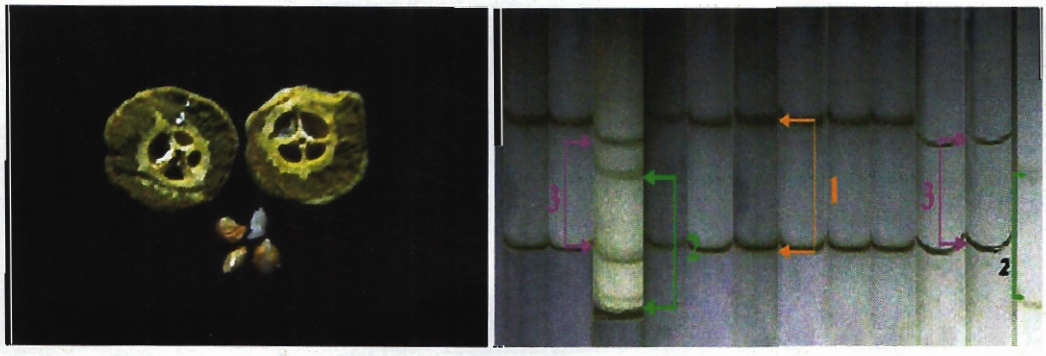
KFRI Project Report No. 383 ISSN 0970-8103

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

E. P. Indira
Forest Genetics & Biotechnology Division
M. Balasundaran
Forest Genetics & Biotechnology Division
K. Mohanadas
Forest Protection Division



Kerala Forest Research Institute
An institution of Kerala State Council for Science, Technology and Environment
Peechi – 680 653, Thrissur, Kerala, India

December 2010

KFRI Project Report No. 383 **ISSN 0970-8103**

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

(Final report of Project KFRI 377/ 02)

E. P. Indira
Forest Genetics & Biotechnology

M. Balasundaran
Forest Genetics & Biotechnology

K. Mohanadas
Forest Protection Division

KFRI

Kerala Forest Research Institute
An Institution of Kerala State Council for Science, Technology and Environment (KSCSTE)
Peechi – 680 653, Thrissur, Kerala, India

December 2010

ABSTRACT OF THE PROJECT PROPSAL

1. Project number : KFRI 377 / 02

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

3. Principal investigator : Dr. E.P. Indira

4. Associate investigators: Dr. M.Balasundaran
Dr. K.Mohanadas

5. Objectives:

i). Population genetic diversity in teak forests in India

ii). Analysis of the impact of human disturbance on genetic diversity

iii). Contemporary gene flow through pollen and seed dispersal.

iv). Identification of effective insect pollinators for teak

6. Duration : 4 years

7. Funding agency : European Union

ACKNOWLEDGEMENT

Sincere thanks are due to Dr. J. K.Sharma, Dr. R Gnanaharan the former Directors and to Dr. K.V. Sankaran, Director, KFRI, for the support and encouragement. The help rendered by the staff of Forest Department of Kerala and other states during the fieldwork is also acknowledged. The strenuous field work and laboratory work conducted by Mr. Pramod N.Nair, Ms. Sabna Prabha, Dr. Rajalakshmi, Mr. Kannan and Mr.Dhanush is appreciable and note worthy.

The authors gratefully acknowledge the financial support from European Union for carrying out the research work. This was a sub project of a general theme "Developing know-how for the improvement and sustainable management of teak genetic resources" carried out by six institutions (Centre for Ecology and Hydrology (UK), Kerala Forest Research Institute (India), Kasetsart University (Thailand), Bogor Agricultural University (Indonesia), Gent University (Belgium), Royal Veterinary and Agricultural University (Denmark). Thanks are due to Dr. Hugo Volkaert for giving us training on Molecular marker development for teak genetic diversity studies as a part of the planning workshop at Kasetsart University at Bangkok, Thailand in 2002. Dr. Volkaert has also developed the molecular markers for teak and has given training to the project staff, for which we are grateful. We thank Dr. Andrew Lowe and Dr. Cecile from CEH, U.K. for giving training on Data analysis during the workshop on Data analysis for Population and Ecological genetics organized by KFRI during December 2003. We also thank Dr. Hubert Wellendorf from Denmark and Dr. Sudarsono from Indonesia for giving us valuable suggestions during the project period.

We thank Dr.T.Surendran and Dr.E.M.Muralidharan from KFRI for the editorial scrutiny and for their suggestions for improving the manuscript.

CONTENTS

ABSTRACT

An account of existing genetic diversity, long and short term dynamics, contemporary gene flow and the mating system are essential for the conservation and sustainable management of teak (*Tectona grandis* L.f.) genetic resources. Hence, seven paired populations of natural teak forests (undisturbed and disturbed) were selected in Kerala, Orissa, Madhya Pradesh and Gujarat. One plantation was also selected in Kerala. Co-dominant DNA markers namely nuclear gene markers and microsatellite markers were used to study the long term dynamics and short term dynamics of the populations and to estimate the population genetic parameters, such as the number of alleles, allelic richness, expected and observed heterozygosity and gene diversity in these teak populations. The impact of human disturbances was also assessed on the above genetic parameters.

The results of the nuclear gene marker studies showed the undisturbed population at Konni to be the most diverse population having maximum number of alleles, allelic richness, expected and observed heterozygosity and gene diversity where as both populations at Khurda (Orissa) had lower number of alleles, allelic richness and lowest gene diversity. Generally Kerala populations had higher gene diversity followed by Valsad (Gujarat) and Jabalpur (Madhya Pradesh). With regard to undisturbed populations, Orissa was genetically far distant from all other populations. In general the total gene diversity, with respect to the fourteen populations examined, was found to be 0.749, of which a large portion (0.550) was within population diversity and only 0.199 was between populations. On analyzing the effect of human disturbance, the present study revealed a reduction in the mean number of alleles, gene diversity and heterozygosity in disturbed populations than their undisturbed pair populations.

The four microsatellite loci employed to examine the short term dynamics showed that Khurda from Orissa to be the richest in number of alleles. Populations in Wayanad and Nilambur had the least number of alleles. Other populations from Kerala also were rich in number of alleles. Maximum gene diversity was seen in undisturbed Khurda population followed by Valsad, while populations at Khurda (Orissa) were found to

exhibit lowest gene diversity on employing nuclear gene markers as noted earlier. This might be due to recent evolutionary changes in consequence of mutations so as to compensate the low gene diversity. Populations from Kerala, including the plantation were found to have high gene diversity. Out of the total 15 populations, inbreeding (*Fis*) was significant in all the populations from North India and in particular, the undisturbed populations from North India showed higher inbreeding. With respect to Kerala populations, disturbed populations had more inbreeding than undisturbed populations except in Nilambur.

The STRUCTURE analysis showed geographical patterns based on the allele frequencies. The analysis using nuclear gene markers showed three clusters, the first cluster comprised of all the populations from Kerala, second included the populations from Gujarat and Madhya Pradesh and the third being the populations from Orissa. Using microsatellite markers the same pattern was obtained except that the populations from Kerala were split in two clusters with the Nilambur provenances separated from the rest of Kerala. In each of the locations, there were no apparent differences between the undisturbed and disturbed pair populations.

To compare the contemporary gene flow through pollen and seed dispersal and the mating system in teak populations with different levels of human interference, two natural teak forests, one highly disturbed and another undisturbed were selected in the Peechi-Vazhani Wildlife Sanctuary of Thrissur District in Kerala State. The individual trees and seedlings on forest floor and progenies through seeds of selected trees were DNA-fingerprinted using seven microsatellite markers in non-denaturing polyacrylamide gel electrophoresis.

The studies revealed that more than 50 per cent of the fruits were without embryos in both the two populations selected. The pollen dispersal was mainly in the distance of below 200 m which indicates that the pollen dilution zone must be at least 200 m in seed orchards to restrict the entry of pollen from outside. The main range of pollen dispersal distance was found to be 151-200 m in disturbed plot and 101-150 m in undisturbed plot.

The reason for the higher pollen dispersal distance in the disturbed plot might be due to the large number of insects observed in this population than the other leading to the increased activity of pollinators. The analysis on seed dispersal showed that the dispersal was mainly in the distance range of 50-100 m.

With regard to mating system, higher out-crossing rate (89-96 %) was seen in the populations. The present study also revealed that an individual mother tree received pollen from many male parents leading to multi-parental mating. Likewise, an individual male parent donated pollen to many mother parental trees leading to a high within population gene diversity through the mixing of alleles while producing the next generation.

The study conducted on pollinators identified Hymenopteran groups of insects as the potential pollinators playing a vital role in teak pollination. The main pollinators are some solitary bees like *Halictus tectonae* Narendran and Jobiraj and Wasps. Two species of solitary bees, *Anthophora zonata* (Linn.) and *A. niveo-cincta* Smith were also found to carry a load of pollen on the under side of their abdomen and hind legs. They were found to be very active, visiting several inflorescences on the same as well as on different trees in a short time. Most of the insect visitors spent their time among the inflorescences of a single tree except bigger wasps which moved rapidly among the adjacent trees.

The results of the present study show that teak populations are genetically diverse and disturbance in natural forests leads to decrease in gene diversity, increased inbreeding and higher population differentiation between parents and their progenies. The findings of this study are useful for the scientific and sustainable management of teak seed orchards/seed stands and germplasm banks as well as for the preparation of efficient and comprehensive strategies for conservation of natural teak populations.

1. INTRODUCTION

Teak (*Tectona grandis* L.f.) belonging to the family Verbenaceae is distributed in South and South East Asia. It is widely cultivated throughout the tropics although its natural distribution is limited to India, Myanmar, Laos and Thailand. Teak has been recognized as a king among the timbers due to its durability, workability, strength and attractive figure and grains. The timber is used in the manufacture of outdoor furniture, boat decks and other articles where weather resistance is desired. Teak timber has excellent physical properties. Heartwood of teak is strong, of medium weight and hardness and outstanding in retaining the shape. It has excellent shock absorbing ability, reasonable shear, tensile strength and low coefficient of expansion and contraction.

Due to its unique wood properties and international acceptance as a plantation species, teak has been widely planted outside its natural range and introduced to many countries. In India, teak improvement programmes were started during early 1960's and through these programmes seed production areas and seed orchards were established throughout the teak growing states in India. During the same period, genetic improvement of teak was initiated in Thailand and later in Indonesia.

In India, it occurs naturally in peninsular region from 8° to 24° N latitudes and 73 ° to 104 °E longitude as well as in Manipur. It grows in the dry and moist deciduous forests in India, south of 24 °N latitude. India is considered as the centre of its genetic diversity (Hedegart, 1976; Dogra, 1981). India has about 8.9 million hectors under natural teak forests and plantations. Natural teak forests have been very much altered during the last 50 to 100 years through uncontrolled logging and movement of planting materials. Due to uncontrolled logging and mixing of germplasm, there has been much concern over the protection of teak genetic resources (Shrestha *et al.*, 2005). The area of natural teak forests has drastically declined and the remaining forests are still under threat from illegal logging and other forms of forest degradation.

Forest fragmentation may lead to genetic isolation of small populations that can cause inbreeding and genetic drift (Bawa, 1990). In isolated populations, genetic drift and some degree of inbreeding may eventually reduce genetic variation (Lacy, 1987; Young *et al.*, 1996). Several studies have reported the effects of fragmentation or other disturbances inducing changes in genetic structure, gene flow and mating patterns of tropical trees (Hall *et al.*, 1996; Nason and Hamrick, 1997; Aldrich *et al.*, 1998).

Tropical trees are thought to be more affected by habitat degradation due to their demographic and reproductive characteristics, including low density, complex self-incompatible breeding systems, high rates of out crossing, and intimate interactions with pollinators and seed dispersers (Cascante *et al.*, 2002; Dick *et al.*, 2003; Ward *et al.*, 2005). An estimation of anthropogenic effect on degradation of forests will be useful for the conservation of forest resources. In the short term, disturbed plant populations are expected to suffer increased disease and pest susceptibility (Barrett and Kohn, 1991), loss of incompatibility alleles, and fixation of deleterious alleles (Huenneke, 1991). In the long term, loss of genetic variation is expected to reduce the fitness of populations to respond to changing selection pressures (Young *et al.*, 1996). Genetic diversity is of prime importance for species persistence for long-lived plants like forest trees that have to cope with high temporal and spatial environmental heterogeneity.

The structure and patterns of genetic diversity in teak populations have been studied using molecular markers (isozymes, RAPD and AFLP) in recent years (Kertadikara and Prat, 1995; Changtragoon and Szmidt, 2000; Nicodemus *et al.*, 2005; Shrestha *et al.*, 2005). All these studies showed that the largest fraction of the genetic diversity is within, rather than among, teak populations. Overall understanding of genetic diversity for teak is still fragmentary and the impact of man made disturbances on the maintenance of the teak germplasm is unknown. Hence, studies were necessary to explore the effect of logging and other human disturbances on gene diversity and related parameters in teak which will be of high value to initiate strategies for conservation and sustainable management of the genetic resources.

In natural forests, teak is a long lived tree that grows to large size. It flowers abundantly most years, but seed set is generally very low. Teak is mainly an out crossing species and is reported to be partially self-incompatible and lack of effective pollinators was reported to be the main reason for very poor seed settings (Hedegart, 1976; Tangmitcharoen and Owens, 1997; Indira and Mohanadas, 2002). The out crossing rates are high and range between 89 per cent and 95 per cent as understood through isozyme studies (Kjaer and Suangtho, 1995)

It is well known that, seed production and germination is one of the most critical problems in planting programmes of this species. Though teak flowers profusely, fruiting is found to be very low. The maximum germination percentage in teak varies from 30 to 50. The low fruiting and germination percentage in teak seed orchards and seed production areas hampered the breeder's efforts considerably. To tackle this problem, studies on breeding system in teak were conducted in different countries which revealed that only about one per cent of the flowers set fruits. Understanding the process of pollination, fertilization, embryo and seed development, fruit maturation and fruit abortion may reveal the reasons for low fruit productivity.

The situation turns out to be highly intricate, when the mechanism of pollination was uncertain and the distance of pollen flow, seed dispersal and percentage of out-crossing rate are also not known, but which are essential for formulating genetic conservation measures and for effective management of seed orchards. In teak, not much study had been conducted on contemporary and long term gene flow as well as migration pattern. It is presumed that the plantations are raised by collecting seeds from very few trees and hence, such plantations are expected to be less diverse. As a result, there may be difference in gene flow through pollen as teak is partially self incompatible. It is also believed that in disturbed forests, some of the insect pollinators may be missing since their habitat is disturbed. The disturbance in habitat of insect pollinators may cause changes in the mating system and gene flow, which will lead to erosion in genetic diversity of the stand.

Factors determining the level and structure of genetic variation within plant species include evolutionary history, population density, mating system and mechanisms of gene flow (Loveless, 1992). Out of these, gene flow is one of the important factors shaping the genetic structure of populations. In recent decades, a number of studies have addressed issues on contemporary gene flow in forest trees, including pollen and seed dispersal, and gene immigration into natural and breeding populations (primarily seed orchards). Gene flow might be considered either beneficial or deleterious from the point of view of a conservation geneticist or a tree breeder. Extensive gene dispersal within local populations promotes panmixis in natural regeneration, thus reducing the potential for inbreeding. However, gene flow may reduce fitness of offspring if genes come from populations maladapted to the habitat of offspring establishment. Furthermore, substantial gene flow limits divergence among populations that might otherwise occur because of genetic drift and directional selection, and may enhance genetic diversity within populations (Burczyk *et al* ., 2004).

Finkeldey (1998) pointed out that inventories using gene markers are the most important experiments for the selection of genetic resources since centers of genetically differentiated populations can be identified by such inventories. Different molecular markers have been developed to investigate the genetic variation of plants and forest trees in the past few decades. Most individuals, except clones, have a nucleotide sequence of DNA that makes them unique and distinguishable from each other. The detection of such differences either through enzyme or DNA based polymorphisms reveals a particular molecular pattern commonly called a "genetic finger print" that can be used for unique identification and discrimination of individual genotypes. Tracking the inheritance of such marker patterns, DNA markers can be used for parentage analysis.

Microsatellites are highly polymorphic DNA markers, which have come into prominence for individual genotyping, and studies of gene flow in forest trees (Butcher *et al*., 1999). Microsatellites are co-dominant markers and are therefore far more informative for genotyping individuals and for linkage mapping than dominant markers such as RAPDs and AFLPs.

Being the background explained earlier, studies were undertaken to study the gene diversity, effect of human disturbance on genetic diversity and contemporary gene flow in Indian teak. This was a sub project of a general theme "Developing know-how for the improvement and sustainable management of teak genetic resources" carried out by six institutions viz., Centre for Ecology and Hydrology (UK), Kerala Forest Research Institute (India), Kasetsart University (Thailand), Bogor Agricultural University (Indonesia), Gent University (Belgium), Royal Veterinary and Agricultural University (Denmark). DNA markers for teak were developed by Kasetsart University, Thailand. Training on software and data analysis was given by CEH, UK. The investigators from RVAU (Denmark) were in the team of establishing and evaluating the International provenance trials in teak during 1978 to 1995 and their experiences were elaborated and utilized for the general analysis of the project. Gent University (Belgium) have analysed the international provenances using AFLP markers. As India, Thailand and Indonesia have teak populations, investigators from these countries have analysed their populations and the conclusions are used for the joint analysis of the influence of anthropogenic disturbance on contemporary gene flow patterns, mating system and genetic diversity to draft guidelines for sustainable management of natural teak forests. The main objectives of the project was i) to trace and quantify genetic diversity of teak within its natural range, the current distribution of genetic diversity within and between populations, historical migration patterns and mating system, ii) to evaluate the amount of contemporary gene flow through pollen and seed dispersal and parentage analysis and iii) to assess the influence of human disturbance on genetic diversity.

Hence, the present project was under taken to study the

i). Population genetic diversity in teak forests in India

ii). Analysis of the impact of human disturbance on genetic diversity

iii). Contemporary gene flow through pollen and seed dispersal.

iv). Identification of effective insect pollinators for teak

2. ASSESSMENT OF GENETIC DIVERSITY IN TEAK FORESTS IN INDIA AND IMPACT OF HUMAN DISTURBANCE ON GENETIC DIVERSITY

2.1. MATERIALS AND METHODS

Selection of populations

Natural teak populations were identified in the forests of Kerala, Orissa, Madhya Pradesh and Gujarat representing South, East, North and West of India respectively. Paired populations with respect to human disturbance (as undisturbed and disturbed) were selected in different forest divisions in these states. The level of disturbance was assessed based on different criteria i.e. number of stumps observed, presence of old logging roads, frequency of forest fires, distance to inhabited area, records of recent disturbance etc.

In Kerala, paired populations were taken from four natural teak growing areas namely, Trichur (Peechi-Vazhani Wildlife Sanctuary), Konni, Nilambur and Wayanad Forest Divisions. From each of the other three States- Orissa, Madhya Pradesh and Gujarat, only two populations (one paired population) were selected (Table 1). They were from Khurda (Orissa), Jabalpur (Madhya Pradesh) and Valsad (Gujarat) Forest Divisions. A 43-year-old teak plantation was also selected at Kuthiran in Pattikkad Range of Peechi-Vazhani Wildlife Sanctuary of Trichur Circle in Kerala.

Sample collection

About 40 to 50 trees in each area were marked randomly and GPS readings were taken. Marked populations were mapped using software MAP Info Professional (Fig.1). Samples of leaves were collected from these selected trees in disturbed and undisturbed populations as well as from the selected plantation at Kuthiran in Trichur Division.

DNA Extraction

The juvenile leaf samples were collected from the trees and seedlings were stored in 2 per cent CTAB buffer at 4^{0}C to avoid degradation of genomic DNA till the extraction. The DNA extraction was done in modified CTAB DNA isolation procedure (Doyle and Doyle, 1990). After

the extraction of DNA, 100 µl dissolved DNA was taken out from the supernatant and stored at -20 ^{0}C till further use.

DNA Visualisation

Two µl of purified DNA was mixed with 3 µl of loading dye (Glycereol and Bromophenol blue) and run on 1.5 per cent agarose gel in 1X TBE buffer with 3 µl of fluorescent dye, Ethidium bromide. It was electrophoresed using horizontal electrophoresis system (Apelex Maxigel Eco2) with a constant volt of 150 for 40 minutes and visualized on UV transilluminator (Ultra Lum). Photographs of the gels were taken with an Epson Camera.

Quantification of DNA

The standard concentration of 30 ng of λ DNA was loaded on 1.5 per cent of agarose gel along with 2 µl of the DNA samples. By using Ultra Lum Total Lab software, the approximate quantity of DNA was found out. Twenty to thirty ng of DNA was found to be enough for PCR amplification. If the DNA concentration is beyond this range, it was sufficiently diluted.

Table. 1. Selected populations

Populations & Forest Divisions	Forest Range	Type of populations	Natural regeneration
1. Division: Peechi - Vazhani Wildlife Sanctuary (Kerala)			
Vazhani	Machad	Undisturbed	Moderate
Thamaravellachal	Peechi	Disturbed	Moderate
2. Division: Nilambur South (Kerala)			
Poochapara	Karulai	Undisturbed	Moderate
Padukka	Karulai	Disturbed	Absent
3. Division: Konni (Kerala)			
Kaduvappara	Naduvathamuzhy	Undisturbed	High
Kattathi	Naduvathamuzhy	Disturbed	Moderate
4. Division: Wayanad (Kerala)			
Tholpetty	Tholpetty	Undisturbed	High
Baveli	Tholpetty	Disturbed	Scanty
5. Division: Khurda (Orissa)			
Balunda	Balugaon	Undisturbed	Moderate
Ranjin Forest	Balugaon	Disturbed	scanty
6. Division: Jabalpur (Madhya Pradesh)			
Ghotgharakpur	Burgi	Undisturbed	High
Disharad	Burgi	Disturbed	Scanty
7. Division: Valsad (Gujarat)			
Sara	Valsad North	Undisturbed	High
Mahwas	Valsad North	Disturbed	Scanty

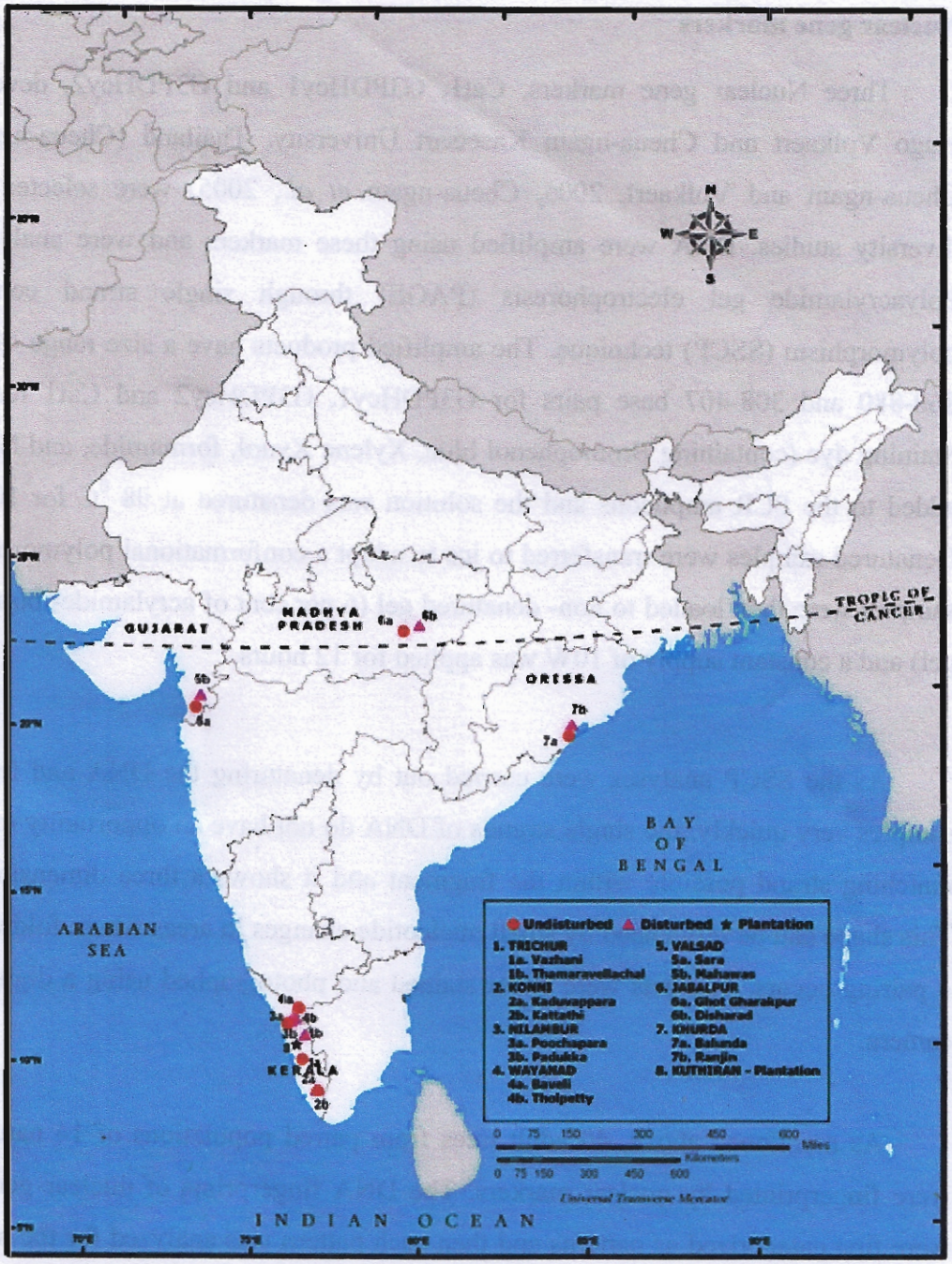


Figure 1. Populations selected in different states

Nuclear gene markers

Three Nuclear gene markers, Cat1, G3PDHcy1 and G3PDHcy2, developed by Hugo Volkaert and Cheua-ngam Kasetsart University, Thailand (Cheua-ngam 2005, Cheua-ngam and Volkaert, 2005, Cheua-ngam *et al.*, 2005) were selected for gene diversity studies. DNA were amplified using these markers and were analyzed using Polyacrylamide gel electrophoresis (PAGE) through single strand conformation polymorphism (SSCP) technique. The amplified products have a size range of 630-645, 860-880 and 308-407 base pairs for G3PDHcy1, G3PDHcy2 and Cat1 respectively. Staining dye (containing Bromophenol blue, Xylene Xynol, formamide, and NaOH) was added to the PCR amplicons and the solution was denatured at 98 ^{0}C for 10 minutes. Denatured samples were transferred to ice to adopt a conformational polymorphism. The samples were then loaded to non- denatured gel (6 per cent of acrylamide- bisacrylamide gel) and a constant supply of 10W was applied for 12 hours.

As the SSCP analyses were carried out by denaturing the DNA and freezing the samples very quickly, the single strands of DNA do not have an opportunity to find their matching strand possible within the fragment and it shows a three dimensional shape. This shape can be influenced by small nucleotide changes in areas where folding and self – pairing occurs. The gels were silver stained and photographed using a digital EPSON camera.

As mentioned above, 40 adult trees from paired populations of 14 natural forests were fingerprinted by nuclear markers. The DNA fingerprints of nuclear gene markers were first categorized as patterns and then each pattern was analyzed for the number and position of bands. Then the alleles were identified by comparing the patterns with the different reference clones and a few doubtful patterns were checked through sequencing. After identification of the alleles, samples from the present study were also used as reference samples in other populations. The bands obtained in the acrylamide gels were scored for allelic polymorphism as well as for heterozygosity and homozygosity.

Microsatellite markers

Hyper variable microsatellite markers designed for teak by Dr. Hugo Volkaert of Kasetsart University, Thailand were used for this study. Microsatellite markers namely AC01, AC28, AC44, AG04, AG14, AG16 and CPIms (EMBL accessions) were used for DNA amplification out of eight markers tested. The amplified products have a size range of 151-250 base pairs. For analyzing all the 15 populations for genetic diversity, four markers namely AC01, AC28, AG04 and AG14 were used. All the seven markers were used for analyzing the mating system and contemporary gene flow.

The PCR amplification reactions were performed in a programmable thermal cycler (PTC–200; MJ Research, USA) equipped with a heated lid to minimize sample fluxing. The amplified products were electrophoresed and separated on 4.5 per cent denaturing poly acrylamide gel at 90W constant for 1 hour 30 minutes in 1X TBE buffer using Sequi–Gen GT Nucleic Acid Sequencing Cell with 38 cm X 50 cm size integral plate chamber (Bio-Rad, USA). The gels were silver stained and photographed using a digital camera.

Allele identification/ scoring of alleles

According to the position of bands in acrylamide gels, alleles were differentiated for each marker (locus) and numbers were given. The low molecular weight fragment moved downwards was marked as allele number 1, the next on the upper side as 2 and so on. After the identification of alleles by comparing with reference samples, the total number of alleles was counted at each locus in each sample and overall samples. As stated earlier the microsatellite markers are co-dominant, so the heterozygous and homozygous individuals were identified for each marker with respect to each individual.

Analysis

Data analyses were done with the softwares namely FSTAT, CERVUS and STRUCTURE.

FSTAT

The number of alleles, allelic richness, gene diversity, inbreeding and genetic differentiation were estimated using FSTAT version 2.9.3.2 (Goudet, 2001). The

estimates of allele frequencies and gene diversity, the proportion of each genotype and their expected proportion are based on the Hardy Weinberg null model. The frequency of alleles for each population was computed as a mean to evaluate population differentiation. The gene diversity was estimated using the observed heterozygosity (Ho), expected heterozygosity (He) and allelic richness.

1. Allelic Richness

Allelic richness is a measure of the number of alleles per locus corrected for differences in population size (Petit *et al.*, 1998) and hence allowed to compare populations with different sample sizes. Estimates of allelic richness per locus and sample (Rs) is estimated through Fstat as

$$R_s = \sum_{i=1}^{n} \left[1 - \frac{\binom{2N - N_i}{2n}}{\binom{2N}{2n}} \right]$$

Where Ni is the number of alleles of type i among the 2N genes in a sample.

2. Gene diversity

Estimates of gene diversity per locus and population/sample are calculated 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.

3. Inbreeding (*Fis*)

Estimates of inbreeding coefficient or heterozygote deficit (*Fis*) for each locus and sample/population is calculated following Weir and Cockerham (1984) as

$$F_{is}^k = 1 - \frac{H_o^k}{H_s^k}$$

where, Ho is observed heterozygosity and Hs is the average expected heterozygosity estimated from each subpopulation and k is the number of sub populations

4. Genetic differentiation (Fst/ Gst'):

Gst' (Nei, 1987) is an unbiased estimator of Fst and is equivalent to θ_{ST} developed by Weir and Cockerham (1984) and it measures the amount of genetic variation in the total samples/ populations that is due to the differences among populations comprising that sample and is calculated as:

$$GST' = \frac{HT' - HS}{HT'} = \frac{DST'}{HT'}$$

where, HT' is total gene diversity and HS' is expected heterozygosity.

CERVUS

Polymorphic Information Content (PIC), Observed heterozygosity and expected heterozygosity were estimated from allele frequencies assuming Hardy-Weinberg equilibrium (Nei, 1987) and an unbiased estimate executed through Cervus version 3 (Marshall *et al.*, 1998).

1. Polymorphic Information Content (PIC)

It 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 highly informative in population-genetic analyses (Botstein *et al.* 1980).

2. Observed Heterozygosity (Ho)

Observed Heterozygosity (H_o) is the value obtained by dividing the number of heterozygotes by total number of individuals. It is estimated as

$$H_o = 1 - \sum_k \sum_i P_{kii} / np$$

where, P_{kii} is the frequency of genotype AiAi in sample k and np is the number of samples.

3. Expected heterozygosity (He)

Expected heterozygosity is calculated using an unbiased formula from allele frequencies assuming Hardy-Weinberg equilibrium (equation 8.4, Nei 1987).

Loci with expected heterozygosity less than 0.5 are in general not very useful for large-scale parentage analysis.

STRUCTURE

The geographical patterns were drawn by the program STRUCTURE using the data on allele frequency in different populations. It implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. The method was introduced by Pritchard *et al.*, (2000) and extended in sequels by Falush *et al.* (2003, 2007).

2.2. RESULTS AND DISCUSSION

Nuclear gene markers

As mentioned in the Materials and methods, the DNA fingerprints of nuclear gene markers were first categorized as patterns and then each pattern was examined for the number and position of bands (Fig.2). Then the alleles were identified and marked by comparing the patterns with the different reference clones. The bands obtained in the acrylamide gels were scored for allelic polymorphism as well as for heterozygosity and homozygosity.

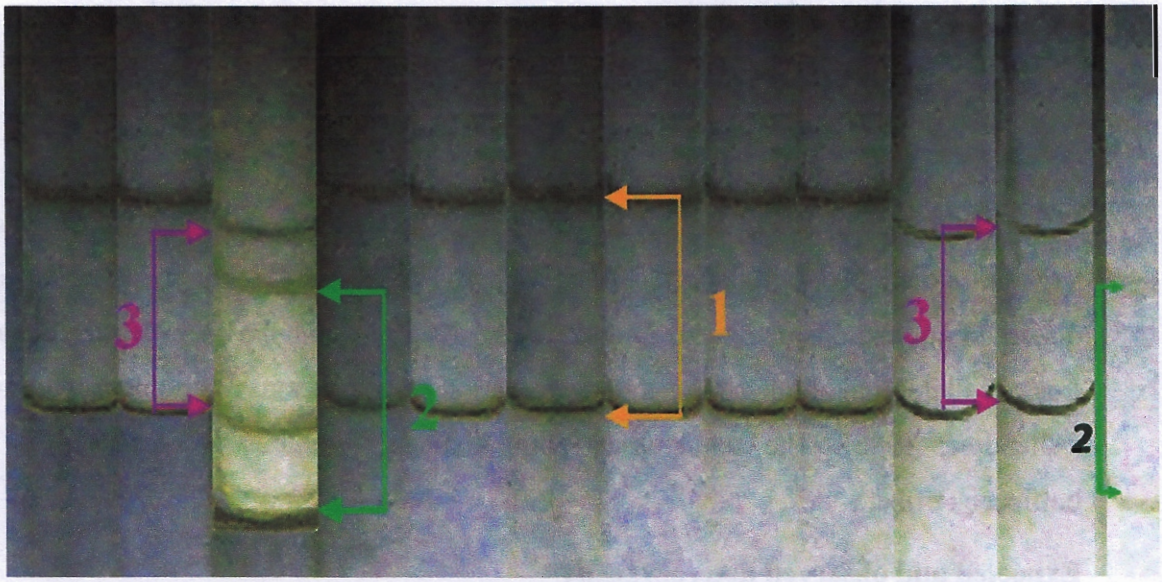


Fig. 2. Allele identification and scoring for heterozygosity

Number of alleles

With respect to undisturbed populations, the results show that Konni had the maximum number of alleles (16) followed by Trichur (13) and Jabalpur (12) (Table 2 & Fig.3). But considering disturbed populations, Valsad had least number of alleles (8) followed by Khurda and Konni (9). The disturbed population at Nilambur showed an increase in the number of alleles (11) over the undisturbed population (10). The decrease in number of alleles in the undisturbed population at Nilambur may be due to the long term isolation of this undisturbed population with very limited number of trees within. In all other paired populations the number of alleles were lower in the disturbed populations.

The disturbed population at Konni had the highest loss of alleles. There was a reduction of 43.7% in the mean number of alleles at Konni due to disturbance.

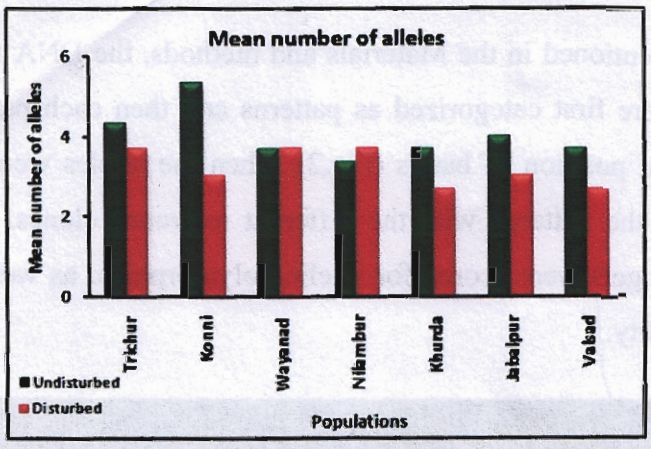


Fig. 3. Effect of disturbance in number of alleles

Allelic richness

Allelic richness was highest in undisturbed Konni population (5.07) followed by Trichur undisturbed population. Allelic richness was found to be reduced in all the disturbed populations compared to their undisturbed pair population except in Nilambur. Loss of alleles and allelic richness in a population is an indicator of population bottleneck (Allendorf, 1986). The undisturbed population at Nilambur is suspected to have bottleneck effect.

Expected and observed heterozygosity

Genetic variation at the level of the individual is measured in terms of heterozygosity. The number and frequency of alleles at a locus, the amount of inbreeding, and the type and intensity of natural selection, all determine the level of heterozygosity within a population. Generally heterozygous genotypes have greater fitness than homozygotes (Young *et al.*, 1996). Expected heterozygosity was highest in Konni undisturbed population (0.699) followed by Wayanad disturbed population (0.628), but it was lowest in Konni disturbed population (0.329) (Table 3). In both undisturbed and disturbed populations at Konni, observed heterozygosity was more (0.723 and 0.685 respectively).

Table 2. Number of alleles and Allelic richness per population

Gene Loci	Konni UD (Kerala)	Konni D (Kerala)	Trichur UD (Kerala)	Trichur D (Kerala)	Nilambr UD (Kerala)	Nilambr D (Kerala)	Wayanad UD (Kerala)	Wayanad D (Kerala)	Khurda UD (Orissa)	Khurda D (Orissa)	Jabalpur UD (M.P.)	Jabalpur D (M.P.)	Valsad UD (Gujrat)	Valsad D (Gujrat)
Alleles for G3PDH cy1	5	4	5	4	3	6	5	5	4	3	4	3	5	3
Alleles for G3PDH cy2	6	2	4	2	3	2	3	3	3	2	4	2	2	2
Alleles for CAT	5	3	4	5	4	3	3	3	4	4	4	4	4	3
Total No. of alleles (for 3 markers)	16	9	13	11	10	11	11	11	11	9	12	9	11	8
Mean number of alleles	5.33	3	4.33	3.67	3.33	3.67	3.67	3.67	3.67	3	4	3	3.67	2.67
Allelic richness	5.07	3	4.25	3.66	3.33	3.67	3.98	3.62	3.43	2.76	3.5	3.26	3.54	2.67

Table 3. Heterozygosity, Gene diversity and Inbreeding in different populations

Gene Loci	Konni UD (Kerala)	Konni D (Kerala)	Trichur UD (Kerala)	Trichur D (Kerala)	Nilambr UD (Kerala)	Nilambr D (Kerala)	Wayanad UD (Kerala)	Wayanad D (Kerala)	Khurda UD (Orissa)	Khurda D (Orissa)	Jabalpur UD (M.P.)	Jabalpur D (M.P.)	Valsad UD (Gujrat)	Valsad D (Gujrat)
Expected heterozygosty	0.699	0.329	0.583	0.625	0.594	0.491	0.619	0.628	0.524	0.479	0.589	0.557	0.611	0.495
Observed heterozygosty	0.723	0.685	0.555	0.406	0.486	0.448	0.408	0.514	0.441	0.455	0.532	0.524	0.566	0.304
Gene diversity	0.703	0.628	0.618	0.579	0.618	0.492	0.670	0.61	0.326	0.24	0.579	0.568	0.614	0.502
Inbreeding	-0.046	-0.08	0.047	0.344*	0.183	0.089	0.306*	0.321*	0.121	0.115	0.04	0.062	0.073	0.373*

* significant at 5% level

Though the disturbed population at Nilambur had more alleles and higher allelic richness, expected and observed heterozygosity were low in this plot compared to the undisturbed pair population. Even though the disturbed population at Konni had lower number of alleles and lowest expected heterozygosity, this population exhibited higher observed heterozygosity. All the disturbed populations had lower expected heterozygosity than their undisturbed pair except at Trichur and Wayanad. Like wise all the disturbed populations had lower observed heterozygosity than their undisturbed pair except at Wayanad and Khurda.

The heterozygous genotypes have greater fitness than homozygotes (Young *et al.*, 1996), and it is the erosion of heterozygosity that reduces fitness of the population. An overall reduction in heterozygosity may lead to increase in (i) the rate of selfing, (ii) the strength of selection of deleterious homozygotes and (iii) inbreeding depression (Hedrick, 1994; Husband and Schemske, 1996).

Gene diversity

The maximum gene diversity was observed at Konni (0.703) and minimum value at Khurda (0.326) (Table 3). All the undisturbed populations in Kerala had shown higher gene diversity (higher than 0.6) than all the other undisturbed and disturbed populations out side Kerala. Gene diversity was lowest in both undisturbed and disturbed populations from Khurda Division of Orissa (0.326 and 0.24). Populations from Jabalpur and Valsad had comparatively higher gene diversity.

Undisturbed populations had higher gene diversity compared to their paired disturbed population in all the locations. The variations in gene diversity of disturbed over undisturbed populations were 2 to 26 per cent (Table 4 and Fig 4.)

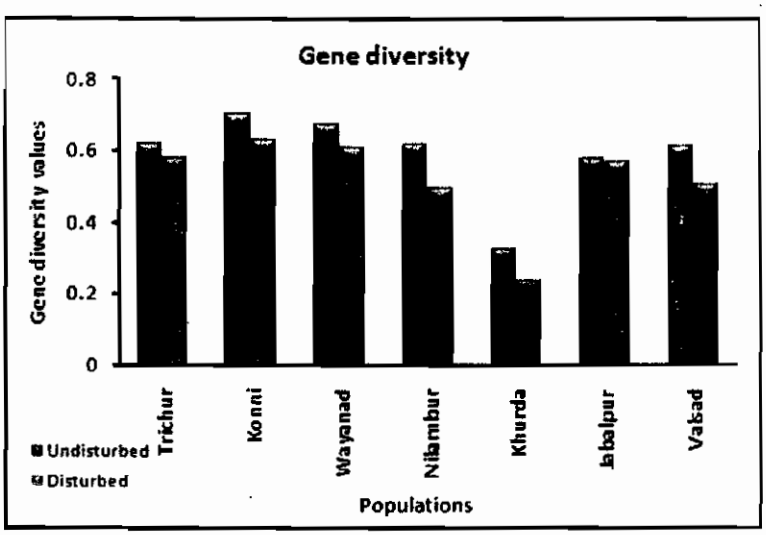
Table 4. Reduction in Gene diversity in disturbed populations

Populations	Undisturbed	Disturbed	Variation*
Trichur	0.618	0.579	-6.3
Konni	0.703	0.628	-10.7
Wayanad	0.670	0.61	-9.0
Nilambur	0.618	0.492	-20.4
Khurda	0.326	0.24	-26.4
Jabalpur	0.579	0.568	-1.9
Valsad	0.614	0.502	-18.2

- (%) *Variation of disturbed over undisturbed population*

Total gene diversity was found to be 0.749 of which a large chunk (0.550) was within population diversity and only 0.199 was between population diversity. This result is in agreement with that of Nicodemus *et al.* (2005), where they reported high within population diversity (78%) on analyzing ten teak populations from Western Ghats and Central Indian regions using RAPD.

Fig. 4. Reduction in gene diversity due to forest disturbance



Inbreeding (Fis)

All the seven paired populations (undisturbed and disturbed) were compared for inbreeding (Fis). There was no sign of inbreeding in disturbed and undisturbed populations at Konni (Figure 5) while it was high in disturbed populations at Valsad and Trichur as well as in both populations at Wayanad (Table 3). Disturbed populations had higher inbreeding rate except in Nilambur and Khurda. Inbreeding within a population is caused by non random mating of the members, in which mating occurs more often than by chance alone, between closely related individuals. As closely related individuals will contain a large proportion of the same alleles due to common descent, their offsprings will have a higher level of homozygosity than expected.

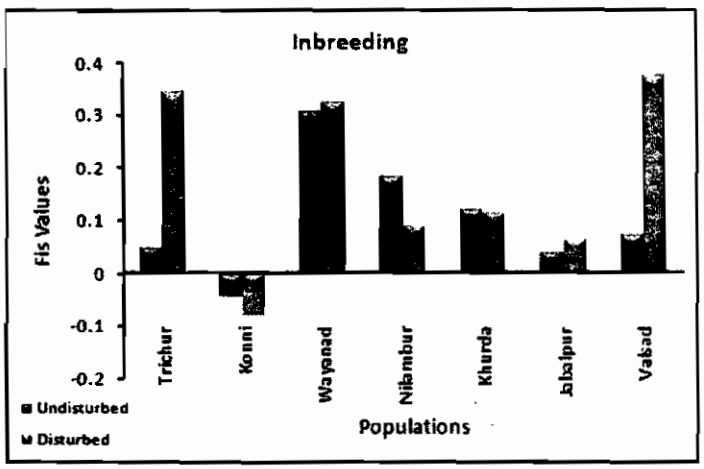


Fig.5. Inbreeding (Fis) in paired populations

Genetic differentiation (Fst/Gst')

The genetic differentiation between undisturbed populations at all locations was found to be significant. The genetic distance between Khurda and all other locations are the highest (0.3631 to 0.5137) (Table 5). Jabalpur and Valsad also show wide distance with Kerala populations (0.2177-0.3310). The UPGMA dendrogram using distance matrices between disturbed teak populations from different seed sources also shows the same pattern (Fig. 6).

Table 5. Differentiation (Fst) between undisturbed populations

Populations	Konni	Trichur (Kerala)	Nilambur (Kerala)	Waynad (Kerala)	Khurda (Orissa)	Jabalpur (M.P.)
Trichur	0.0865					
Nilambur	0.0373	0.1236				
Wynad	0.0553	0.1061	0.1447			
Khurda	0.3631	0.4890	0.4388	0.4246		
Jabalpur	0.2608	0.2441	0.3310	0.2492	0.5322	
Valsad	0.2177	0.2216	0.2451	0.2838	0.5137	0.1166

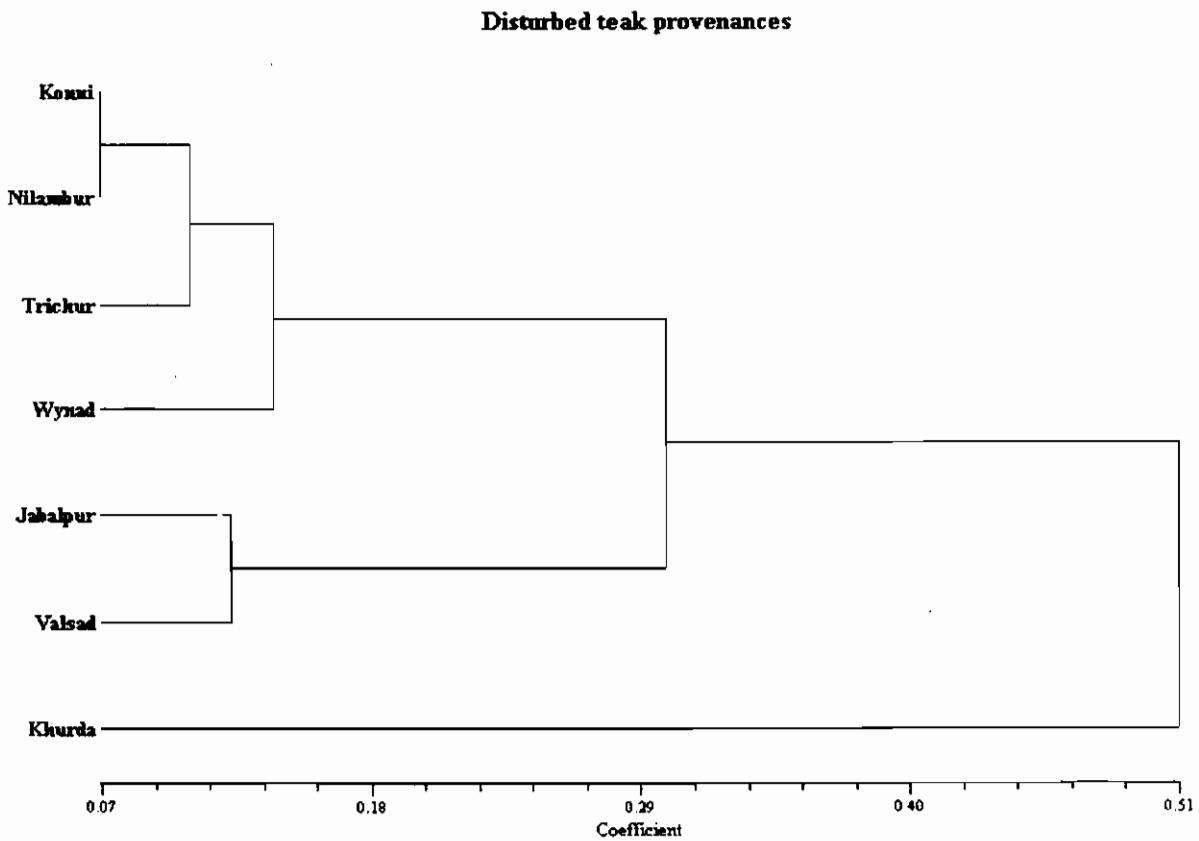


Fig. 6. Dendrogram based on genetic distance between populations

Genetic Structure

The STRUCTURE analysis revealed geographical patterns based on the allele frequencies. In the present study, the analysis shows three clusters, one comprising of Madhya Pradesh and Gujarat, the second of Orissa and the third of Kerala populations

(Fig.7). In each of the locations, there are no apparent differences between disturbed and undisturbed populations.

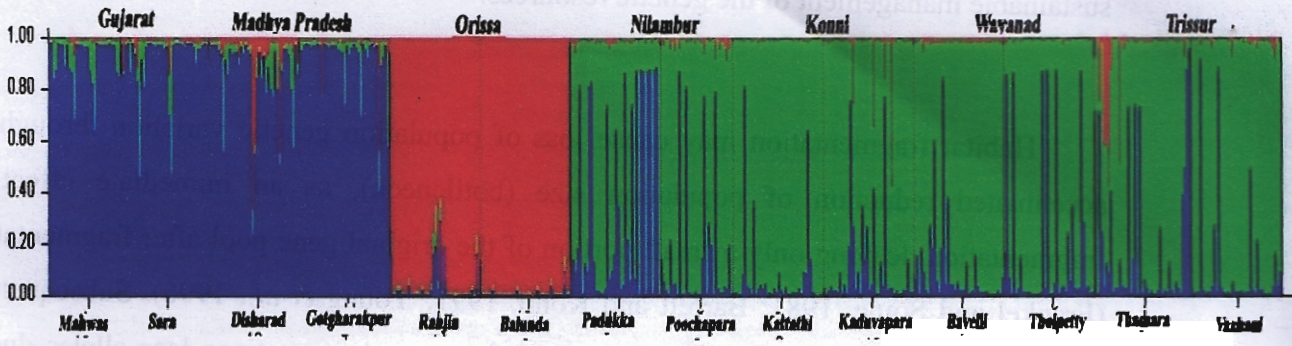


Fig 7. Bar diagram showing geographical patterns in the allele frequency

When the effects of human disturbance is taken in to consideration, the results obtained from the present study after evaluating the long term dynamics clearly indicate that disturbances such as logging activities and the frequent fires lead to a decrease in gene diversity, allelic richness and heterozygosity along with the loss of individual trees and habitat.

Genetic diversity is important for the fitness and the adaptive changes. So loss of genetic diversity will affect the fitness and evolutionary adaptability of teak. A reduction in diversity through the loss of alleles reduces a population's ability to respond to biotic challenges (e.g. pathogens) and to changes in the abiotic environment. Evidences were given by many researchers that fragmentation may induce changes in genetic structure, gene flow and mating patterns of tropical tree populations (Hall *et al.,* 1996; Nason and Hamrick, 1997; Aldrich and Hamrick, 1998; Aldrich *et al.,* 1998). Tropical trees are thought to be particularly vulnerable to the effects of habitat degradation due to their demographic and reproductive characteristics, including low density of occurrence, complex self-incompatible breeding systems, high rates of out crossing (Cascante *et al.*, 2002) and intimate interactions with pollinators and seed dispersers. Quantifying the impact of habitat degradation on remnant forest stands will not only help to assess genetic and some ecological consequences of these changes but will also help to (i) to ascertain

the conservation value of remnant stands, (ii) to estimate future repercussions of tropical deforestation, and (iii) to formulate the strategies for effective conservation and sustainable management of the genetic resources.

Habitat fragmentation may cause loss of population genetic variation through an accentuated reduction of population size (bottleneck), as an immediate effect of fragmentation, leaving only a small portion of the original gene pool after fragmentation (Frankel and Soule, 1981; Barrett and Kohn, 1991; Young *et al.*, 1996). Subsequently, populations which remain small and isolated for several generations lose alleles due to random genetic drift, reducing the levels of genetic variation within populations (Barrett and Kohn, 1991; Young *et al.*, 1996).

Microsatellite markers

Number of alleles

The alleles were marked in each population according to its position in the acrylamide gel as explained earlier (Fig.8.). As the microsatellite markers are co-dominant, identification of heterozygote and homozygote individuals was possible. The number of alleles present in each population per locus is noted in Table 6. Mean number of alleles is given in Fig 9. Relatively good levels of multi allelism were observed at all the four microsatellite loci studied.

Altogether 32 alleles were seen in four microsatellite markers. Seven rare alleles were observed, out of which one allele each in AG04 and AG14 were absent in the disturbed populations. It is observed that all the markers have more than 5 alleles where, AC01 has 9 alleles, AC28 has 8, AG04 has 7 and AG14 has 8 alleles. Hence, they are suitable for assessment of genetic diversity and other related parameters. The mean number of alleles per population in general was in a range of 4.75 to 6.75. Undisturbed natural population from Trichur had the maximum number of alleles, followed by disturbed natural population and plantation from Trichur. Khurda from Orissa also was rich in number of alleles (Table 6). There was no evident reduction in alleles due to disturbance (Fig.9). Mean number of alleles was quite high in plantation at Trichur which is contrary to the belief that plantations are less diverse. The high polymorphism exhibited in the plantation may be because of mixing of the seeds from many trees and locations for its establishment. Polymorphic information content was found to be more than 0.5 (Table 6). PIC was highest at Khurda and least at Wayanad.

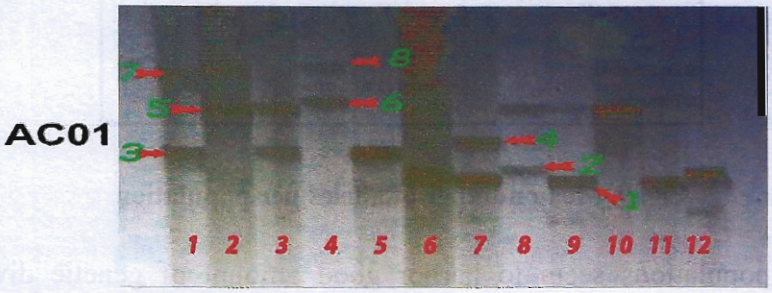


Fig. 8. Identification of alleles

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

Population	Tcr1 UD	Tcr2 D	Kon1 UD	Kon2 D	Way1 UD	Way 2 D	Nil1 UD	Nil2 D	Khr1 UD	Khr 2D	Jab 1 UD	Jab 2 D	Valsd1 UD	Valsd 2 D	Planta tion
AC01	6	7	5	8	6	4	5	5	6	8	9	7	7	8	7
AC28	6	5	4	4	3	3	4	3	6	4	4	7	5	7	6
AG04	7	6	4	4	5	4	4	4	5	5	2	2	4	2	5
AG14	8	7	6	6	7	6	6	7	6	7	4	6	5	4	6
Mean for each population	**6.75**	**6.25**	**4.75**	**5.5**	**5.25**	**4.25**	**4.75**	**4.75**	**5.75**	**6**	**4.75**	**5.5**	**5.25**	**5.25**	**6**
Mean for each location	**Trichur**		**Konni**		**Wynad**		**Nilambur**		**Khurda**		**Jabalpur**		**Valsad**		**Plantation**
	6. 5		**5. 13**		**4.75**		**4.75**		**5. 88**		**5.13**		**5. 25**		**6**
Mean PIC	0.564		0.58		0.482		0.517		0.619		0.509		0.565		0.548

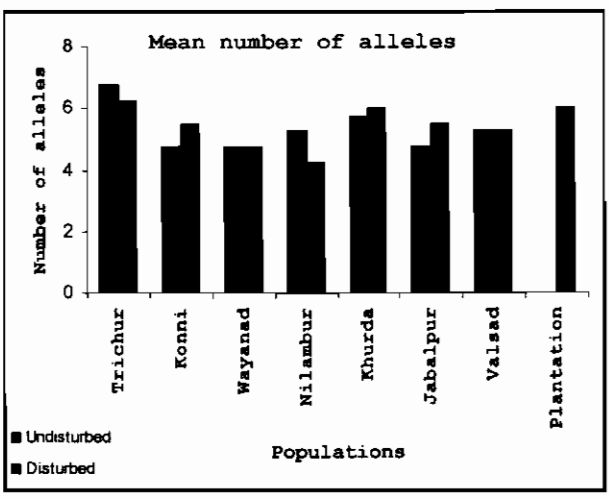


Fig. 9. Mean number of alleles per population

The teak populations seem to harbor good amount of genetic diversity within population. It is generally known that genetic diversity within population is influenced by the mating system and the method of seed dispersal (Hamrick *et al.*, 1992).

Expected and Observed Heterozygosity

The maximum expected heterozygosity (0.718) was obtained in undisturbed population at Khurda and lowest at Wayanad. Expected heterozygosity (He) was more only in four out of seven undisturbed populations, Trichur, Khurda, Jabalpur and Valsad (Table 7). Observed heterozygosity (Ho) was more in undisturbed populations at all locations where maximum observed heterozygosity (0.584) was seen in the Nilambur undisturbed population, which is higher than the expected heterozygosity in that population. Observed heterozygosity was lowest in Jabalpur (Madhya Pradesh) followed by that in Valsad.

Table 7. Expected and Observed Heterozygosity

Populations	He (Expected heterozygosity)	Ho (Observed heterozygosity)
Trichur UD	0.604	0.572
Trichur D	0.591	0.552
Konni UD	0.637	0.571
Konni D	0.645	0.559
Wayanad UD	**0.521**	0.521
Wayanad D	0.537	0.485
Nilambur UD	0.547	**0.584**
Nilambur D	0.593	0.484
Khurda UD	**0.718**	0.522
Khurda D	0.625	0.519
Jabalpur UD	0.643	0.326
Jabalpur D	0.477	**0.254**
Valsad UD	0.654	0.348
Valsad D	0.570	0.337

Inbreeding

Out of the total 15 populations, inbreeding (*Fis*) was significant in all the populations from North India (Table 8). But undisturbed populations from North India showed higher inbreeding. With respect to Kerala populations, disturbed populations have more inbreeding than undisturbed populations except in Nilambur. As noted earlier, this population at Nilambur with a very few individual trees may have bottle neck effect through an accentuated reduction of population size.

Table 8. Inbreeding

Populations	Inbreeding
Trichur UD	0.068
Trichur D	0.074
Konni UD	0.105
Konni D	0.134
Wayanad UD	0.005
Wayanad D	0.09
Nilambur UD	0.185*
Nilambur D	-0.051
Khurda UD	0.267*
Khurda D	0.175*
Jabalpur UD	0.504*
Jabalpur D	0.472*
Valsad UD	0.464*
Valsad D	0.416*
Plantation	-0.005

** Significant at 5 % level*

Gene diversity

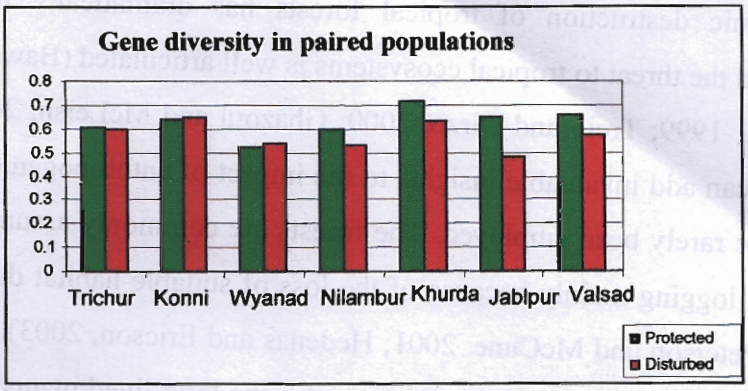
The results show that the estimated gene diversity was lower in disturbed populations than undisturbed populations in all the locations except Konni and Wayanad (Table 9 and Fig.10). The differences between paired populations are in the range of 1.4 to -25.6%. In general, with respect to gene diversity, variation of disturbed over undisturbed populations was more in populations in other states compared to Kerala. Within Kerala populations, Nilambur has shown higher variation.

Table 9 Gene diversity

Area	Population 1 -UD	Population 2 -D	Variation *
Trichur	0.608	0.596	-2.0
Konni	0.639	0.647	1.4
Wayanad	0.526	0.538	2.3
Nilambur	0.594	0.532	-10.4
Khurda	0.719	0.629	-12.5
Jabalpur	0.645	0.48	-25.6
Valsad	0.657	0.575	-12.5
Plantation (Trichur)	0.636		

* (%) *Variation of disturbed over undisturbed population*

Fig. 10. Reduction in gene diversity



Population genetic structure

Based on the allele frequencies, the STRUCTURE analysis show geographical patterns. In the present study the analysis shows four clusters, one comprising Madhya Pradesh and Gujarat, second one Orissa, third one Kerala populations except Nilambur and fourth one Nilambur (Fig.11). Within Kerala, Nilambur stands separately which may be due to the recent evolutionary changes. Other populations from Kerala namely Trichur, Wayanad, and Konni show genetic similarity. But in each of the locations, there are no apparent differences between disturbed and undisturbed populations.

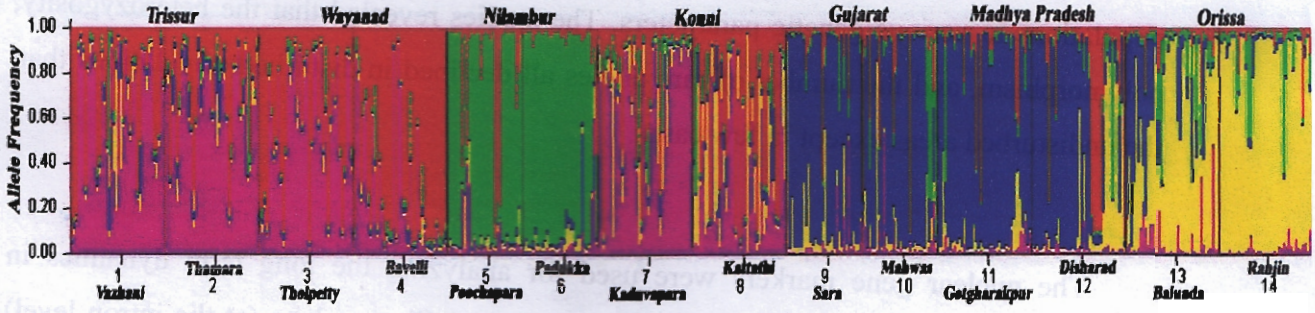


Fig.11. Bar diagram showing geographical patterns in the allele frequency

Effect of Anthropogenic disturbances

The genetic consequences of forest management receive more attention recently, but are still restricted to conifers and some tropical species like the genus Eucalyptus and

conflicting results are presented for different species (Buiteveld *et al.,* 2007). Anthropogenic destruction of tropical forests has dramatically increased in recent decades, and the threat to tropical ecosystems is well articulated (Bawa and Seidler, 1998; White *et al.,* 1999; Trejo and Dirzo, 2000; Ghazoul and McLeish, 2001). While genetic techniques can add invaluable insights to the impact of anthropogenic disturbances, such studies have rarely been employed. The forests are commonly assumed to be negatively affected by logging mainly because of the loss of suitable habitat due to the removal of old trees (Peterson and McCune, 2001; Hedenas and Ericson, 2003). Genetic diversity is of prime importance for species persistence for the long-lived plants like forest trees that have to cope with high temporal and spatial environmental heterogeneity. Detrimental anthropogenic impact on the gene pool of forest trees calls for conservation of genetic resources. The loss of rare alleles could diminish the potential of populations to successfully adapt to and survive the ongoing environmental change. It is required to take steps for the preservation and secure of rare alleles in breeding programs. Evidences were given by many researchers that fragmentation may induce changes in genetic structure, gene flow and mating patterns of tropical tree populations (Hall *et al.,* 1996; Nason and Hamrick, 1997; Aldrich and Hamrick, 1998; Aldrich *et al.,* 1998).

The present study showed marked differences between disturbed and undisturbed populations in various genetic parameters. The studies revealed that the heterozygosity, polymorphism, and the number of rare alleles all declined in disturbed populations than the undisturbed areas except in few cases.

The nuclear gene markers were used for analyzing the long term dynamics in genetic structure of populations where as the microsatellite markers (at the intron level) are to study the short term dynamics. Mutation rate of microsatellites is much higher, approaching 10^{-3} per generation. Because of this high mutation rate, microsatellites have the potential to provide information about recent evolutionary events. Nuclear gene markers clearly indicated that disturbances such as logging activities and the frequent fires lead to a decrease in allelic richness, heterozygosity and gene diversity. Genetic

diversity is important for the fitness and the adaptive changes. So loss of genetic diversity will affect the fitness and evolutionary adaptability of teak.

Using nuclear gene markers, it could be found that both the populations in Orissa have low allelic richness and genetic diversity than all other populations. But using SSR markers, Orissa populations have the highest gene diversity. In order to compensate the low gene diversity seen at Khurda with respect to nuclear gene markers, recent mutations might have occurred at the intron level for better adaptation. There are now several reports suggesting that particular life history characteristics of some tree species like mating system, pollen and seed dispersal mechanisms may help mitigate against genetic diversity loss in post fragmentation and disturbance landscapes (Dick, 2001; White *et al.*, 2002). Populations at Konni were found to be highly diverse on using both the markers.

The structure analysis shows three clusters, one for Kerala populations, second comprising Madhya Pradesh and Gujarat and third cluster for Orissa. But using microsatellite markers, the same clustering pattern could be seen except that Nilambur stands separately as a fourth cluster. Hence, a change in the allele frequencies occurred recently in Nilambur, may be due to some adaptations or selections.

Almost all disturbed populations had lower expected and observed heterozygosity than their undisturbed pair except very few cases. The heterozygous genotypes have greater fitness than homozygotes (Young *et al.*, 1996), and it is the erosion of heterozygosity that reduces fitness of the population. An overall reduction in heterozygosity may leads to increase in (i) the rate of selfing, (ii) the strength of selection of deleterious homozygotes and (iii) inbreeding depression (Hedrick, 1994; Husband and Schemske, 1996). It is prompting to presume that genetic consequences of forest management depend on the species including their life history traits and also on local environmental conditions, which are partly the results of a regionally specific management (Buiteveld *et al.*, 2007).

One of the theoretical effects expected from fragmentation is the increase of genetic divergence among populations due to genetic drift and reduction of gene flow (Young *et al.*, 1996). In fact, adult plants from disturbed population also exhibited genetic divergence in relation to adults of the more preserved sites (Ribeiro *et al.*, 2005). The present study, using adult trees, also has shown a significant genetic differentiation between disturbed and undisturbed populations.

Tropical trees are thought to be particularly vulnerable to the effects of habitat degradation due to their demographic and reproductive characteristics, including low density of occurrence, complex self-incompatible breeding systems, high rates of out crossing (Cascante *et al.*, 2002) and intimate interactions with pollinators and seed dispersers. Quantifying the impact of habitat degradation on remnant forest stands will not only help to assess genetic and some ecological consequences of these changes but will also help to (i) ascertain the conservation value of remnant stands, (ii) estimate future repercussions of tropical deforestation, and (iii) to formulate the strategies for effective conservation and sustainable management of the genetic resources. Continuing deforestation will eventually eliminate any possible contribution remnant populations can provide to the genetic diversity of the last, large, natural population. This study concludes that small, isolated and disturbed populations that are also of important genetic value, can not persist as genetically diverse, and that it is of paramount importance to protect as much natural forests (disturbed as well as undisturbed) as possible to maintain sustainable populations of teak.

3. MATING SYSTEM AND CONTEMPORARY GENE FLOW THROUGH POLLEN AND SEED DISPERSAL

3.1. MATERIALS AND METHODS

a) Genetic structure of populations

Two natural teak populations selected for the studies on contemporary gene flow are located in Peechi-Vazhani Wildlife Sanctuary (Table 1). The highly disturbed population is at Thamaravellachal (Peechi Range) at a latitude of 10^0 30' N and longitude of 76^0 22' E and undisturbed population at Vazhani (Machad Range) at a latitude of 10^0 37' N and longitude of 76^0 19' E (Fig.12). These populations are in two different forest ranges, but in nearby areas.

There were 190 adult teak trees in the continuous patch of 10 hectares of disturbed natural forest at Thamaravellachal during survey period, but the number of trees got reduced to 174 during the tenure of the study. The 7.5 hectares of undisturbed forest at Vazhani contained 102 mature teak trees.

All the adult teak trees above 30 cm gbh (girth at breast height) in the populations were paint marked for identification. The GPS coordinates of each tree were recorded and the plots were mapped using the software MapInfo Professional. One hundred naturally grown seedlings on the forest floor, were randomly selected in the disturbed natural teak plot at Thamaravellachal and they were also tagged and mapped to study the pattern of seed dispersal. The seedlings were one or two years old with in a height range of 50 to 75 cm on an average.

Sample Collection

Juvenile leaf samples were collected from all the adult trees marked in each population for DNA extraction and fingerprinting the parent trees. Leaves were also collected from the selected hundred seedlings on the forest floor at Thamaravellachal to

DNA fingerprint and there by detecting their parents for acquiring details on seed dispersal.

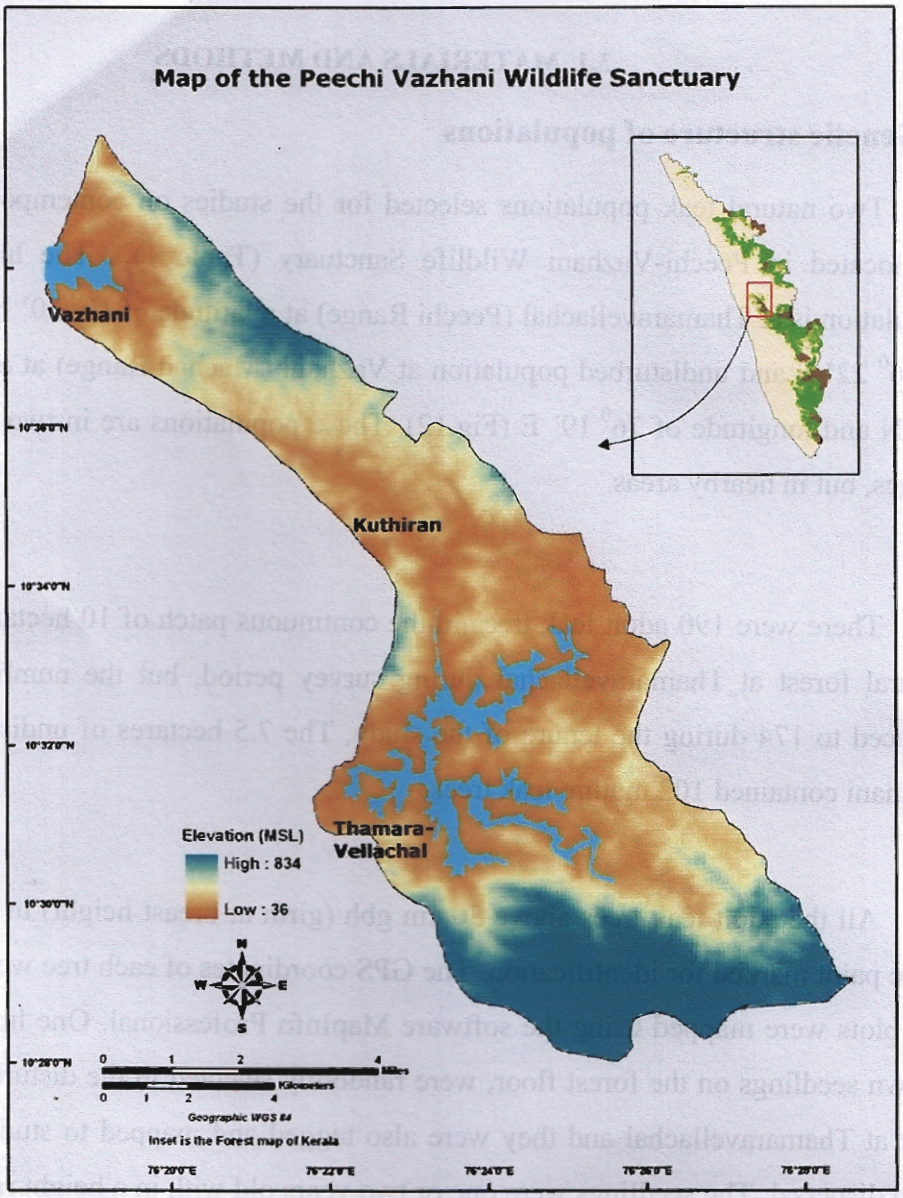


Fig. 12. Peechi- Vazhani Wildlife Sanctuary showing the location of plots

Seeds were collected from 9 randomly selected fruit bearing trees from each of the two populations and put for germination to DNA fingerprint and subsequent paternity analysis, which will help in collecting details on pollen dispersal. Juvenile leaf samples were collected from the germinated seedlings (progenies) and kept in 2 per cent CTAB buffer for DNA extraction. DNA extraction was done following the method described

earlier. Since seed germination was very sparse, embryos were used for DNA extraction. But DNA could not be extracted using CTAB and hence, the DNA extraction kit (Qiagen DNeasy plant kit-Westburg, Netherlands) was used for extraction from embryos.

Molecular analysis

The molecular analysis helps to describe the genetic structure and to identify true parentage of the seedlings and seeds collected from the selected populations. The microsatellite markers used for the present study were highly polymorphic and species specific. The discrimination power of markers to identify true parents depends basically on allelic richness or allele frequency and heterozygosity. Hence, the molecular analysis in teak was carried out with 7 microsatellite markers and their polymorphism was checked with allelic richness and PIC.

Allele identification/scoring of allele and analysis

As mentioned earlier, all the 174 adult trees, 180 progenies from 9 mother trees as well as 100 seedlings on forest floor from the disturbed teak population were DNA fingerprinted. Likewise all the adult trees and progenies from 9 mother trees from each of the undisturbed plot and plantation were also DNA fingerprinted. The bands were scored for allelic polymorphism as explained earlier. Number of alleles per locus was varied and ranged from two to nine. All the genetic parameters such as allelic richness, genetic diversity, heterozygote deficit or inbreeding coefficient (*F*is) and population differentiation (*F*st) usually determine the genetic structure. These parameters were estimated as explained earlier under genetic diversity studies.

b) Mating system, Parentage analysis, Pollen and Seed dispersal

Factors determining the level and structure of genetic variation within plant species include evolutionary history, population density, mating system and mechanism of gene flow. In this section, mating system and contemporary gene flow through pollen and seed dispersal in teak populations are discussed.

For parentage analysis, working with a larger number of loci is the only way to reduce the probability that at least one non-parent will carry a set of alleles identical by descent that are compatible with the offspring at all loci. The average PIC value and allelic richness of the selected populations indicated that the resolving power of the loci was sufficient and the output was suitable for unbiased estimation of individual reproductive success and its parentage, as well as for pedigree reconstruction.

Methods and analysis

In teak, fruits may contain a maximum of four seeds. More than 300 fruits from each of the population were cut open. They were scored as fruits with 0, 1, 2, 3 and 4 embryos based on the number of embryos/seeds present in fruits.

The alleles for each of the 7 loci were scored with respect to all the individuals in the parental populations and progenies as described earlier. The heterozygous and homozygous individuals for each maker were identified in all the selected populations. The genotypic fingerprints of each of the seedlings/embryos were compared with all the adult trees in respective plots in order to find out the potential pollen parents or pollen donors (Fig. 13). After allelic/genotypic fingerprinting, these data were analysed for identifying the parents. The data collected with respect to all the adult trees and their progenies in each population was used for parentage analysis through Cervus version 3. Cervus analyses genetic data from co-dominant genetic markers and assumes that markers are autosomal and that the species is diploid. It also assumes that markers are inherited independently of each other without any linkage.

Parentage analysis

Pollen flow

The genotypic fingerprints of each of the seeds/embryos (180 and 109 progenies respectively in disturbed plot and undisturbed plot) were compared with all the adult trees (174 and 102 potential parents respectively in disturbed and undisturbed plot) to find out the potential pollen parents or pollen donors. By comparing each and every allele of the progeny with all the potential parents in the plot, the male parent could be identified

through maximum likelihood method. Like wise the pollen parents of all the progenies were identified. After identification of pollen parents, the distance between seed parent and the pollen parent was measured.

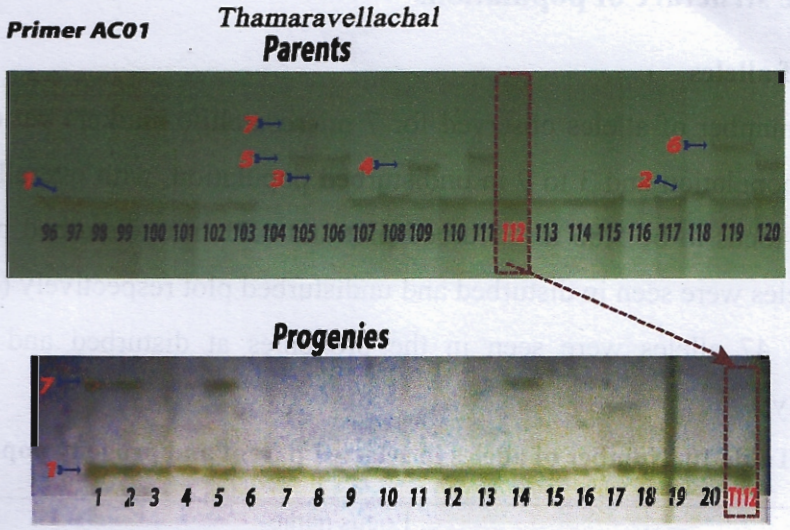


Fig. 13. Comparison of alleles from progenies with mother parent

Seed movement

Following the same method through the analysis of the fingerprints, both the parents of the hundred seedlings on the forest floor were also identified. The closest parent was taken as the mother parent, which is the normal procedure followed usually. When the haplotype of the offspring is compatible with the nearest reproductive tree, it is a probable assumption that the nearest reproductive tree is the seed parent of the offspring (Konuma *et al.*, 2000). Recent studies in *Shorea leprosula* by Fukue *et al.* (2007) indicated that most immigrant seedlings originated from neighbouring mother trees. Once the parents were identified, the distance of seeds moved from the mother parent could be measured; thereby the contemporary gene flow through pollen and seed dispersal was evaluated.

The percentage of natural crossing and selfing as well as other forms of inbreeding was estimated from the data generated through parentage analysis.

3.2. RESULTS AND DISCUSSION

a) Genetic structure of populations

Number of alleles

The number of alleles observed for 7 microsatellite markers varied from 2 to 8 in disturbed population and 3 to 9 in undisturbed population, with overall mean number of alleles per locus to be 5.86 in disturbed plot and 6.71 in undisturbed plot. A total of 41 and 45 alleles were seen in disturbed and undisturbed plot respectively (Table 10). A total of 38 and 47 alleles were seen in the progenies at disturbed and undisturbed plot respectively.

Table 10. Number of alleles in selected parent and progeny populations

Population type	Locus name							Total
	AC01	AC28	AG04	AG14	AG16	AC44	CPIMS	
Parent populations								
Disturbed	8	5	5	7	6	7	2	41
Undisturbed	7	5	8	8	8	6	3	45
Progeny populations								
Disturbed	7	5	5	6	6	6	3	38
Undisturbed	8	5	8	8	9	6	3	47

Allelic richness and Polymorphic information content (PIC)

The estimate for allelic richness revealed that it was lower in disturbed teak forest (5.28) than undisturbed natural teak forest (6.26). The average polymorphic information content (PIC) in disturbed natural teak forest was 0.586 and that in undisturbed forest was 0.501.

Generally in forest trees, the allelic richness and percentage of polymorphism are reported to be influenced by local environmental conditions and by gene flow (Prus-Glowacki and Stephan, 1994).

Heterozygosity and gene diversity

The mean observed heterozygosity in disturbed and undisturbed forests were 0.476 and 0.584 respectively. Expected heterozygosity was 0.529 and 0.622 in disturbed and undisturbed forests. Gene diversity estimated were 0.563 in disturbed population and 0.614 in undisturbed population. The difference in gene diversity between parent populations and their progenies revealed that there is a decrease in gene diversity in progenies of disturbed forest (by 0.079), whereas in the undisturbed population the gene diversity in progenies was increased (0.010) slightly (Table 11).

Table 11. Gene diversity and heterozygosity

Population type	Observed heterozygosity	Expected heterozygosity	Gene diversity	
			Parents	progenies
Disturbed forest	0.4764	0.5293	0.563	0.484
Undisturbed forest	0.5843	0.6215	0.614	0.624

Inbreeding coefficient (*Fis*)

The heterozygote deficiencies or the inbreeding coefficients within populations (*Fis*) were estimated using Fstat. The average inbreeding estimate was 0.070 (7 %) in disturbed population and 0.018 (1.8 %) in undisturbed population. Only in disturbed population significant inbreeding was noted. Numerous factors, such as inbreeding, null alleles (non amplifying alleles) and occurrence of population substructure (Wahlund effect) have been established as reasons for heterozygote deficiency in populations (Nei, 1987).

Inbreeding within a subpopulation is caused by the non random mating of the members of that subpopulation, in which mating occurs more often than by chance alone,

between closely related individuals. As closely related individuals will contain a large proportion of the same alleles due to common descent, their offsprings will have a higher level of homozygosity and conversely, a lower level of heterozygosity than expected.

Population differentiation (*Fst*) between parents and progenies

Fst or *Gst'* is estimated to examine the population differentiation, which measures variation in allele frequencies among populations. The *Fst* values showed a higher population differentiation existing between parents and progenies (seeds) in disturbed plot (0.024), while it was low (0.01) in undisturbed plot.

In order to estimate the distance of seed movement seedlings on the forest floor were also analysed in disturbed plot at Thamaravellachal. Here the genetic differentiation between parents and the seedlings on the forest floor was found to be 0.015 (Fst).

b) Mating system, Parentage analysis, Pollen and Seed dispersal

Details of mating system and contemporary gene flow in selected teak populations were brought out from the pollen dispersal and seed dispersal studies. Parentage estimation was carried out using likelihood analysis through Cervus and the data were taken to evaluate the contemporary gene flow and mating system.

Seed setting

Seed setting efficiency in selected teak populations is presented in Table 12. In the disturbed population, seed setting was found to be a maximum of 47.39 per cent. Around 38.89 per cent fruits had only one embryo while, 6.21 per cent fruits had two embryos, 1.63 per cent had three embryos, 0.65 per cent had four embryos (Fig. 13) and rest of the fruits were seedless. Seed setting in the undisturbed population was found to be the low (19.3 per cent). In this population, the percentage of fruits with single embryo was found to be 18.2 per cent followed by fruits with two embryos (1.1%) and seeds producing three and four embryos were absent. The presence of pollinators was far less in the undisturbed population compared to that of disturbed plot which is reflected in the fruit productivity.

Fig.13. Four seeds in one teak fruit

Table 12. Seed setting efficiency in selected teak populations

Population type	No. of fruits cut opened	0 embryo	1 embryo	2 embryo	3 embryo	4 embryo	Percentage of seeds per fruit
Disturbed	**306**	**151**	**119**	**19**	**5**	**2**	**47.39**
Undisturbed	**456**	**368**	**83**	**5**	**0**	**0**	**19.3**

Pollen flow

Once the pollen parent was identified, the pollen dispersal was traced in natural teak forest at Thamaravellachal and Vazhani, representing disturbed and undisturbed systems. 180 fruits/ progenies from disturbed and 109 from undisturbed were used for this study as mentioned earlier.

Disturbed population

Pollen dispersal

The result of the analysis pointed out the identity of pollen parents and thereby the distance of pollen transfer could be calculated. The maximum distance of pollen flow was 414 m and the minimum distance excluding selfing was 14.4 m. Main distance range of pollen flow was found to be 151- 200 m (Table 13 and Fig. 143) Here, out of the total 180 progenies, 42 have their male parents within the main distance range (151-200 m,

which contributes to 23.33 per cent of the total progenies. A total of 17.78 per cent of the progenies had their male parents within a range of 101-150 m. Only one of the progenies had the pollen parent in the maximum distance of 414 m which represent only 0.56 per cent of the total progenies (Table 13). Most of the progenies (70%) had their pollen parents below 200 m distance.

Mating system (Crossing and selfing)

It is understood from the studies that, cross pollination explicitly dominated in the sampled trees (Table 14). Out of the total 180 progenies, 173 were cross fertilized (96.11%) and rest was self fertilized. The increased percentage of cross pollination helps the teak to be genetically highly diverse.

Fertility pattern

Female fertility pattern in this disturbed plot showed that female parents received pollen from all directions (Fig. 15). It was also revealed that most of the individual mother trees were pollinated by many pollen donor trees. The number of pollen donors to a mother tree ranged from six to ten to produce 10 progenies (Table 15). Out of the total nine mother trees, four received pollen from 10 different trees to produce 10 seeds, by which all the seeds turned to be dissimilar and diverse. Out of the total 26 multi-seeded fruits, each of the 23 fruits had seeds with different pollen parents indicating that many of the flowers are pollinated by multiple male parents. Hence, seeds even within one fruit are non identical.

Tree number165 has donated pollen to seven different female parental trees and produced a maximum of 11 seeds. Tree number101 and 106 had produced 5 and 6 progenies by donating pollen to 5 different females. Likewise tree number 98 and 79 produced seven progenies by crossing with six and three different female parents respectively. Tree number 2 had produced six progenies through crossing with two different females.

Table 13. Progenies having their pollen parents in 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

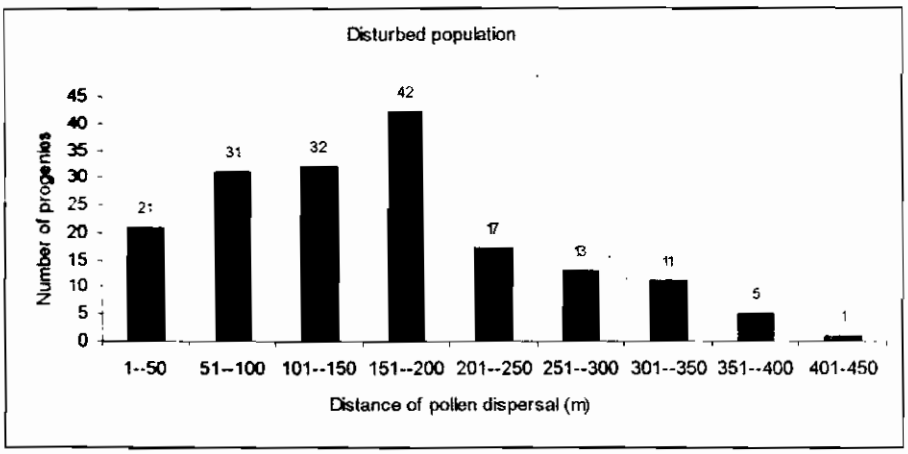


Fig. 14. Number of pollen dispersed in different distance classes

Table 14. Pattern of pollination in the sampled progenies

Pollination pattern	Number of progenies	Percentage of progenies
Cross pollinated	173	96.11
Self pollinated	7	3.89
Grand total	180	100.00

Thus. a total of 91 pollen donor trees (52.3%) contributed pollen to produce 180 progenies. Out of the total 91 male parents, 68 per cent trees crossed with only one female parent. Each of the 23 per cent male donors crossed with two female trees, 5 per cent with three female trees, 2 percent with five female parents, and 1 per cent each with six and seven female parental trees. A maximum of seven different female parents were pollinated by a single male parent (Fig. 16).

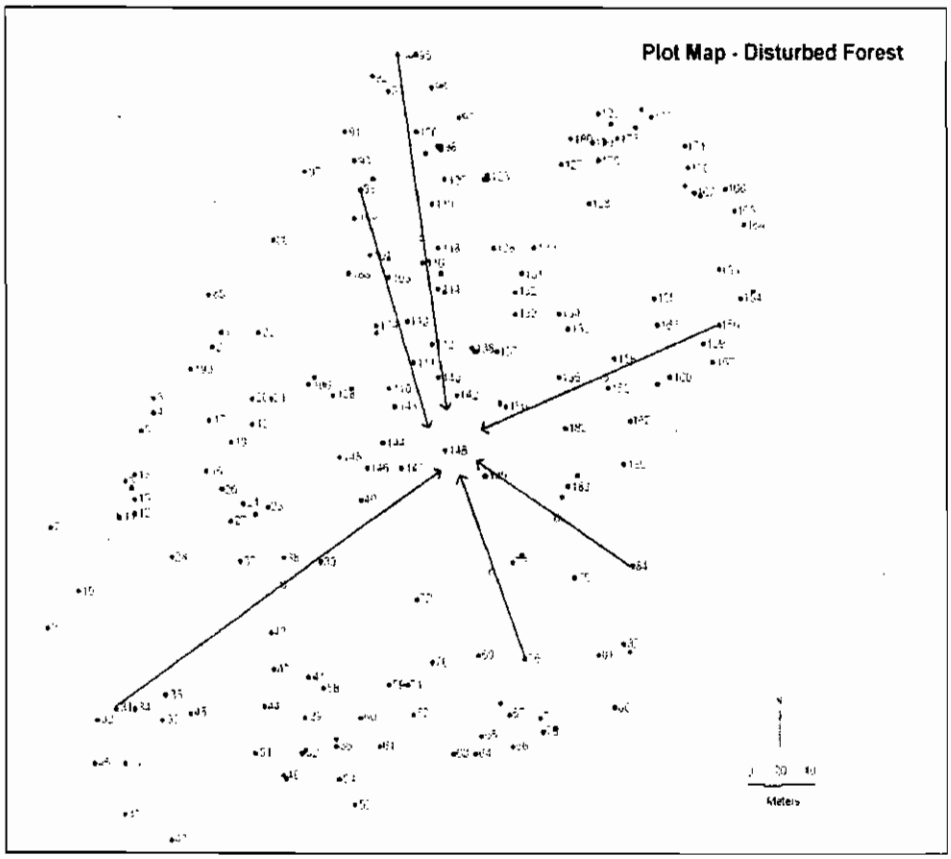


Fig. 15. Pollen flow from different pollen donors to mother tree No. 148

Table 15. Number of pollen donors to produce 10 seeds by each mother tree

Mother tree Number	151	112	178	11	148	42	100	20	124
Total number of pollen donors	9	10	9	10	10	8	6	10	7

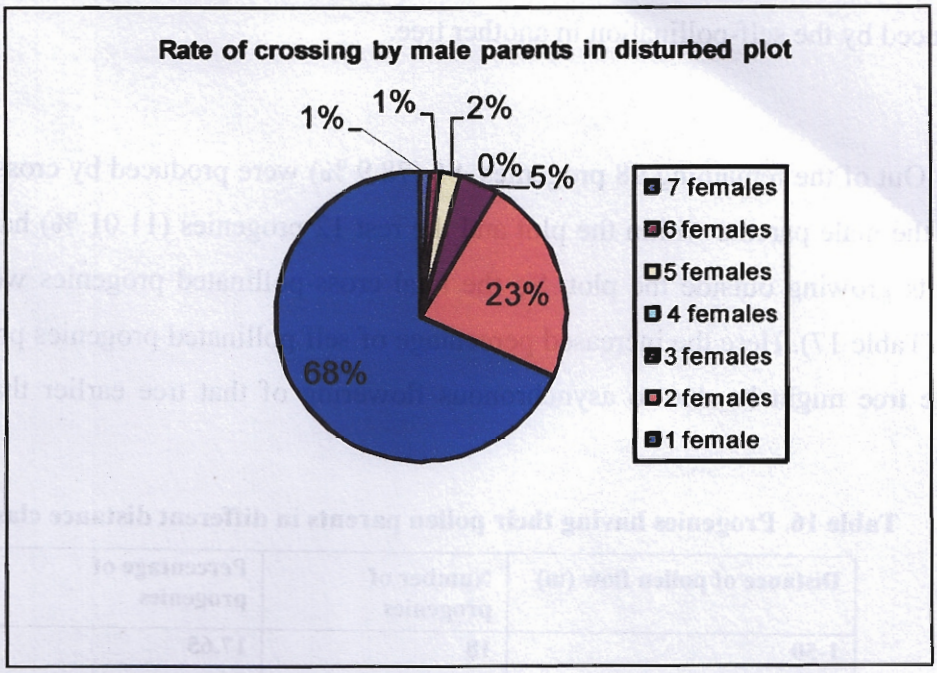


Fig. 16. Percentage of male parents crossed with one or more females

Undisturbed population

Pollen dispersal

The analysis of 109 adult teak trees and 102 progenies from undisturbed population showed that, the pollen transfer was mainly in the range of 101-150m. Twenty seven progenies (26.47%) had their male parents within 101-150 m and 22 progenies had their pollen parents within 151-200 m. In this population only two of the progenies (1.96%) had their male parents in the maximum distance range of 251- 300 m, here the maximum distance was 282 m. The minimum distance of pollen transfer was 4 m excluding selfing and 2.94 per cent of the total progenies had their male parents in the distance range of 201-250 m (Table 16 and Fig. 17). Most of the progenies (79.42%) had their pollen parents with in 200 m distance.

Mating system (*Crossing and selfing*)

Studies on mating system revealed that, out of 109 progenies analysed, 11 were self-pollinated and the percentage was 10.09. Out of these 11 selfed progenies, seven

were produced by the self-pollination confined to a single tree and the rest four were produced by the self-pollination in another tree.

Out of the remaining 98 progenies, 86 (78.9 %) were produced by cross-pollination with the male parents within the plot and the rest 12 progenies (11.01 %) had their male parents growing outside the plot. So the total cross-pollinated progenies were 89.9 per cent (Table 17). Here the increased percentage of self pollinated progenies produced by a single tree might be due to asynchronous flowering of that tree earlier than the other trees.

Table 16. Progenies having their pollen parents in different distance classes

Distance of pollen flow (m)	Number of progenies	Percentage of progenies
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

Table 17. Pattern of pollination in the progenies

Pollination pattern	Number. of progenies	Percentage of progenies
Total Cross pollinated	98	88.91
Self pollinated	11	10.1
Grand total	109	100.00

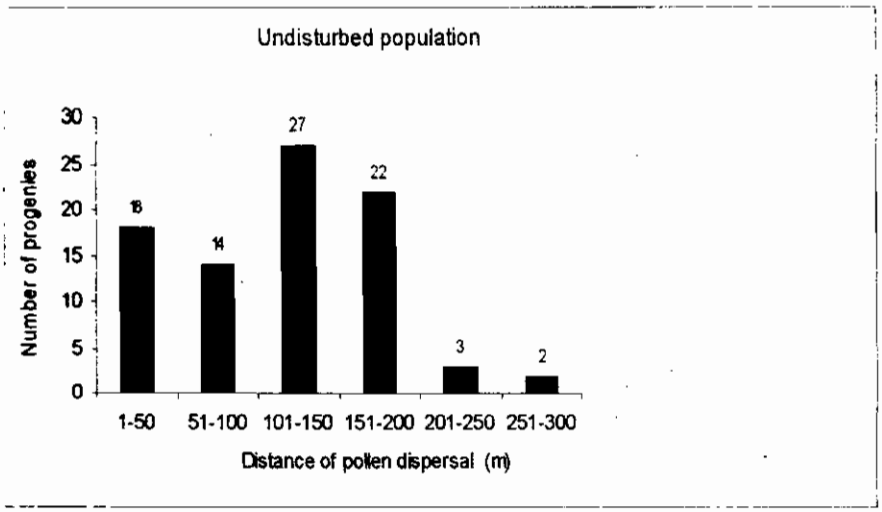


Fig. 17. Number of pollen dispersed in different distance classes

Fertility pattern

It is again understood that in the undisturbed natural teak forest also, individual mother trees were pollinated by many pollen donor trees growing on different sides of the plot (Fig. 18). The number of pollen donor trees ranged from 3-10 to produce 10 progenies by each of the nine mother trees (Table 18). But only one mother parent produced 10 dissimilar progenies by receiving pollen from 10 different trees. Male fertility pattern showed that individual pollen parents donated pollen to many mother parent trees. A single tree (tree number 51) had donated pollen to produce a maximum of eight seeds, out of which seven were self pollinated ones. Out of 51 male pollen donors, tree number 50 and 57 had donated pollen to three different mother parents to produce five progenies and tree number 12 had donated pollen to two different mother parents and produced five progenies.

In this plot, out of 102 trees, 50 per cent (51 trees) contributed pollen to the 9 mother parents to produce 86 crossed progenies and 11 self-pollinated progenies. Out of these 51 trees, 33 pollen parents (65 %) donated pollen to only one female parent, 15 pollen parents (29 %) donated pollen to two female parents and 3 pollen parents (6 %) donated pollen to three different female parents. Only a maximum of three different

female parents were pollinated by a single pollen parent (Fig. 19), where as, in disturbed plot, a maximum of seven female parents were pollinated by a single pollen donor.

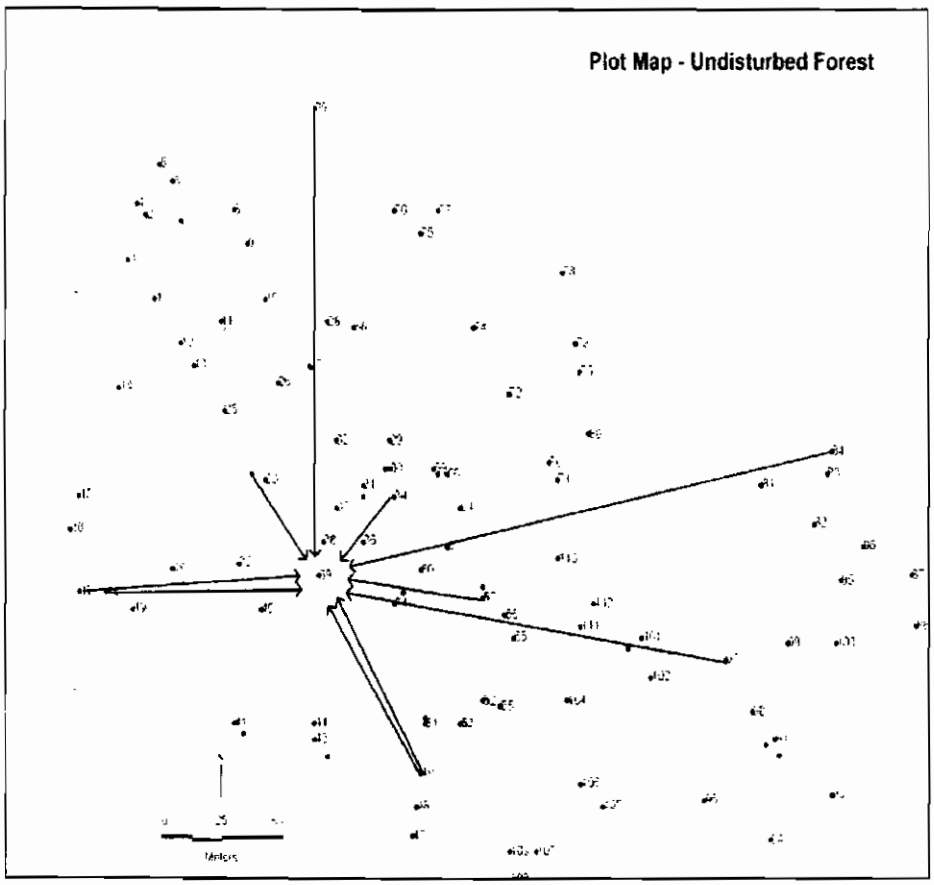


Fig. 18. Pollen flow from different pollen donors to mother tree no. 39

Table 18. Number of pollen donors to produce 10 seeds by each mother tree

Mother tree Number	12	78	39	34	58	51	33	17	27
Total number of pollen donors	8	8	10	6	9	3	6	8	9

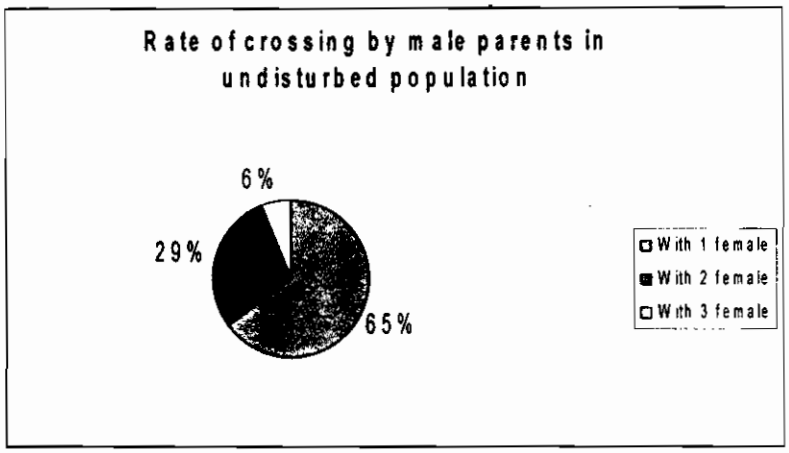


Fig. 19. Percentage of male parents crossed with one or more females

Seed dispersal

The DNA fingerprinting of 100 seedlings on the forest floor followed by parentage analysis showed that the main distance of seed dispersal from their mother parents was within a range of 51 to 100 m. Maximum number of seeds (33 per cent) were dispersed in this distance range. Twenty three per cent of the seeds were dispersed to 101-150 m range. Four seeds were transferred to the maximum distance range of 251-300 m (Table 19 and Fig. 20). The maximum distance of seed dispersal observed in this plot was 291 m and minimum distance was 8 m. Few seedlings were seen very close to the mother trees. Through seed dispersal analysis, 80 pollen parents have pollinated 65 female parents to produce 100 progenies.

Table 19. Seeds dispersed at different distance classes

Distance of seed dispersal (m)	**Percentage of seedlings**
1—50	8
51—100	**33**
101—150	23
151—200	16
201—250	10
251—300	4

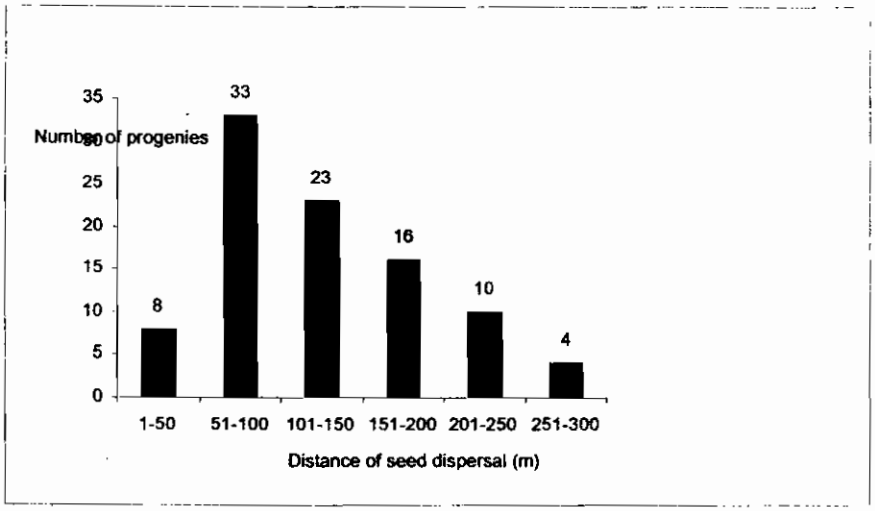


Fig. 20. Number of seeds dispersed in different distance classes

Comparison between undisturbed and disturbed populations with respect to mating system and pollen flow

During the last 50 to 100 years, the extent of natural teak populations has been drastically reduced and the teak genetic resources have been altered due to anthropogenic activities. The impact of these anthropogenic disturbances on the teak genetic resources is little known though it is presumed to be disastrous. According to Schuster and Mitton (2000), the pattern of mating system and gene flow via pollen dispersal within a population strongly influence the genetic structure. An understanding on the extent of seed setting is also important for teak improvement programmes. Hence, a comparison of contemporary gene flow through pollen and seed dispersal, mating system and seed setting prevailing in teak populations with different disturbance status was made.

The amount of contemporary gene flow was evaluated through pollen and seed dispersal. The pollen transfer was taking place to all directions of the plots. In the disturbed plot, no progenies were found to be pollinated from outside the plot. In undisturbed natural teak forest 11.01 per cent progenies were produced as a result of pollination from trees outside the plot.

The pollen dispersal studies showed that the main range of pollen dispersal distance was 151-200 m in disturbed plot and 101-150 m in undisturbed plot. The maximum distance of pollen transfer was found to be more in disturbed natural teak forest (414 m) than undisturbed (300 m). One of the reasons for the increase in the distance of pollen transfer in disturbed population appeared to be the presence of large number of insects, including bees and other pollinators observed in this plot. The two factors responsible for mating distance in tropical trees are the performance of pollinators and flowering tree density (Konuma *et al.*, 2000). The committed impact of population density, pollinator abundance and composition change over the range of species on the out crossing rate and pollen dispersal at a landscape level was already discussed (Franceschinelli and Bawa, 2000; Dick *et al.*, 2003; Degen *et al.*, 2004).

The pollen dispersal analysis from the two teak populations showed that the pollen dispersal was mainly with in a distance below 200 m. It indicates that the pollen dilution zone must be more than 200 m in seed orchards to restrict pollen from outside. The analysis on seed dispersal showed that the dispersal was mainly in the distance range of 50-100 m. This indicated that, in natural teak population, the distance of pollen flow is more than the seed movement. Hence, remarkable contribution in maintaining genetic diversity happens to be from pollen transfer. For partially or fully out crossed species, gene flow via pollen is generally believed to occur at much greater rates than gene flow via seed (Levin and Kerster, 1974; Handel, 1983). In most studies on tropical plant species, gene flow through pollen acts over longer distances than through seed dispersal, with most seeds getting dispersed only over a short distance or just dropping under the parent plant (Dick, 2001; Burczyk *et al.*, 2004; Difazio *et al.*, 2004; Trapnell and Hamrick, 2004).

The present study revealed that the pollen and seeds are transferred to all directions of the plots resulting in thorough mixing of alleles in the teak populations. This mixing may help in spreading an advantageous allele from its localized area or sub population to the whole population (Fisher, 1937; Barton and Whitlock, 1997) resulting in genetically diverse populations. Voigt (2005) found that the gene exchange *via* both pollination and

seed dispersal influences the genetic structure of plant populations. The extent and distribution of genetic variation within tree species are of fundamental importance to their evolutionary potential and chances of survival (Changtragoon, 2004).

Higher out crossing rate (89-96 %) was observed among the teak trees in all the populations. These results are in agreement with the earlier report on teak through isozyme studies (89-95 %) by Kjaer and Suangtho (1995). An individual mother tree received pollen from many male parents and even up to 10 pollen donors to produce 10 seeds (100% seeds of the individual tree are non identical) as well as seeds within an individual fruits are also non identical due to multi-parental mating, which ensures very high variations within family. This also revealed that there was very high mixing between trees to produce the next generation, which will surely lead to high genetic variations. Hence, the mating system analysis again proved that teak is mainly an out crossing species and 90 per cent of the total seeds produced are from cross pollination, thus indicating that seeds from seed orchards or seed stands must be genetically diverse without much inbreeding.

Comparatively higher selfing rate of 10.09 percentage (7 self fertilized progeny from a single tree) in undisturbed teak population might be due to the asynchrony in flowering time leading to reproductive isolation as reported in *Ceiba pentandra* (Gribel *et al.*, 1999), *Dicorynia guianensis* (Latouche-Halle *et al.*, 2004) and *Shorea leprosula* (Fukue *et al.*, 2007). The variation in selfing rate could also be due to the differences in flowering plant densities (Murawski and Hamrick, 1992). In the present study, there is every chance of equal or more rate of self pollination, but self incompatibility leads to flower and fruit abortion restricting the number of selfed seeds to the minimum.

The analysis using the embryos/seeds from individual mother trees in the disturbed natural teak plot showed 96.11 per cent outcrossing rate. The analysis of the seedlings on the forest floor also showed 97 per cent cross pollination. So the estimation of out crossing rate was not influenced by different developmental stages such as seeds or seedlings in teak (inbreds had low survival capacity in few other species), which

confirmed the report of early acting self incompatibility during pollen tube entry to ovule through micropyle (Indira and Mohanadas, 2002). Bittencourt and Semir (2005) as well as Hufford and Hamrick (2003) documented changes in outcrossing rate with developmental stages of seeds due to late acting self incompatibility system in other species. So the mating system analysis proved that teak do not manifest self incompatibility later in the seedling stage with respect to survival, which confirmed the earlier reports even though those reports were not through DNA marker studies.

The fertility pattern of all the populations showed that the flowers of individual teak trees were pollinated by many male parents (up to 10 to produce 10 seeds) as a result of multi-parental mating. In disturbed natural teak forest each of the 4 female parents received pollen from 10 male donors whereas in undisturbed plot only one female tree received pollen from 10 male parents. Hence, it is sure that contemporary gene flow is quite high in all the plots and a thorough mixing of alleles is taking place within teak populations.

Nason *et al.* (1998) found that mother trees in *Ficus dugandii* had numerous pollen donors and observed up to 11 pollen donors to produce 15 fruits and a single male parent donated pollen to 11 female trees, that leading to high genetic diversity. Likewise, in the present study in disturbed population, tree number 165 has been found to be the most effective pollen donor, which donated pollen to seven different females and produced 11 progenies. Effective pollen donation was lower in both undisturbed and plantation populations, where male parents donated pollen only to a maximum of three females.

The overall picture showed that pollen flow was higher in disturbed natural teak population at Thamaravellachal, where pollen reception from different male parents by female parents as well as effective pollen donation (up to seven female trees) were more (Fig.21). Comparison of the fertility pattern of the populations showed that the multi-parental mating by female parents as well as male parents (Table 20 and Table 21) were

found to be more in disturbed natural forest, which might be due to the abundance of pollinators observed in the plot.

Table 20. Multi-tree mating by female parents

Mother parents	Number of Pollen donors*								
	M 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M 9
in disturbed plot	**9**	**10**	**9**	**10**	**10**	**8**	**6**	**10**	**7**
in undisturbed plot	**8**	**8**	**10**	**6**	**9**	**3**	**6**	**8**	**9**

* ***No. of pollen donors to produce 10 seeds*** M- ***Mother parent***

Table 21. Multi-tree mating by male parents

Population type	Percentage of total pollen parents mating with different number of female parents						
	1 female parent	2 female parents	3 female parents	4 female parents	5 female parents	6 female parents	7 Female parents
Disturbed	**68**	**23**	**5**	**0**	**2**	**1**	**1**
Undisturbed	**65**	**29**	**6**	**0**	**0**	**0**	**0**

The percentage of more than one-seeded fruits was also high in disturbed plot. In undisturbed plot, more than two seeded fruits were absent which may be due to low pollinator activity. The percentage of stigmas pollinated and the number of pollen on stigma were found to be significantly reduced during rainy days due to low pollinator activity (Mohanadas *et al.*, 2002). However, since gene diversity was more in the undisturbed parent population, it was high in the progenies also. But in disturbed plot, gene diversity was less among the parents and in the subsequent generation it was again decreased.

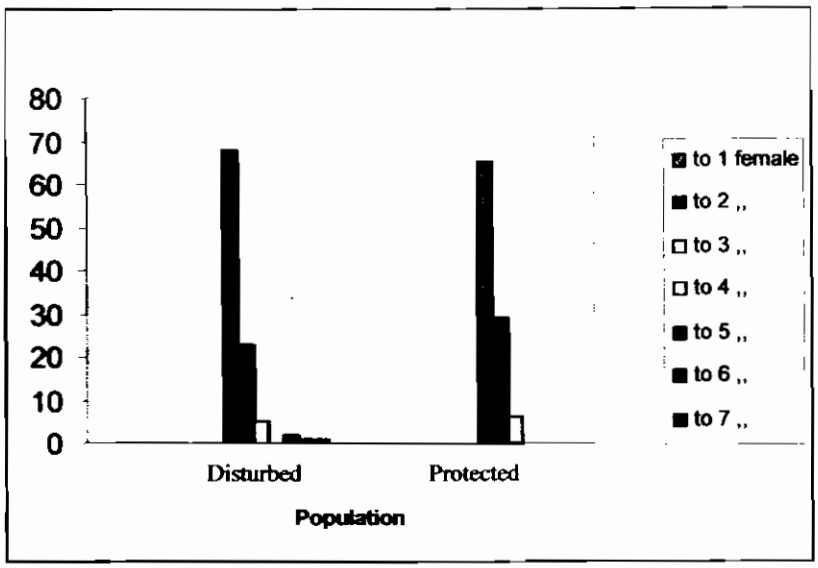


Fig. 21. Percentage of male parents donated pollen

In the selected teak populations, fruits with more than one seed were seen and seeds within one fruit had different male parents indicating that even a single flower is pollinated and fertilized by many pollen donors. There are possibilities for same pollinators visiting many trees or same flowers pollinated by many pollinators. The activity of pollinators reflected in the proportion of pollen donors. The high proportion of pollen donors indicates active movement of pollinators. Data on multi-parental mating in which a female parental tree received pollen from many different pollen donors also supported the above assumption. In the disturbed plot, single males donated pollen even up to 7 females, in undisturbed and plantation plots, a single pollen donor donated pollen to a maximum of three females only. Normally the maximum germination percentage in teak varies between 30–50 per cent. Experiences gained from the teak growing regions in India, Myanmar, Thailand, Laos and Indonesia indicated that the germination of teak is very low and sporadic with the germination period of 10–50 days after sowing (Suangtho, 1980; FAO, 1956a; FAO, 1956b; Kaosa–ard, 1986; Kumaravelu, 1993; Phengduang, 1993). The low and sporadic germination is due to strong dormancy behaviour of teak seeds which cause a low percentage of plants in natural and nursery production.

From all the populations selected, more than 50 per cent of the fruits collected were without embryos. Lack of effective pollinators was reported to be the main reason for very poor seed settings (Hedegart, 1976; Palupi and Owens 1997; Tangmitcharoen and Owens, 1997; Indira and Mohanadas, 2002; and Mohanadas *et al.,* 2002). The low seed setting could also be due to the low pollen-ovule ratio found in many cases especially in days of heavy rain (Indira and Mohandas, 2002). Uma Shaanker and Ganeshaiah (1990) found that the pollen deposition patterns regulate the seed number per fruit in multi ovulated species.

Evidence was found by many workers that fragmentation or disturbance may induce changes in genetic structure, gene flow and mating patterns of tropical tree populations (Hall *et al.*, 1996, Nason and Hamrick, 1997, Aldrich and Hamrick, 1998, Aldrich *et al.*, 1998). The present study revealed that due to disturbance of natural teak forests, the gene diversity is decreasing with high population differentiation and low gene flow (between parents and progenies) along with significant inbreeding.

4. IDENTIFICATION OF EFFECTIVE INSECT POLLINATORS FOR TEAK

The study on pollinators was carried out to identify the insect species that visit teak flowers in natural habitats and to quantify the teak flower visits. Observations were conducted and descriptions made on the foraging behaviour of the important flower visiting insects to evaluate the pollination efficacy. The observations were made in the natural forests, degraded forests and plantations.

MATERIALS AND METHODS

Teak trees were selected in natural forests and plantations. Ladders and towers were used to get an access to the flowering branches. Observations were recorded on the insects visiting the flowers and their specimens were collected. Collection of insects was carried out over three flowering seasons. Study of pollen grains on insects' body parts was carried out. For a few selected species, more in depth study of their potential as pollinators has been conducted. Presence of pollen was examined on insect legs, abdomen and thorax under a Stereo Microscope.

RESULTS AND DISCUSSION

A list of insects visiting/pollinating the teak flowers is given in Table 22 and photographs of few insects are given in Plate I and II. An indication of their behaviour is also given as frequency of visits. Based on the visual observation and presence of pollen on the insects body parts, insects were classified as visitors or pollinators and is presented in Table 22.

The insect activity increased from the morning hours as the temperature increased, continued till noon and then it gradually decreased. The most active period was from 9am to 12 noon. Of the various orders of insects represented include Hymenoptera, Diptera, Coleoptera, Lepidoptera, Hemiptera and Thysanoptera. Of the variety of species represented, the dominant one was from Hymenoptera. These include solitary bees and wasps. The Hymenopteran groups of insects were identified as the potential pollinators and in general they play a vital role in teak pollination. The main pollinators are some solitary bees like *Halictus tectonae* Narendran and Jobiraj and Wasps. Two species of solitary bees, *Anthophora zonata* (Linn.) and *A. niveo-cincta* Smith were also found to

carry a good quantity of pollen on the under side of their abdomen and hind legs. They were found to be very active, visiting several inflorescences on one tree as well as on different trees in a short time.

There were a variety of insects visiting the trees and most of them spent their time among the inflorescences of a single tree. The only exceptions were bigger wasps which moved very fast among the inflorescences of a single tree as well as to the adjacent trees. Rainy conditions that dominate during flowering time of teak brought down the insect activity in general and the role of insects in cross pollination. During peak flowering season on sunny days around 95% flowers were found to be pollinated with an average pollen load of 8.25 while on rainy days only 45% were found to be pollinated with low pollen load of 1.9 (Indira and Mohanadas, 2002). This was because of the low insect activity during heavy rain.

Table 22. List of insect visitors/pollinators of teak flowers

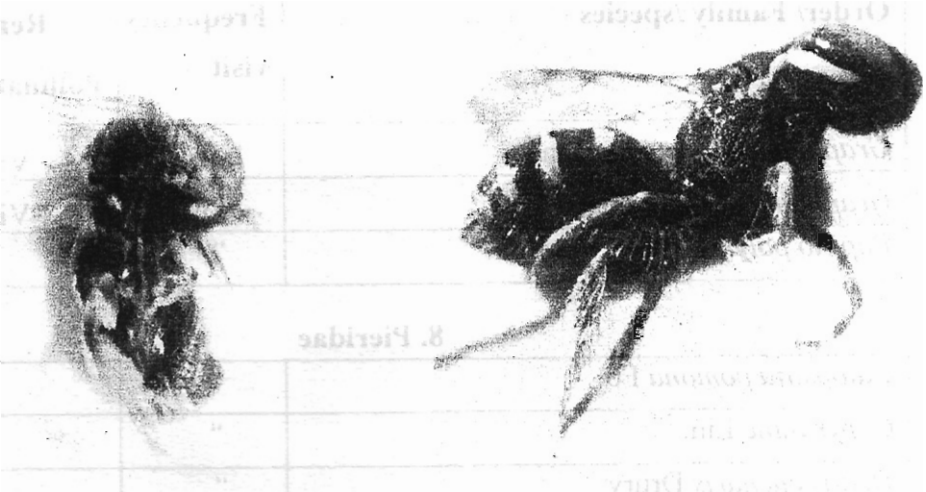
Order/ Family /species	**Frequency visit**	**Remarks Pollinator/visitor**
HYMENOPTERA		
1. Apidae		
Anthophora niveocincta Smith	High	Pollinator
Anthophora zonata Lin.	"	"
Apis cerana indica Fabricius	"	"
Apis florea Lin.	"	"
2. Apinae: Meliponini		
Lisotrigona mohandasi Jobiraj and Narendran	"	"
Melipona iridipennis Dall.	"	"
Trigonisca sp.	"	"
3. Anthophoridae		
Ceratina hieroglyphica Smith	Low	Pollinator
Nomada sp	"	"

Order/ Family /species	Frequency visit	Remarks Pollinator/visitor
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.	"	"
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)	"	"

Order/ Family /species	Frequency visit	Remarks Pollinator/visitor
8. Sphecidae		
Chalybion bengalense Dalbhom	"	"
Sphex sericeus Fb.	"	"
Sphex sp.	"	"
LEPIDOPTERA RHOPALOCERA		
1. Acraeidae		
Acraea violae Fb.	Low	Visitor
2. Danaidae		
Euploea core Cramer	"	"
Tirumala limniace leopardus (Butler)	"	"
3. Erycinidae		
Abisara echerius (Stoll)	L	Visitor
Udaspes sp.	"	"
4. Lycaenidae		
Caleta caleta Hewitson	"	"
5. Hesperidae		
Celaenorrhinus leucocera Kollar	"	"
Potanthus sp	"	"
Tagiades litigiosa Moschler	"	"
6. Nymphalidae		
Cupha erymanthis Drury	"	"
Hypolimnas bolina Lin.	Low	Visitor
Junonia almana *Lin.*	"	"
Junonia iphila Fruh slovfer	"	"
Junonia stygia	"	"
Neptis hylas Moore	"	"
7. Papilionidae		

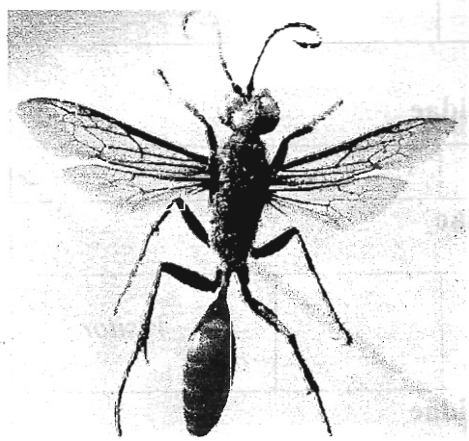
Order/ Family /species	Frequency visit	Remarks Pollinator/visitor
Graphium agamemnon Lin.	Low	Visitor
Graphium doson Felder	Low	Visitor
Papilio polytes Lin.	"	"
8. Pieridae		
Catopsilia pomona Fb	"	"
C. pyranthe Lin.	"	"
Delias eucharis Drury	"	"
9. Satyridae		
Ythima huebneri Kirby	"	"
HETEROCERA		
1. Hyblaeidae		
Hyblaea puera Cramer	"	
2. Pyralidae		
Syngamia floridalis Zell.	"	*Visitor*
3. Syntomidae		
Eucromia polymena *Lin.*	"	"
HEMIPTERA		
1. Pentatomidae		
Tessaratoma *sp.*	Low	Visitor

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



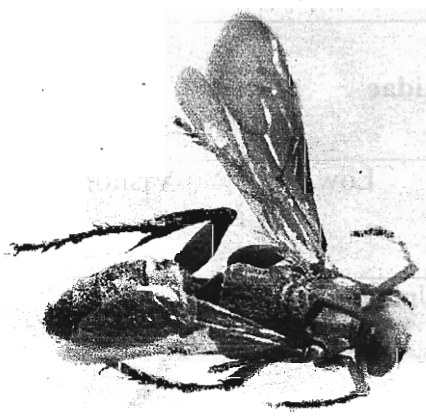
Halictus tectonae (Halictidae)

Ceratina heiroglyphica Smith (Anthophoridae)



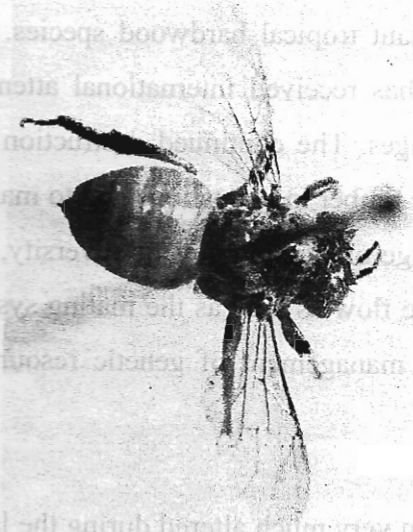
Sphex sericea Fabricius (Vespidae)

Nomia curvipes Fabricius (Halictidae)

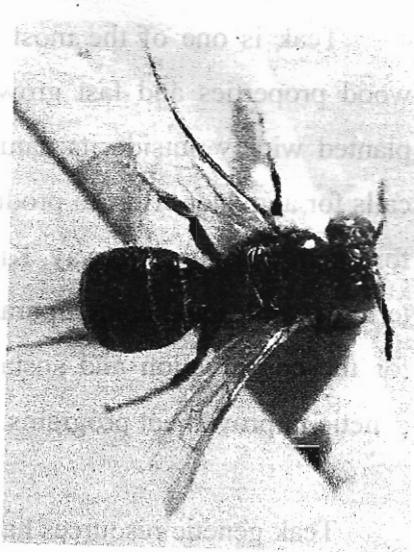


Megachila sp ((Megachilidae)

Plate 1. Photographs showing few pollinator insects



Apis cerana Fabricius (Apidae)



Nomia basalis Smith(Halictidae)

Megachila sp. (Megachilidae)

Heriades sp. (Colletidae)

Plate 2. Photographs showing few pollinator insects

5. SUMMARY

Teak is one of the most important tropical hardwood species. Due to its unique wood properties and fast growth, it has received international attention and has been planted widely outside its natural ranges. The continued destruction of tropical forests calls for a greater effort to produce the timber in plantations and to manage the remaining forests in a sustainable way. Knowledge of existing genetic diversity, the long and short term dynamics and contemporary gene flow as well as the mating system form the basis for the conservation and sustainable management of genetic resources as well as for genetic improvement programs.

Teak genetic resources have been very much altered during the last century through uncontrolled logging and movement of planting materials. The impact of the anthropogenic disturbances on the teak germplasm is unknown. Tropical trees are thought to be more affected by habitat degradation due to their demographic and reproductive characteristics, including low density of occurrence, complex self-incompatible breeding systems, high rates of outcrossing and intimate interactions with pollinators and seed dispersers.

The overall understanding of genetic diversity in teak is fragmentary. Genetic analysis helps the evaluation of the overall pattern and partitioning of genetic variation in different populations across the country. Hence, to study the long term dynamics and short term dynamics of population genetic parameters in teak, nuclear gene markers and microsatellite markers were used respectively.

Seven paired populations of natural teak forests (undisturbed and disturbed) were selected in Kerala, Orissa, Madhya Pradesh and Gujarat. One plantation was also selected in Kerala. Co-dominant DNA markers were used to estimate the population genetic parameters, such as the number of alleles, allelic richness, expected and observed heterozygosity and gene diversity in these teak populations. The impact of human disturbances was also assessed on the above genetic parameters. Forty trees per population were DNA fingerprinted using three co-dominant nuclear gene markers

through denaturing Poly Acrylamide Gel Electrophoresis (PAGE) following Single Strand Confirmation Polymorphism (SSCP) technique and also employing four co-dominant microsatellite markers through non-denaturing PAGE method.

The results of the present study employing three nuclear gene markers (to study the long-term dynamics) showed these markers to be highly polymorphic and informative. Undisturbed population at Konni was found to be the most diverse population having maximum number of alleles (16 alleles), allelic richness, expected and observed heterozygosity (0.699 and 0.723 respectively) and gene diversity (0.703) where as both populations at Khurda (Orissa) had lower number of alleles, allelic richness and lowest gene diversity (0.326 and 0.24). Undisturbed population in Trichur was also rich in number of alleles (13) followed by Jabalpur (12). Generally Kerala populations had higher gene diversity followed by Valsad (Gujarat) and Jabalpur (Madhya Pradesh).

With regard to undisturbed populations, Orissa was genetically far distant from all other populations as from Madhya Pradesh (Fst 0.532), Gujarat (0.5157) and Kerala (0.36-0.489). The genetic distance from Kerala populations to Madhya Pradesh and Gujarat were in the range of 0.2177 to 0.331. However, the distance between Madhya Pradesh and Gujarat was low (0.1166). The genetic distance among Kerala populations were also low (0.037-0.145). In general the total gene diversity, with respect to the fourteen populations examined, was found to be 0.749, of which a large portion (0.550) was within population diversity and only 0.199 was between populations.

On analyzing the effect of human disturbance, the present study using nuclear gene markers revealed a reduction in the mean number of alleles in disturbed populations than their undisturbed pair populations, except at Nilambur and Wayanad. The disturbed populations showed 10.7 to 40 per cent of reduction in mean number of alleles and a decrease of 2 to 26 per cent in gene diversity than in their undisturbed pair population. In almost all populations, heterozygosity was also lower in disturbed populations.

The four microsatellite loci employed to examine the short term dynamics appeared to be informative and more than five alleles were obtained in all the markers. Undisturbed natural population from Trichur had the maximum number of alleles, followed by disturbed natural population and plantation from Trichur. Khurda from Orissa was also rich in number of alleles. Populations in Wayanad and Nilambur had the least number of alleles.

The maximum expected heterozygosity (0.718) was obtained in undisturbed population at Khurda and lowest at Wayanad. Maximum observed heterozygosity (0.584) was seen in the Nilambur population. Observed heterozygosity was lowest in Jabalpur (Madhya Pradesh) and then Valsad. Maximum gene diversity was seen in undisturbed Khurda population followed by Valsad using microsatellite markers, while populations at Khurda (Orissa) were found to exhibit lowest gene diversity on employing nuclear gene markers. This might be due to recent evolutionary changes in consequence of mutations so as to compensate the low gene diversity. Populations from Kerala, including the plantation were found to have high gene diversity.

Out of the total 15 populations, inbreeding (*Fis*) was significant in all the populations from North India and in particular, the undisturbed populations from North India showed higher inbreeding. With respect to Kerala populations, disturbed populations have more inbreeding than undisturbed populations except in Nilambur.

On analyzing the effect of human disturbance employing microsatellites, it was seen that the estimated gene diversity to be lower in disturbed populations than undisturbed populations in all the locations except Konni and Wayanad. Observed heterozygosity (Ho) was lower in disturbed populations at all locations. There was no evident reduction in alleles due to disturbance.

The STRUCTURE analysis showed geographical patterns based on the allele frequencies. The analysis using nuclear gene markers showed three clusters, the first cluster comprising all the populations from Kerala, second consists of the populations

from Gujarat and Madhya Pradesh and the third is being the populations from Orissa. Using microsatellites the same pattern was obtained except that the populations from Kerala were split in two clusters with the Nilambur Division separated from the rest of Kerala. The separation of the Nilambur populations could be due to the recent evolutionary changes as these were changes at intron level. In each of the locations, there are no apparent differences between the undisturbed and disturbed pair populations.

The present study clearly indicates that human disturbance leads to genetic erosion in terms of number of alleles, allelic richness, gene diversity and heterozygosity. Erosion of genetic variation due to disturbance may have significant long-term evolutionary consequences and is also of immediate concern if genetic changes directly affect individual fitness. Hence, an optimum population size should be retained for better management of teak genetic resources. This study has provided some insights into the genetic variation within and between populations and the effect of human disturbance on genetic structure of teak populations.

As part of teak improvement programmes, seed production areas and seed orchards have been established in India and elsewhere. However the seed orchards, in general, are low productive. Hence, researchers in Thailand, Indonesia and India studied the breeding system including pollinator activity. Though, these studies generated very useful data, some lacunae exist. Patterns of pollen flow and contemporary gene flow are useful to draft guidelines for establishment and sustainable management of seed orchards, and *in situ* or *ex situ* germplasm conservation areas. This information is also required for estimating the viable population size and tree density, which are necessary for the maintenance of genetic diversity. But there was not much information regarding the distance of pollen flow and seed dispersal as well as contemporary gene flow and mating system in teak.

Hence, to compare the contemporary gene flow and mating system in teak populations with different levels of human interference, two natural teak forests, one highly disturbed and another undisturbed were selected in the Peechi-Vazhani Wildlife

Sanctuary of Thrissur District in Kerala State. All the adult teak trees (100 to 200 trees) in the selected sites and one hundred randomly selected seedlings on the forest floor (to study the seed dispersal) were marked and mapped using the software Mapinfo Professional. Seeds were also collected from nine randomly selected mother trees from each of the plots to estimate the pollen flow. The individual trees and seedlings on forest floor and progenies through seeds were DNA-fingerprinted using seven microsatellite markers in non-denaturing polyacrylamide gel electrophoresis.

The total number of alleles for all the loci obtained was 40 and 45 in disturbed and undisturbed natural teak forests respectively. The allelic richness and the average PIC of the selected populations confirm the resolving power of the loci for unbiased estimation of individual reproductive success and for tracing parentage.

The genetic structure of teak populations revealed that all the teak populations harbour good amount of gene diversity and it was lower in disturbed teak forest population (0.563) than the undisturbed teak forest population (0.614). With regard to inbreeding coefficient, 7 per cent (0.070) inbreeding or heterozygote shortfall was observed in the disturbed plot whereas in undisturbed plot only 1.8 per cent (0.018) inbreeding was noted. In disturbed population, genetic differentiation (Fst) between parents and progenies was high (0.024) compared to the value of 0.01 obtained for undisturbed population.

In the two populations selected, more than 50 per cent of the fruits were without embryos, which might be due to the low pollen ovule ratio reported earlier. The contemporary gene flow was evaluated through the components of pollen and seed dispersal. The main range of pollen dispersal distance was found to be 151-200 m in disturbed plot and 101-150 m in undisturbed plot. The reason for the higher pollen dispersal distance in the disturbed plot might be due to the large number of insects observed in this population than the other leading to the increased activity of pollinators. It confirms the earlier findings that low pollinator activity to be one of the most important reasons for low fruit productivity in teak. The pollen dispersal analysis from these teak

populations showed that the pollen dispersal was mainly in the distance of below 200 m. It indicates that the pollen dilution zone must be at least 200 m in seed orchards to restrict the entry of pollen from outside. The analysis on seed dispersal showed that the dispersal was mainly in the distance range of 50-100 m. This indicated that, in natural teak population, the distance of pollen flow was more than the seed movement. Hence, the remarkable contribution in maintaining genetic diversity happens to be by pollen transfer.

With regard to mating system, higher out-crossing rate (89-96 %) was seen in the populations. The present study also has revealed that an individual mother tree received pollen from many male parents leading to multi-parental mating. Likewise, an individual male parent donated pollen to many mother parental trees leading to a high within population gene diversity through the mixing of alleles while producing the next generation. This observation supported the high gene diversity estimated within population and also confirmed to the tree breeders' expectation that seeds from orchards or seed stands could be genetically diverse.

The study conducted on pollinators identified Hymenopteran groups of insects as the potential pollinators playing a vital role in teak pollination. The main pollinators are some solitary bees like *Halictus tectonae* Narendran and Jobiraj and Wasps. Two species of solitary bees, *Anthophora zonata* (Linn.) and *A. niveo-cincta* Smith were also found to carry a load of pollen on the under side of their abdomen and hind legs. They were found to be very active, visiting several inflorescences on the same as well as on different trees in a short time. Most of the insect visitors spent their time among the inflorescences of a single tree except bigger wasps which moved rapidly among the adjacent trees.

The results of the present study show that teak populations are genetically diverse and disturbance in natural forests leads to decrease in gene diversity, increased inbreeding and higher population differentiation between parents and their progenies. The findings of this study are useful for the scientific and sustainable management of teak seed orchards/seed stands and germplasm banks as well as for the preparation of efficient and comprehensive strategies for conservation of natural teak populations.

6. CONCLUSIONS

1. On employing nuclear gene markers, Konni from Kerala was found to be the most diverse and populations from Orissa to be the least diverse.
2. On employing microsatellite markers, populations from Orissa were found to be the most diverse populations. Konni also was genetically diverse.
3. Population structure analysis showed three geographic clusters (on employing nuclear gene markers), one comprising Kerala populations, another containing populations from Madhya Pradesh and Gujarat and the third with Orissa. The same pattern was obtained on using microsatellite markers, except that the cluster for Kerala split in to two, one containing Nilambur alone and the other comprising the remaining populations (Wynad, Trichur, Konni) from Kerala.
4. Human disturbances lead to genetic erosion in terms of number of alleles, allelic richness, gene diversity and heterozygosity.
5. More than 90 percent out crossing was seen in teak. Multi-parental mating was observed in teak where individual flowers receive pollen from many donors and individual pollen parents distribute pollen to many other trees.
6. The main range of pollen dispersal distance was found to be 151-200m in disturbed plot and 101-150m in undisturbed plot.
7. Pollinators have an active role in deciding the distance of pollen dispersal and multi-parental mating. As the potential pollinators, Hymenopteran groups of insects were playing a vital role in teak pollination.
8. Lack of pollinators affects seed setting.
9. The seed dispersal was found mainly in the distance range of 50-100 m.

7. LITERATURE CITED

Aldrich, P., and Hamrick, J. L. 1998. Reproductive dominance of pasture trees in a fragmented tropical mosaic. *Science* 281: 103 –105.

Aldrich, P., Hamrick J. L., Chavarriaga, P., and Kochert, G. 1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. *Molecular Ecology* 7: 933 –944.

Allendorf, F.W. 1986. Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology* 5: 181–190.

Barrett, S.C.H., and Kohn, J.R. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. *In*: Falk DA, Holsinger KE, (Eds). Genetics and conservation of rare plants. New York: Oxford University Press: Pp 3–30.

Barton, N.H., Whitlock, M.C. 1997. The evolution of metapopulations. *In*: Hanski, I A., Gilpin, M.E. (Eds.). Metapopulation Biology: Ecology, Genetics and Evolution. Academic Press, San Diego. Pp. 183–210.

Bawa, K.S. 1990. Plant - pollinator interaction in tropical rain forests. *Annual Review of Ecology and Systematics*. 21: 399–422.

Bawa, K.S and Seidler, R. 1998. Natural forest management and conservation of biodiversity in tropical forests. *Conservation biology* 12: 46 – 55.

Bittencourt, N.S., Semir, J. 2005. Late-acting self-incompatibility and other breeding systems in *Tabebuia* (Bignoniaceae). *International Journal of Plant Sciences* 166: 493–506.

Botstein B., White R.L., Skolnick, N. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Human Genetics*. 32: 314 – 331.

Buiteveld, J., Vendramin,G.G., Leonardi,S., Kamer,K.,Geburek,T. 2007. Genetic diversity and differentiation in European beech . (Fagus Sylvatica L.) stands varying in management history. *Forest Ecology and management* 247: 98-106.

Burczyk, J., Lewandowski, W., Chalupka, W. 2004. Local pollen dispersal and distant gene flow in Norway spruce (*Picea abies* [L.] Karst). *Forest Ecology and Management* 197: 39–48.

Butcher, P.A., Glaubitz, J.C., Moren, G.F. 1999. Application for microsatellite markers in the domestication and conservation of forest trees. *Forest Genetic Resources No. 27.* FAO, Rome. Pp. 34 – 42.

Cascante, A., Quesada, M., Lobo, J.A and Fuchs, E.J. 2002. Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. *Conservation Biology* 16: 137–147.

Changtragoon, S and Szmidt, A.E. 2000. Genetic diversity of teak . (*Tectona Grandis*) in Thailand revealed by Random Amplified Polymorphic DNA (RAPD) markers. *In*: IUFRO conference on tropical species breeding and genetic resources: Forest genetics for next millennium, International conference center, Durban, South Africa 8 – 13 October 2000. Pp 82 – 83.

Changtragoon, S. 2004. The potential for using molecular markers to facilitate gene management and the *in situ* and *ex situ* conservation of tropical forest trees. Asia Pacific Forest Genetic resources programme (APFORGEN) pp. 305–313.

Cheua-ngam, J. 2005: Development of Nuclear Gene DNA Based Markers for Population Genetic Analysis in Teak (*Tectona grandis* L.f.). M.Sc. Thesis, Kasetsart Univ. 79p.

Cheua-ngam, J. and Volkaert, H. 2005. Development of *Catalase* gene Nuclear DNA-based marker for population genetic analysis in Thai teak (*Tectona grandis* L.f.). *Kasetsart J. (Nat. Sci.)* 40: 91 – 98.

Cheua-ngam, J.; Apisitvanich, S.; Toojinda, T.; Volkaert, H. 2005. Development of nuclear gene DNA based markers for population genetic analysis in teak (*Tectona grandis* L.f.). Proceedings of 43rd Kasetsart University Annual Conference, Thailand (Plants). pp. 402-408.

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

Dick, C.W., 2001. Genetic rescue of remnant tropical trees by an alien pollinator. Proc. of the Royal Society of London Series B – Biological Sciences 268: 2391–2396.

Dick, C.W., Etchelecu, G and Austerlitz, F. 2003. Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* 12: 753–764.

Difazio, S.P., Slavov, G.T., Burczyk, J., Leonardi, S., Strauss, S.H., 2004. Gene flow from tree plantations and implications for transgenic risk assessment. In: Walter, C., Carson, M. (Eds.), Forest Biotechnology for 21st Century. Research Signpost, Trivandrum, Kerala, India, pp. 405-422.

Dogra, P. D.1981. Forest Genetics - Research and application in Indian Forestry II: *The Indian Forester* 107: 263-279.

Doyle, J.J., Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Falush, D., Stephens, M., and Pritchard, J. K. 2003. Inference of population structure: Extensions to linked loci and correlated allele frequencies. *Genetics* 164:1567 – 1587.

Falush, D., Stephens, M., and Pritchard, J. K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*.

FAO.1956a. Country reports on Teak Forestry: India, FAO, Rome, Italy. Pp. 21-48.

FAO.1956b. Country reports on teak forestry: Indonesia, FAO, Rome, Italy. Pp. 49-105.

Finkeldey, R., 1998. An Introduction to Tropical Forest Genetics. Lecture Notes. Institute of Forest Genetics and Forest Tree Breeding. Georg-August University, Goettingen. Germany, 221 p.

Fisher, R.A., 1937. The wave of advance of advantageous genes. *Annals of Eugenics* 7: 355–369.

Franceschinelli, E.V., Bawa, K.S. 2000. The effect of ecological factors on the mating system of a South American shrub species (*Helicteres brevispira*). *Heredity* 84: 116–123.

Frankel, O.H and Soule, M.E. 1981. Conservation and evolution. Cambridge University Press, UK.

Fukue, Y., Kado, T., Lee, S.L., Ng, K.K.S., Norwati, M., Tsumura, Y. 2007. Effects of flowering tree density on the mating system and gene flow in *Shorea leprosula* (Dipterocarpaceae) in peninsular Malaysia. *Journal of Plant Research* 120: 413-420.

Ghazoul and Mcleish M. 2001. Reproductive ecology of tropical forest trees in logged and fragmented habitats in Thailand and Costa Rica. *Plant Ecology* 153: 335 – 345.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices . (version 2.9.3). http://www.unil.ch/izea/softwares.

Gribel, R., Gibbs, R.P.E., Queiróz, A.L., 1999. Flowering phenology and pollination biology of *Ceiba pentandra* (Bombacaceae) in Central Amazonia. *Journal of Tropical Ecology* 15: 247–263.

Hall, P., Walker, S and Bawa, K. 1996. Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. *Conservation Biology*, 10: 757 – 768.

Hamrick, J.L., Godt, M.J.W., Sherman–Broyles, S.L., 1992. Factors influencing level of genetic diversity in woody plant species. *New Forests* 6: 95-124.

Handel, S., 1983. Pollination ecology, plant population structure and gene flow. *In*: Real, L. (Ed.), Pollination biology. Academic Press, Florida, pp. 163–211.

Hearne, C M., Ghosh, S., and Todd, J.A. 1992. Microsatellites for linkage analysis of genetic traits. *Trends in Genetics*. 8: 288- 294.

Hedegart, T. 1976. Breeding systems variation and genetic improvement of teak (*Tectona grandis* L.f.). In: Burley, J., Styles, B.T., (Eds.), Tropical trees, variation, breeding and conservation, Linnean society symposium series 2. Academic press, London. pp.109-122.

Hedenas, H., and Ericson, L. 2003. Response of epiphytic lichens on *Populus tremula* in a selective cutting experiment. *Ecological Applications* 13: 1124 – 1134.

Hedrick, P.W.(1994. Purging inbreeding depression and the probability of extinction: full sib mating. *Heredity* 73: 363 – 372.

Hufford, K.M., Hamrick, J.L., 2003. Viability selection at three early life stages of the tropical tree, *Platypodium elegans* (Fabaceae, Papilionoideae). *Evolution* 57: 518–526.

Huenneke LF. 1991. Ecological implication of genetic variation in plant population. In: Falk DA, Holsinger KE (Eds.) *Genetics and Conservation of rare plants* Oxford University press, Oxford. pp 31–44.

Husband, B.C. and Schemske, D.W. 1996. Evolution of the magnitude of inbreeding depression in plants. *Evolution* 50: 54 – 70.

Indira, E.P., Mohanadas, K. 2002. Intrinsic and extrinsic factors affecting pollination and fruit productivity in teak (*Tectona grandis* L.f.). *Indian Journal of Genetics and Plant Breeding* 62: 208–214.

Kaosa-ard, A., 1986. Teak (*Tectona grandis* L.f.) nursery techniques with special reference to Thailand. DANIDA Forest Seed Centre, Seed Leaflet No 4A, 42p.

Ketadikara, A.W.S and Prat. 1995. Isozyme variation among teak (*Tectona Grandis* L.f.) provenances. *Theoretical and Applied Genetics* 90: 103 – 110.

Kjaer, E.D and Suangtho, V. 1995. Outcrossing rate of teak. (*Tectona Grandis* L.f.) *Silvae Genetica* 44: 175 – 177.

Konuma, A., Tsumura, Y., Lee, C.T., Lee, S.L., Okuda, T. 2000. Estimation of gene flow in the tropical-rain forest tree *Neobalanocarpus heimii* (Dipterocarpaceae), inferred from paternity analysis. *Molecular Ecology* 9: 1843-1852.

Kumaravelu, G., 1993. Teak in India. *In*: "Teak in Asia". Technical Document GCP/RAS/I34/ASB, FORSPA Publication 4, FAO-RAPA, pp. 27-36.

Lacy, R.C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection and population subdivision. *Conservation Biology* 1: 143–158.

Latouche-Hallé, C., Ramboer, A., Bandou, E., Caron, H., Kremer, A., 2004. Long-distance pollen flow and tolerance to selfing in a neotropical tree species. *Molecular Ecology* 13: 1055–1064.

Levin, D. A., Kerster, H.W., 1974. Gene flow in seed plants. *Evolutionary Biology* 7: 139-220.

Lira, C.F., Codoso, S.R.S., Ferreiva, P.C.G., Cordoso, M.A., Provan, J. 2003. Long term population isolation in the endangered tropical tree species *Caesalpinia echinata* revealed by chloroplast microsatellites. *Molecular Ecology* 12: 3219 – 3225.

Loveless, M. D., 1992. Isozyme variation in tropical trees: patterns of genetic organization. *New Forests* 6: 67-94.

Marshall, T. C., Slate, J., Kruuk L.E.B., Pamberton, J.M., 1998. Statistical confidence for likelihood based paternity inference in natural populations. *Molecular Ecology* 7: 639–655.

Mohanadas, K., Mathew, G., Indira, E.P., 2002. Pollination ecology of teak in Kerala. *KFRI Research Report No. 225*, KFRI, Peechi, 36p.

Murawski, D.A., Hamrick, J.L., 1992. The mating system of *Cavanillesia platanifolia* under extremes of flowering-tree density: a test of predictions. Biotropica 24: 99–101.

Nason, J.D and Hamrick, J.L. 1997. Reproductive and genetic consequences of forest fragmentation: two case studies of Neotropical canopy trees. *Heredity* 88: 264 –276.

Nason, J.D., Herre, E.A., Hamrick, J.L. 1998. The breeding structure of a tropical keystone plant resource. *Nature* 391: 685–687.

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press: New York.

Nicodemus, A., Nagarajan, B., Narayanan, C., Varghese, M. and Subramanian, K. 2005. RAPD variation in Indian teak population and its implications for breeding and conservation. *In:* Bhat *et al.* (Eds.), *Proceedings of the International Conference on Quality Timber Products of Teak from Sustainable Forest Management*, Kerala Forest Research Institute, Peechi. Pp.321 – 330.

Palupi, E.R. and Owens, J.N. 1997. Pollination, fertilization, and embryogenesis of teak (*Tectona grandis* L.f.). *International Journal of Plant Sciences* 158(3): 259–273.

Peterson, E.B., and McCune, B. 2001. Diversity and succession of epiphytic macrolichen communities in low-elevation managed conifer forests in Western Oregon. *Journal of Vegetation Science* 12: 511 – 524.

Petit, R.J., Mousadik, A. and Pons, O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* 12: 844 – 855.

Phengduang, V. 1993. Teak in Laos PDR. *In*: "Teak in Asia". Technical Document GCP/RAS/I34/ASB, FORSPA Publication 4, FAO-RAPA, Pp. 41-50.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945 – 959.

Prus-Glowacki, W., Stephan, B.R., 1994. Genetic variation of *Pinus Sylvestris* from Spain in relation to other European populations. *Silvae Genetica* 43: 7-14.

Schuster, W.S.F., Mitoon, J.B. 2000. Paternity and Gene dispersal in limber pine (*Pinus flexilis james). Heredity* 84: 348- 361.

Shrestha, M.K., Volkaert, H. and Van Der Straeten, D. 2005. Assessment of genetic diversity in *Tectona grandis* using amplified fragment length polymorphism markers *Canadian Journal of ForestResearch* 35: 1017–1022.

Suangtho, V. 1980. Factors controlling teak (*Tectona grandis* L.f.) seed germination and their importance to Thailand. M.Sc. Thesis, Australian National University, Canberra, Australia.

Tangmitcharoen, S and Owens, J. N. 1997: Floral biology, pollination, pistil receptivity, and pollen tube growth of teak (*Tectona Grandis* L.F) *Annals of Botany* 79: 227- 241.

Trapnell, D.W., Hamrick, J.L. 2004. Partitioning nuclear and chloroplast variation at multiple spatial scales in the neotropical epiphytic orchid, *Laelia rubescens*. *Molecular Ecology* 13: 2655-2666.

Trejo, I and Dirzo, R. 2000. Deforestation of seasonally dry tropical forest: national and local analysis in Mexico. *Biological Conservation* 94: 133 – 142.

Uma Shaanker, R., Ganeshaiah, K.N., 1990. Pollen grain deposition patterns and stigma strategies in regulating seed number per pod in multi ovulated species. *In*: Bawa, K.S., Hadley, M. (Eds.), *Reproductive ecology of tropical forest plants. Man and the Biosphere Series* 7: 165-178.

Voigt, F.A., 2005. Influence of pollination and seed dispersal on the genetic population structure of two *Commiphora* species in Madagascar and South Africa. Ph. D. Thesis, Johannes Gutenberg University of Mainz, Germany.

Ward, M., Dick, CW., Gribel, R., Lemes, M., Caron, H., and Lowe, A.J. 2005. To self, or not to self... A review of outcrossing and pollen - mediated gene flow in neotropical trees. *Heredity* 95: 246–254.

Weir, B.S. and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.

White, G.M., Boslier, D.H and Powell, W. 1999. Genetic variation within a fragmented population of *Swietenia humilis* Zuccarini. *Molecular Ecology* 8: 1899-1909.

White, G.M., Boshier, D.H. and Powell, W. 2002. Increased pollen flow counter acts fragmentation in a tropical dry forest: an example from *Swietenia humilis* Zuccarini. *Proceedings of National Academy of Science* USA 99 (4): 2038 – 2042.

Young, A., Boyle, T., and Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* 11: 413-418.