E-2.4 Molecular Phylogeny and Genetic Diversity of Dipterocarpaceae in Malaysia

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Total Budget for FY1993-1995 22,431,000Yen (FY1995 6,535,000Yen)
Key Words Dipterocarpaceae, Molecular phylogent, Genetic diversity, Evolution

Abstract

A molecular phylogeny of the Dipterocarpaceae subfamily Dipterocarpoideae was constructed using restriction fragment length polymorphisms of polymerase chain reaction amplified specific genes in chloroplast DNA. The phylogenetic trees clearly separate species with two different base chromosome numbers. Our conclusions agree with a phylogeny derived from wood anatomy data analysis, and Symington's and Ashton's taxonomic classifications. We selected five mother trees which are isolated from the other same species trees in Pasoh reserved forest to investigated the outcrossing rate of Neobalanocarpus heimii.. That resulted that the outcrossing rate of this species was quite high, its 1.065 (SD=0.212). RAPD data also supported this result. We have investigated the genetic variation of mitochondrial DNA (mtDNA) and RAPD in Hopea species to clarify the genetic diversity and speciation of Dipterocaraceae. The five species could be separated into three groups using mtDNA variation data. This grouping resembles the taxonomic subsections of Hopea species. Hopea species have rather smaller diversities (their overall average is 0.0104 ± 0.0009) than Shorea species (their oveall avearge is 0.0189 ± 0.0012) in RAPD analysis.

Introduction

Tropical rainforests which are rich in species diversity are being reduced by human activity. However, the information on their genetic diversity and phylogenetic relationship are limited. It is very important for conversation of the forests to understand the genetic diversity, phylogenetic relationship and mating system of tropical rainforests tree species. Dipterocarps tree species which consist of the one of major species in the tropical rainforest and are very valuable for forestry. Therefore, we have studied the molecular phylogeny and genetic diversity of dipterocarps tree species using a molecular marker.

Materials and methods:

1. Molecular phylogeny

The leave tissues were collected from 30 species in seven genera of Dipterocarpaceae, which are including the following genera, *Shorea, Hopea Noebalanocarpus, Parashorea, Dipterocarpus, Dryobanops, Anisoptera, Upuna, Vatica* and *Cotylelobium* . Total DNAs were extracted from the leaves using the modified CTAB method. Eleven chloroplast genes were amplified by PCR (polymerase chain reaction) and RFLP (restriction fragment length polymorphism) analysis was done to detect site change of each genes among 30 species. Phylogenetic tree was constructed using PAUP ver2.0 and NJ methods.

2. Mating systems

We selected five mother trees which are isolated from the other same species trees in Pasoh reserved forest to investigated the outcrossing rate of *Neobalanocarpus heimii*. The leaves and twigs of them and their seedlings were collected. Total DNAs were extracted from the leave samples and RAPD analysis was carried out. Isozyme analysis was also done using the inner bark of each twig samples. Outrcrossing rate was estimated by the method of Ritland and Jain (1981).

3. Genetic diversity of populations of *Hopea* species.

We collected leaves from 192 individuals of 13 populations of nine *Hopea* species which are *H. dyeri, H. dyeri, H. glaucescens, H. myrtiforia, H. latifolia, H. odorata, H. myrtiforia, H. Pierrei,* and *H. beccariana* in Peninsula Malaysia. Total DNAs were extracted from the leaves using the modified CTAB method. We have screened the polymorphism of mitochondrial DNA using RFLP analysis. The RAPD analysis also carried out to investigate the genetic diversity between and within populations and species.

Results and Discussion

1. Molecular phylogeny

A molecular phylogeny of the Dipterocarpaceae subfamily Dipterocarpoideae was constructed using restriction fragment length polymorphisms of polymerase chain reaction amplified specific genes in chloroplast DNA. Fully 141 site changes were detected among ten genera and 30 species in 11 different genes: *rbcL, psbA, psbD, rpoB, rpoC, petB, atpH, 16S, psaA, petA* and *trnK* (Table 1). Phylogenetic trees constructed by Wagner parsimony and neighbor-joining methods, using Upuna as the outgroup, displayed five monophytelic groups that included *Upuna: Hopea-Shorea-Parashorea-Neobalanocarpus; Dryobalanops; Dipterocarpus; Anisoptera-Vatica-Cotylelobium*; and *Upuna*. (Fig. 1). The phylogenetic trees clearly separate species with two different base chromosome numbers. The first group is $x = 7$ and the other is x

= 11. Therefore, the x = 7 group is thought to be in a synapomorphic character state. *Parashorea lucida* is a sister to most *Shorea* species. *Neobalanocarpus heimii* and *Hopea* form a clade of a sister to two *Shorea* species, and *Cotylelobium* and *Vatica* are closely related species. Our conclusions agree with a phylogeny derived from wood anatomy data analysis, and Symington's and Ashton's taxonomic classifications.

2. Mating systems

Isozyme analysis was done to investigate a outcrossing rate of this species. Five isozyme loci (*EST*-1, *Gpi*-1, *Pgm*-2, *Shd*-1 and *Ugp*-1) were investigated and two or three alleles were detected in each locus. We calculated the outcrossing rate using Ritland and Jain (1981) model. That resulted that the outcrossing rate of this species was quite high, its 1.065 (SD=0.212) (Table 2). RAPD data also supported this result.

3. Genetic diversity of populations of *Hopea* species.

We have investigated the genetic variation of mitochondrial DNA (mtDNA) and RAPD in *Hopea* species to clarify the genetic diversity and speciation of Dipterocaraceae. Screening of mtDNA polymorphism was done using the combinations of 18 restriction endonucleases and three kinds of mtDNA genes which are *cox*I, *cox*III and *nad*5. We detected intra-specific variation in the combinations of *cox*III-*Pst*I and *cox*III-*Bam*HI. Seven populations representing 5 species were then investigated using these two combinations. The five species could be separated into three groups using mtDNA variation data (Table 3). This grouping resembles the taxonomic subsections of *Hopea* species.

Hopea species have rather smaller diversities (their overall average is 0.0104 ± 0.0009) than Shorea species (their oveall avearge is 0.0189 ± 0.0012) in RAPD analysis (Table 4). This difference might suggest that Hopea species have slightly small effective population sizes and/or slightly small mutation rate due to shorter generation time because nucleotide diversity is proportional to the product of these two quantities (Nei 1987). Furthermore, the interspecific gene flow can increase the nucleotide diversity within species. Interspecific genetic divergence ranged 0.0108 to 0.0209. Interspecific genetic divergence between *Hopea* species seems to be slightly larger than that for Shorea species.

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Table 1. The mean estimates of nucleotide substitutions (%), the corresponding standard deviations, site change between species, surveyed sequence and sizes of amplified fragments for each gene.

Gene	Fragment size	Site change	Surveyed sequencea (%)	Nucleotide substitution	S.D.
*rbc*L	1387 bp	15	143 bp (10.3%)	4.05	0.0229
*rpo*B	1286 bp	17	124 bp (9.6%)	3.09	0.0197
*pet*B	1634 bp	23	133 bp (8.1%)	6.22	0.0476
*psb*A	939 bp	3	79 bp (8.4%)	6.48	0.0096
*psb*D	1042 bp	8	85 bp (8.2%)	3.11	0.0254
*atp*H	385 bp	3	24 bp (6.2%)	6.21	0.0682
16S	1375 bp	6	78 bp (5.7%)	1.87	0.0176
*rpo*C	3603 bp	33	220 bp (6.1%)	4.21	0.0321
*psa*A	2218 bp	8	86 bp (3.9%)	4.05	0.0237
*pet*A	2314 bp	8	46 bp (2.0%)	8.57	0.0790
*trn*K	2569 bp	17	108 bp (4.2%)	6.83	0.0390
Total	18752 bp	141	1126 bp (6.0%)	3.83	0.0627

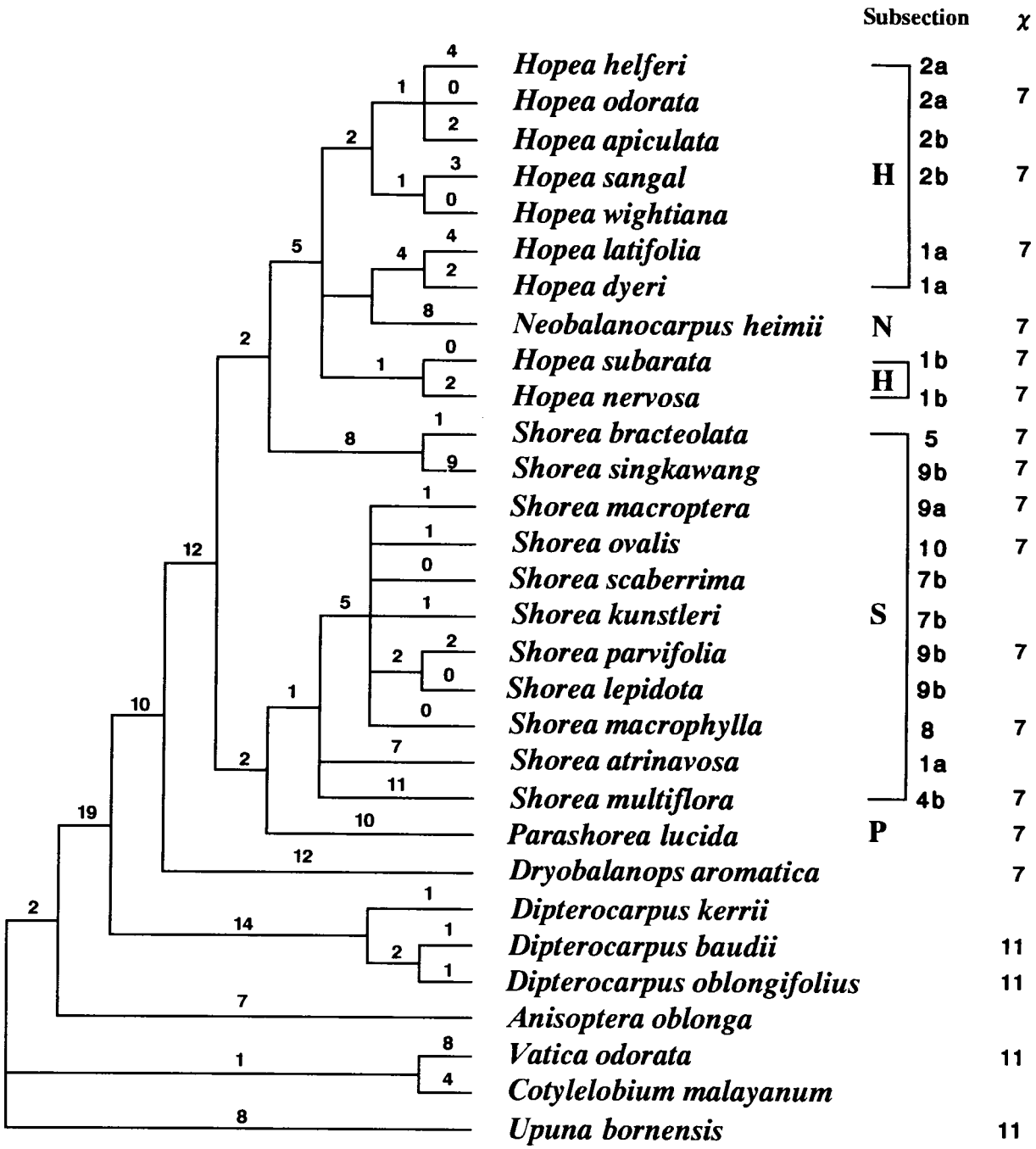

Fig. 1 Consensus tree of Wagner persimonious trees of Dipterocarpaceae using PCR-RFLP analysis of chloroplast genes.