No.E-2.3.1 Genetic Diversity of Dipterocarpaceae in Tropical Forest

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Abstract We tried to clarify the genetic diversity of Dipterocarpacea species using a molecular genetic maker. Plylogenetical relationship between 21 species of seven genera was investigated using RFLP (Restriction Fragment Length Polymorphism) of chloroplast DNA. When phylogenetic tree was constructed based on these data, the result was supported Ashton's classification based on morphological trait. Mating system of *Neobalanocarpus heimii* was also surveyed using gene makers, namely, allozyme and RAPD (Random Amplified Polymorphic DNA). In this case, five isozyme loci were used for estimation of outcrossing rate. This result was 1.065 (SD; .212), thus, this species was mostly a outcrossing species. The RAPD data also supported the result.

Key Word Diperocarpacea, Tropical Forest, Molecular Phylogeny, Mating System, Genetic Diversity

1.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. We need to know the genetic diversity, phylogenetic relationship and mating system of tropical rainforests tree species for proper conversation of the forest. Dipterocarp tree species which constitute a major commercially valuable for their hardwood timber. Therefore we tried to clarify the genetic diversity of dipterocarp tree species using a molecular genetic maker. In this study, we tried to clarify to the genetic feature of dipterocarps species using RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphism DNA) and isozyme. Meanwhile, molecular phylogeny of dipterocarps species also investigated using RFLP of chloroplast DNA. We tried to construct a conservation method of tropical forest based on these result.

2.Materials and methods

(1) Molecular phylogeny

Seven genera 21 species of dipterocarps were used for molecular phylogeny study (Table 1). Total DNAs were extracted from the leave samples using slightly modified CTAB method. The DNAs were digested by 20 kind of restriction endonucleases, *Bam*HI, *Hin*dIII, *Eco*RI, *Hae*III, *Hin*fI, *Pvu*II, *Eco*RV, *Sca*I, *Pst*I, *Sal*I, *Xba*I, *Bgl*II, *Kpn*I, *Sac*I, *Xho*I, *Dra*I, *Rsa*I, *Hha*I, *Sty*I. Electrophoresis of the DNAs was done using 0.7% agarose gel for 18 hours at 15V and the DNAs were transferred to nylon membrane (Hybond-N). Southern hybridization was carried out using DIG system (Boehringer Mannheim Co.Ltd.). Tobacco chloroplast DNA clones were used as probes. Detected fragments were scored, and a

phylogenetic tree was constructed using PHYLIP (Felsenstein 1985) and PAUP (Swofford 1990).

(2)Mating system

We selected five mother trees in Pasoh forest reserve to investigate outcrossing rate of *Neobaranocarpus heimii*. These mother trees were isolated from other same species trees and also have some seedlings. We collected leaves and twigs samples from the mother trees and seedlings (Table 2). Total DNAs were extracted from leaves samples using modified CTAB method. A RAPD analysis was carried out using these DNAs. Isozyme analysis was also done using the inner bark of each twig samples.

3.Results and discussion

(1)Molecular phylogeny of Dipterocarpaceae

We investigated genetic relationship between species and genus of Dipterocarpaceae at chloroplast DNA level. Because chloroplast DNA is conservative among species and also is suitable genetic marker to study a phylogeny. Tobacco chloroplast DNA clones were used for probes because all sequence of this species already have been published (Sugiura et al. 1986) and this genome is a typical type among land plants. We analyzed 110 combinations of restriction endonucleases and probes. *Vatica* species was put into outgroup when phylogentic tree was constructed, because this species is said to be a old type based on morphological traits. Bootstrap analysis was carried out to check the probability of each branch (Fig. 1). The result was following; *Shorea* and *Hopea* species were advanced group, *Anisoptera* was closed related to *Vatica* species, and this tree was mostly coincide to Ashton classification based on morphological traits.

(2)Mating system of *Neobalanocarpus heimii*

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 investigate and two to three alleles were detected in each locus. We calculate the outcrossing rate using Ritland and Jain (1981) model. The result was that the outcrossing rate of this species was quietly high, 1.065 (SD; 212) (Table 3). A RAPD analysis was carried out using 10 kind of random primers. RAPD data also supported this result. However, RAPD marker is mostly a dominant maker, thus it is difficult to identify the difference between a dominant homozygote and a heterozygote. But if we can identify between them, RAPD maker will give us many information.

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Table 1 Materials for molecular phylogeny.

Species		Species	
Shorea	*macroptera*	*Dipterocarpus*	*kunstlleri*
Shorea	*acuminata*	*Dipterocarpus*	*baudii*
Shorea	*maxwelliana*	*Dryobalanops*	*lanceolata*
Shorea	*assamica*	*Dryobalanops*	*aromatica*
Shorea	*ovalis*	*Dryobalanops*	*oblongfolia*
Shorea	*atrinervosa*	*Vatica*	*odorata*
Shorea	*sumatrana*	*Vatica*	*wallichii*
Shorea	*materialis*	*Vatica*	*bella*
Hopea	*odorata*	*Anisoptera*	*oblonga*
Hopea	*sangal*	*Neobalanocarpus*	*heimii*
Hopea	*nervosa*		

Table 2 Materials for mating system of *Neobalanocarpus heimii*.

Mother tree	No. of Pasoh Forest Reserve	Numbers of seedling
Mother tree 1	--	35
Mother tree 2	25A	24
Mother tree 3	173	15
Mother tree 4	40	26
Mother tree 5	--	25

Table 3 Outcrossing rate of *Neobalanocarpus heimii*.

Method	Outcrossing rate	Standerd Deviation
A. Multilocus	1.065	.212
B. Single locus	1.005	.052
A - B	0.060	.206
F value	0.443	

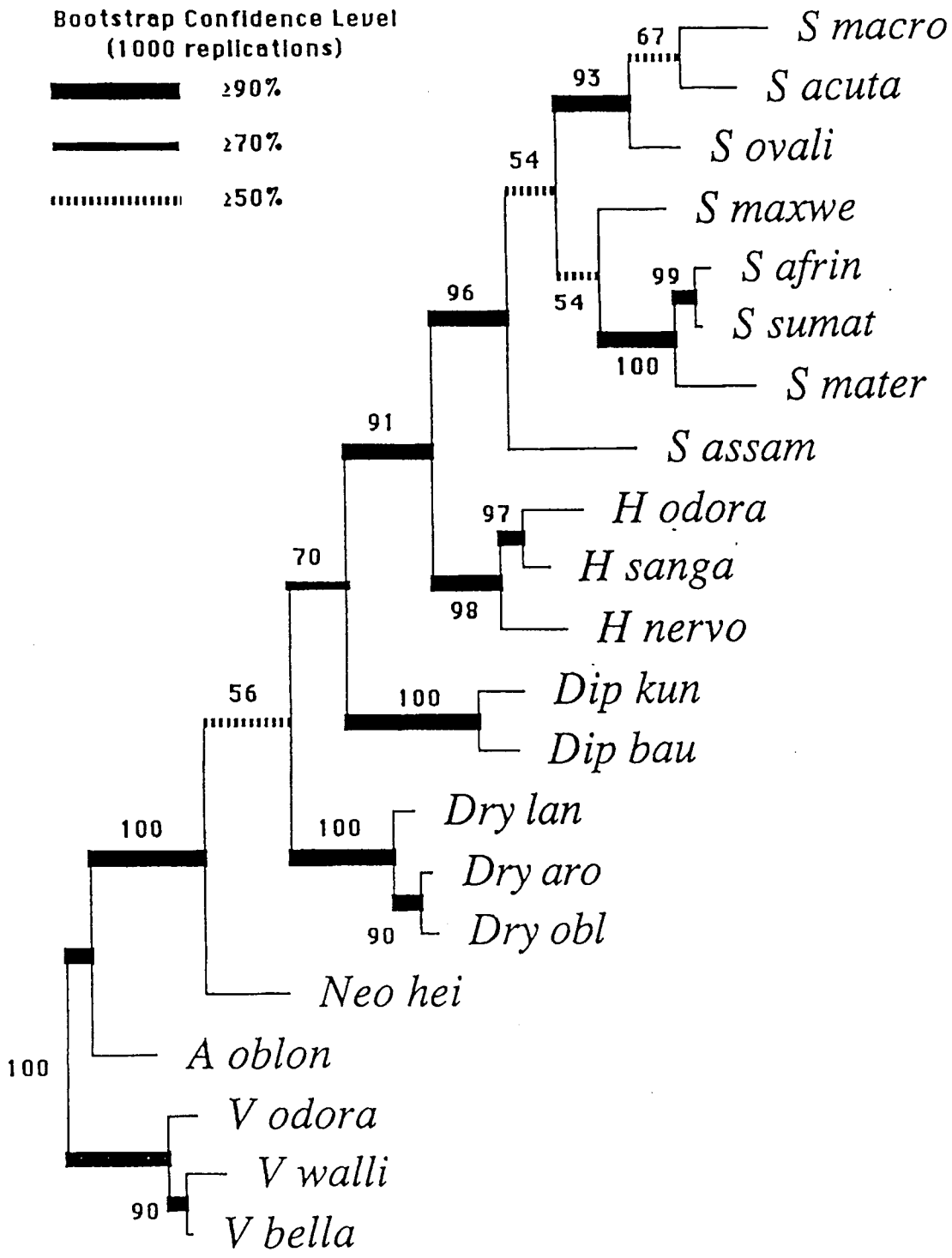

Fig. 1 Phylogenetic tree of Dipterocarpaceae using RFLP of chloroplast DNA. Numbers of each branch indicated the result of bootstrap analysis.