20	0.20	0.20	-
OffspringCP3	0.51	TreeCP2	0.91	0.65	-
OffspringCP4	0.21	TreeCP10	0.87	0.07	-
OffspringCP5	0.35	TreeCP2	0.75	0.65	-
OffspringCP6	0.47	TreeCP15	0.54	0.27	-
OffspringCP7	0.20	TreeCP19	-0.10	0.00	
OffspringCP8	0.25	TreeCP20	0.64	0.64	-
OffspringCP9	0.45	TreeCP1	1.36	1.12	-
OffspringCP10	0.16	TreeCP2	0.41	0.05	-
OffspringCP11	0.54	TreeCP2	1.37	1.08	-
OffspringCP12	0.56	TreeCP15	1.27	0.36	-
OffspringCP13	0.10	TreeCP19	-0.05	0.00	
OffspringCP14	0.47	TreeCP2	0.73	0.07	-
OffspringCP15	0.53	TreeCP15	0.76	0.40	-
OffspringCP16	0.25	TreeCP20	0.82	0.82	-
OffspringCP17	0.47	TreeCP15	1.13	0.40	-
OffspringCP18	0.46	TreeCP19	1.96	1.41	+
OffspringCP19	0.51	TreeCP15	0.48	0.40	-
OffspringCP20	0.13	TreeCP10	-0.68	0.00	

Table 4. Parent-Offspring analysis among samples of randomly selected adult trees and seedlings in the Ceddie, southern Sulawesi teak forest (Model: parent identified in Table 3 as known parent)

Offspring ID	Known parent ID	Prob. non-exclusion	Candidate parent ID	LOD	Delta	Confidence
OffspringCP1	TreeCP12	0.09	TreeCP1	0.16	0.16	-
OffspringCP2	TreeCP20	0.03	TreeCP10	-1.78	0.00	
OffspringCP3	TreeCP2	0.14	TreeCP15	0.06	0.06	-
OffspringCP4	TreeCP10	0.01	TreeCP10	-0.66	0.00	
OffspringCP5	TreeCP2	0.35	TreeCP2	0.78	0.66	+
OffspringCP6	TreeCP15	0.60	TreeCP12	0.69	0.11	-
OffspringCP7	TreeCP19	0.20	TreeCP19	-0.71	0.00	
OffspringCP8	TreeCP20	0.03	TreeCP1	0.84	0.84	+
OffspringCP9	TreeCP1	0.10	TreeCP10	0.22	0.22	-
OffspringCP10	TreeCP2	0.05	TreeCP20	0.13	0.13	-
OffspringCP11	TreeCP2	0.42	TreeCP12	0.86	0.01	-
OffspringCP12	TreeCP15	0.18	TreeCP10	2.11	0.59	-
OffspringCP13	TreeCP19	0.21	TreeCP19	-0.80	0.00	
OffspringCP14	TreeCP2	0.17	TreeCP12	1.83	0.68	+
OffspringCP15	TreeCP15	0.18	TreeCP2	1.56	1.56	*
OffspringCP16	TreeCP20	0.25	TreeCP20	0.85	0.85	+
OffspringCP17	TreeCP15	0.16	TreeCP2	1.94	1.94	*
OffspringCP18	TreeCP19	0.31	TreeCP12	0.88	0.03	-
OffspringCP19	TreeCP15	0.04	TreeCP1	0.42	0.42	-
OffspringCP20	TreeCP10	0.01	TreeCP20	-2.12	0.00	

Table 5. Parent-Offspring analysis among samples of randomly selected adult trees and seedlings in the Massepe, southern Sulawesi teak forest (Model: Both parent unknown)

Offspring ID	Prob. non-exclusion	Candidate parent ID	LOD	Delta	Confidence
OffspringMS1	0.97				
OffspringMS2	0.30	TreeMS1	0.23	0.02	-
OffspringMS3	0.39	TreeMS7	1.04	0.87	-
OffspringMS4	0.10	TreeMS5	2.03	0.96	-
OffspringMS5	0.60	TreeMS15	0.41	0.41	-
OffspringMS6	0.46				
OffspringMS7	0.46				
OffspringMS8	0.35	TreeMS5	0.36	0.04	-
OffspringMS9	0.69	TreeMS6	0.53	0.10	-
OffspringMS10	0.80				
OffspringMS11	0.15	TreeMS7	1.13	1.13	-
OffspringMS12	0.20	TreeMS4	0.62	0.62	-
OffspringMS13	0.86				
OffspringMS14	1.00				
OffspringMS15	0.81	TreeMS2	0.92	0.32	-
OffspringMS16	0.41	TreeMS1	0.64	0.01	-
OffspringMS17	0.09	TreeMS6	2.01	0.85	-
OffspringMS18	0.52	TreeMS1	0.84	0.21	-
OffspringMS19	1.00				
OffspringMS20	0.40	TreeMS7	1.00	0.04	-

Table 6. Parent-Offspring analysis among samples of randomly selected adult trees and seedlings in the Massepe, southern Sulawesi teak forest (Model: parent identified in Table 5 as known parent)

Offspring ID	Known parent ID	Prob. non-exclusion	Candidate parent ID	LOD	Delta	Confidence
OffspringMS1		0.97				
OffspringMS2	TreeMS1	0.09	TreeMS11	-0.18	0.00	
OffspringMS3	TreeMS7	0.39	TreeMS7	0.99	0.99	+
OffspringMS4	TreeMS5	0.03	TreeMS11	1.40	0.51	-
OffspringMS5	TreeMS15	0.44	TreeMS7	-0.11	0.00	
OffspringMS6		0.46				
OffspringMS7		0.46				
OffspringMS8	TreeMS5	0.10	TreeMS13	-0.02	0.00	
OffspringMS9	TreeMS6	0.41	TreeMS7	0.66	0.11	-
OffspringMS10		0.80				
OffspringMS11	TreeMS7	0.15	TreeMS7	1.21	1.21	+
OffspringMS12	TreeMS4	0.20	TreeMS4	0.65	0.65	+
OffspringMS13		0.86				
OffspringMS14		1.00				
OffspringMS15	TreeMS2	0.81	TreeMS2	0.65	0.39	-
OffspringMS16	TreeMS1	0.41	TreeMS1	0.65	0.01	-
OffspringMS17	TreeMS6	0.06	TreeMS11	1.86	1.22	+
OffspringMS18	TreeMS1	0.33	TreeMS6	1.17	0.59	-
OffspringMS19		1.00				
OffspringMS20	TreeMS7	0.40	TreeMS7	1.02	0.04	-

Table 7. Parent-Offspring analysis among samples of randomly selected adult trees and seedlings in the Welongge, southern Sulawesi teak forest (Model: Both parent unknown)

Offspring ID	Prob. non-exclusion	Candidate parent ID	LOD	Delta	Confidence
OffspringWS1	0.59	TreeWS11	0.97	0.68	-
OffspringWS2	0.26	TreeWS9	0.94	0.94	-
OffspringWS3	0.45	TreeWS7	0.78	0.18	-
OffspringWS4	0.38	TreeWS9	1.81	1.81	+
OffspringWS5	0.58				
OffspringWS6	0.68				
OffspringWS7	0.47	TreeWS20	1.13	0.58	-
OffspringWS8	0.57				
OffspringWS9	0.72	TreeWS7	0.18	0.18	-
OffspringWS10	0.85				
OffspringWS11	0.63	TreeWS7	0.83	0.83	-
OffspringWS12	0.54				
OffspringWS13	0.79				
OffspringWS14	0.85				
OffspringWS15	1.00				
OffspringWS16	1.00				
OffspringWS17	0.87				
OffspringWS18	0.51				
OffspringWS19	0.41	TreeWS20	0.93	0.66	-
OffspringWS20	0.87				

Table 8. Parent-Offspring analysis among samples of randomly selected adult trees and seedlings in the Welongge, southern Sulawesi teak forest (Model: parent identified in Table 7 as known parent)

Offspring ID	Known parent ID	Prob. non-exclusion	Candidate parent ID	LOD	Delta	Confidence
OffspringWS1	TreeWS11	0.22	TreeWS17	(0.51)	-	
OffspringWS2	TreeWS9	0.15	TreeWS20	(0.72)	-	
OffspringWS3	TreeWS7	0.15	TreeWS11	1.38	1.38	+
OffspringWS4	TreeWS9	0.29	TreeWS20	0.45	0.45	-
OffspringWS5		0.58				
OffspringWS6		0.69				
OffspringWS7	TreeWS20	0.47	TreeWS20	1.19	0.60	-
OffspringWS8		0.57				
OffspringWS9	TreeWS7	0.08				
OffspringWS10		0.85				
OffspringWS11	TreeWS7	0.23	TreeWS7	(0.77)	-	
OffspringWS12		0.54				
OffspringWS13		0.79				
OffspringWS14		0.85				
OffspringWS15		1.00				
OffspringWS16		1.00				
OffspringWS17		0.87				
OffspringWS18		0.51				
OffspringWS19	TreeWS20	0.20	TreeWS9	0.80	0.80	+
OffspringWS20		0.87				

Partner 4 (Scientific coordinator) Kasetsart University

Apichart Vanavichit – Agricultural Biotechnology and Genetic Engineering Research Centre, Kasetsart University, Kampaengsaen Campus, 73140, Kampaengsaen, NakornPathom, Thailand. Tel: (+66) 34 281 494. Fax: (+66) 34 282 494. Email: apichart@dna.kps.ku.ac.th
Hugo Volkaert – Centre for Agricultural Biotechnology, Kasetsart University, Kampaengsaen Campus, 73140, Kampaengsaen, NakornPathom, Thailand. Tel: (+66) 34 282 494 ext 305. Fax: (+66) 34 282 494 ext 117. Email: hugo.v@ku.ac.th

Scientific team
Dr. Hugo Volkaert, Ms. Jongkon Cheua-ngam (M. Sc. student), Ms. Piyaporn Phansak (technician)

Workpackage 1: Development of practical protocols and assays for studying genetic diversity in teak.

DNA extraction protocols.
Isolation and characterisation of microsatellite markers.
A library of teak DNA fragments enriched for microsatellites has been constructed and screened. Sixty nine microsatellite sequences have been deposited with EMBL DNA database. A set of teak accessions has been screened to identify the different alleles that can be detected and assess the general level of polymorphism. Ten microsatellite loci have been identified as most promising. The protocol for microsatellite assessment still has to be adjusted to allow multiple loadings in a single gel, to speed up the genotyping process.
A new library of microsatellite fragments has been constructed, to isolate trinucleotide repeats. Several of these microsatellite inserts have been retained as polymorphic markers with potential for practical application. They have not yet been submitted to EMBL, but that will be done in the near future.

Characterisation of specific cytoplasmic and nuclear genetic markers.
Conserved PCR amplification primers have been developed for nuclear and chloroplast genes.

A set of teak accessions from different origins (4 populations from India, 2 from Thailand and 2 from Indonesia) from the international provenance trial at PhaaNokKhao (managed by the Royal Forest Department), has been screened for cytoplasmic and nuclear gene allelic variants.

Initially, polymorphism had been detected for seven of the chloroplast loci that have been investigated: *rpl*16, *trn*T-L, *mat*K, *psa*A, *rpo*A, *pet*B and *ndh*A. No polymorphism was detected in the screening population for two other plastidic markers (*psb*A, *rbc*L) and one mitochondrial marker studied. The polymorphisms were observed on SSCP analysis gels, and thus deemed reliable. However, when trying to pinpoint the particular mutations responsible

for the polymorphism through cloning of PCR amplified fragments, the results were ambiguous. Using direct sequencing of PCR amplified fragments to confirm the mutations, it turned out that allmost all of the polymorphisms observed in cloned fragments were due to misincorporation of nucleotides by the *Taq* DNA polymerase during PCR amplification and only a very small number of polymorphic nucleotide positions could be confirmed. Therefore, three additional loci have been screened in 2004: *pet*A, *ndh*F and *psb*C/D, to have a larger base of molecular polymorphisms. Polymorphisms at these new loci have not yet been confirmed by direct sequencing.

Detailed protocols for scoring of the alleles have been finalised and have been made available on the TEAKDIV website. A protocol to amplify PCR products for direct sequencing has been added in the updated procedures.

The following nuclear genes families are currently being investigated for polymorphisms: isocitrate dehydrogenase (IDH, 1 locus), aspartate amino transferase (AATcy, a cytoplasmic isoform), alcohol dehydrogenase (ADH2), glyceraldehyde 3-phosphate dehydrogenase (G3PDHcp, a plastid isoform and G3PDHcy1 and G3PDHcy2, two putative cytoplasmic isoforms), catalase (CAT1), Terminal Flower1 / Centroradialis / Flowering Locus T homolog (TFL1). In addition, microsatellites have been found in introns for three nuclear genes: alchol dehydrogenase (ADH1), cystein proteinase inhibitor (CPI) and chitinase (CHIT).

Different alleles for each locus have been cloned and sequenced and confirmed by direct sequencing. Detailed protocols for scoring of the alleles have been finalised and have been made available on the TEAKDIV website. A protocol to amplify PCR products for direct sequencing has been added in the updated procedures.

1.5 Development of a catalogue of available alleles and allelic clone library.

Sequencing of alleles from Thailand, India and Indonesia

Workpackage 2 Assessment of genetic diversity in teak over its natural range – Phylogeography

Collection of samples, Analysis using chloroplast and nuclear DNA fragments.

Workpackage 3 Population genetic diversity in teak forests

Collection of samples, Analysis using nuclear gene DNA fragments.
Samples were collected from Thailand, and from a provenance trial growing at PhaNokKhao. Through the provenance trial, accessions were obtained from Allapally, Masale, Konni, Nilambur (southwestern India), Purunakote and Bakbahal (eastern India), Bangsri and (Java). These samples were used to obtain DNA sequences from Indian and Indonesian origin.

Workpackage 4 Gene flow through pollen and seed dispersal.

2 populations in MaeYom National Park, Phrae (permission to collect granted)
1 population in Salaween National Park, MaeHongSon (permission to collect granted)
2 populations in MaeWong National Park, KamPhaengPhet (permission to collect)
Dr. Endah Palupi, who had collected samples from the teak seed orchard in Cepu, Java, used the facilities at the Kamphaengsaen lab to performa all PCRs and polyacrylamide gel electrophoresis analysis.

Workpackage 5 Analysis of the impact of human disturbance on genetic diversity

Analysis of the relevant data from WP3.

Workpackage 6 Identification of effective insect pollinators in teak

Work on this package has been done by Dr. Suwan Tangmitcharoen, the assigned couterpart in the Department of National Parks, Wildlife and Plant Protection.

Workpackage 7 Analysis of the genetic diversity data
The data that have been gathered in the previous workpackages will be analysed for several aspects of population genetics and gene flow. Through discussion with end-users, reports will be prepared dealing with several aspects of the use of teak genetic resources and its conservation.

Activities for this workpackage have not yet started because of a delay in obtaining the genotyping data.

Partner 5 (Contractor) Universiteit Gent

Dominique Van Der Straeten – Department of Molecular Genetics, Gent University, K.L. Ledeganckstraat 35, 9000, Gent, Belgium. Tel: (+32) 9 264 5185. Fax: (+32) 9 264 5333. Email: dominique.vanderstraeten@rug.ac.be

Scientific team

Dr. Dominique Van Der Straeten, principal investigator

Dr. Madan Shrestha, postdoctoral fellow

WP2

Samples from India, Indonesia and Thailand were supplied by partner 4 (Hugo Volkaert). AFLP procedure was used to analyze these samples

Workpackage 3 Population genetic diversity in teak forests

Samples from Thai populations were supplied by partner 4 (Dr. Hugo Volkaert) and were analyzed using AFLP method

Partner 6 (Contractor) KVL

Hubert Wellendorf - Royal Veterinary University, Sektion Arboretet, Institut for Oekonomi, Skov og Landskab, KVL, Kirkegaardsvej 3A, 2970, Hoersholm, Denmark. Tel: (45) 35 283 628. Fax: (45) 35 283 629. Email: hwe@kvl.dk

Personnel:
Dr. Hubert Wellendorf, Dr. Elise Skov.

WP4

5. EXPLOITATION AND DISSEMINATION ACTIVITIES

Refereed papers published

Lowe AJ, Menozzi P (2003) Spatial dynamics and natural regeneration: Dynamics and conservation of genetic diversity in forest ecosystems. *Forest Genetics* 9:336-337

Davies S, White A and Lowe AJ (Submitted) An investigation into long distance seed dispersal on resulting organelle population genetic structure and colonisation rate: a model analysis. *Heredity*.

Shrestha, M. K., Volkaert, H. and Van Der Straeten, D. 2005. Assessment of genetic diversity in *Tectona grandis* L.f. using AFLP markers. Canadian Journal of Forest Research (Accepted)

Books

Lowe AJ, Harris SA and Ashton P (2004). *Ecological Genetics: Design, Analysis and Application*. Blackwells Publishing, Oxford, UK. 344 pp.

Conference posters

Jongkok Cheuangam and Hugo Volkaert (2003) TEAKDIV – Developing know-how for the improvement and sustainable management of teak genetic resources. BioThailand 2003, Pattaya, Thailand, 17th 19th July, 2003. Published in: Proccedings of the 2nd International Conference on Medicinal Mushrooms and the International Conference on Biodiversity and Bioactive compounds. Pp. 529-532

Lowe, A., Davies, S. Cheua-ngam, J. Volkaert, H., E.P., I., Balasundaran, M., Sudarsono, Palupi, E., Wellendorf, H. Skov, E., van der Straeten, D. and Shrestha, M. (2003) TEAKDIV – Developing know-how for the improvement and sustainable management of teak genetic resources. Accompanying paper to be published in Proc. International Conference on Quality Timber products of teak from sustainable Forest Management held at KFRI, Peechi during 2-5th December 2003

Number of visiting scientists

Dr. Sasaki Japanese Tree breeding Center

8. DATA SHEET FOR FINAL REPORT

1.Dissemination activities	Totals
Number of communications in conferences (published)	2(1)
Number of communications in other media (internet, video etc)	website
Number of publications in refereed journals (published)	3 (1)
Number of articles/books (published)	1 (0)
Number of other publications	0

2.Training	
Number of Ph.D.s	3
Number of M.Sc.s	1
Number of visiting scientists	0
Number of exchanges of scientists (>3 months)	0

3.Achieved results	
Number of patent applications	0
Number of patents granted	0
Number of companies created	0
Number of new prototypes/products developed	0
Number of new tests/methods developed	nuclear marker 0
Number of new norms/standards developed	0
Number of new software/codes developed	0
Number of production processes	

4.Industrial aspects		
Industrial contacts		no
Financial contribution by industry		none
Industrial partners	large	none
	small	none