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Population-genetical Studies on *Dryobalanops* (Kapur) Species

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Summary : The genetic variation, differentiation and mating system of some species of *Dryobalanops* (Kapur) in Brunei Darussalam were investigated using a putative allozyme (*Lap*) locus coding leucine amino peptidase as marker gene.

Three populations were investigated in *D. aromatica* (Kapur Peringgi). The two populations investigated in main land showed similar allele frequencies. The other population in Temburong district showed different allele frequencies from those in main land.

On the investigation of genetic differences in the three species (*D. aromatica*, *D. lanceolata*, and *D. rappa*), *D. rappa* was quite different from the other two species.

Genotype frequencies and allele frequencies were investigated in natural regenerated seedlings of *D. aromatica* forest. Hardy-Weinberg equilibrium was kept between them. Most of the surviving seedlings seemed to have originated from the seeds produced by random mating.

1 Introduction

Tropical rain forest has been considered as rich in gene-diversity as well as species-diversity, and its conservation is an urgent problem for human existence in future. The advances in ecological and population genetics are indispensable as a grounding knowledge for gene conservation and forest regeneration in *Dipterocarpaceae* which is characteristic of the Asian tropical rain forest. Only a few studies, however, have been conducted in this research field.

The development of gel electrophoresis and histochemical detection of enzyme activity has made it possible to clarify the genetic differences between individuals or populations at the allelic level. Recently, these techniques have been used to progress in studies on variation and evolution in forest trees, that is, long-lived perennial plants. A large amount of data has been accumulated on electrophoretically detectable isozyme variations in various conifers (MITTON, 1983). Researchers are, at present, greatly interested in acquiring information regarding the genetic structure of populations, for example, the distribution of genetic variation within and between populations, genetic differentiation within a species, and gene flow and mating systems of populations. Isozymes as marker genes help to solve these problems practically, by the use of theories in population genetics.

In this study, we tried to apply the isozyme technique to some *Dryobalanops* (Kapur) species, that are important tree species of *Dipterocarpaceae* in Brunei Darussalam. We investigated the genetic variation, differentiation, and mating system of them at allelic level.

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2 Genetical Differentiation in *D. aromatica* (Kapur Peringgi)

Genetic variations and differences among populations have been investigated in many kinds of species, and genetic differentiations were observed within species.

In this study, we clarified the genetic variation and differentiation of *D. aromatica*, at the allelic level by using the one allozyme locus encoding leucine amino peptidase as gene marker. We also investigated the genetic structure of natural populations.

2.1 Materials and Methods

Three natural stands were used for this study (Fig. 1). Twenty-four seedlings were chosen at random from each stand, and their leaf blades were collected from the crown tops. All samples were brought to the Research Centre in Sungai Liang under low temperature (below 0℃) condition, and were stored at -30℃ for later use.

Leaf blades were disrupted with Tris-HCl buffer individually, and the extracts were analyzed by polyacrylamide slab gel electrophoresis after refining by centrifuging (SHIRAISHI *et al.*, 1987). Enzyme activities were detected on leucine amino peptidase (LAP)(SHIRAISHI, 1988). The genotypes of individuals were determined by their electrophoretic patterns of the enzyme system.

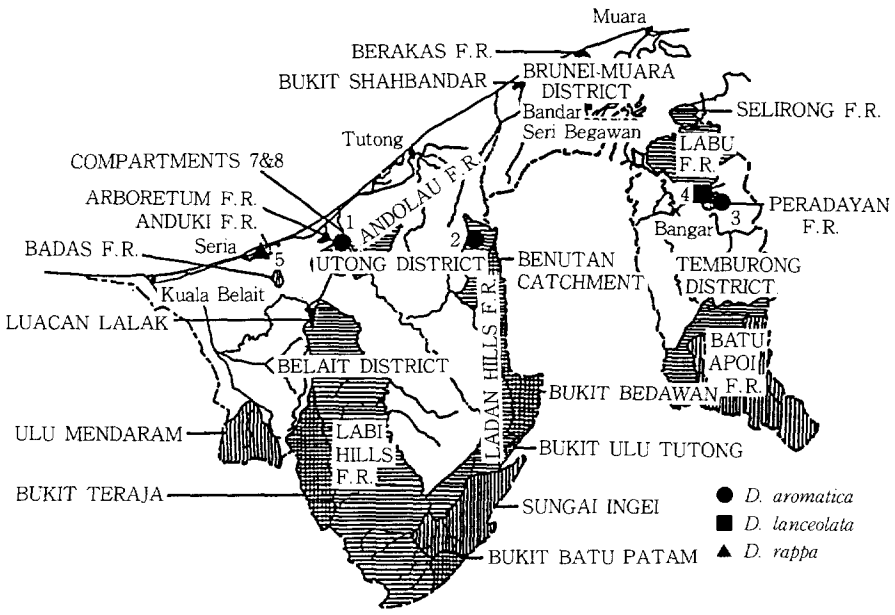

Fig. 1. Location of five populations studied
1,2,3; *D. aromatica*, 4; *D. lanceolata*, 5; *D. rappa*

2.2 Results and Discussion

2.2.1 Isozyme variations

Seven phenotypes (1, 4, 5, 6, 8, 9, 10 in Fig. 2) could be recognized in LAP isozyme, and a putative monomeric locus *(Lap)* encoding this enzyme was found. Four alleles, Lap^a, Lap^b, Lap^c, and Lap^d, were observed in this locus. The electrophoretically determined marker locus was utilized to investigate the genetic variations of the three natural populations, and to compare their genetic compositions.

2.2.2 Genetic structure of natural populations

The three natural populations examined are located as shown in Fig. 1. One stand was selected for a sampling site on each of the Andulau Hills (Andulau), Ladan Hills (Lamunin), and Pera-dayan F.R. (Temburong).

The allele frequencies of each population at *Lap* locus were estimated from observed genotype frequencies (Table 1). The mean frequency of each allele of the three populations was obtained with an arithmetical mean.

The allele frequency estimates of *Lap* were different among populations. The frequencies of Lap^b were 0.65 at Temburong, 0.66 at Andulau, and 0.71 at Lamunin, and a clear difference was not observed among populations. The frequencies of Lap^d varied from 0.15 at Lamunin to 0.31 at Temburong, however, a clear geographical cline was not observed. Lap^a and Lap^c alleles could not be detected at Andulau and Temburong, respectively.

Theoretically, genetic differentiation of populations is accumulated due to the genetic

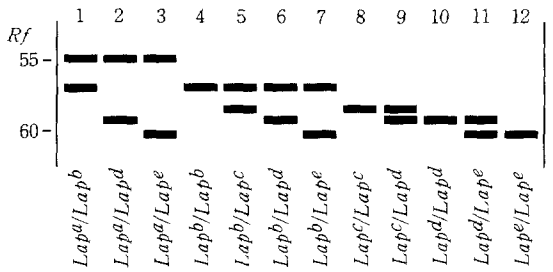

Fig. 2. Phenotypes of LAP isozymes observed in *Dryobalanops* and their genotypes

Table 1. Estimated allele frequencies and heterozygosities at *Lap* in three populations

Population	Allele frequency				Heterozygosity
	Lap^a	Lap^b	Lap^c	Lap^d	*(H)*
Andulau	0.00	0.66	0.17	0.17	0.50
Lamunin	0.02	0.71	0.12	0.15	0.46
Temburong	0.04	0.65	0.00	0.31	0.48
Average	0.02	0.67	0.10	0.21	0.481

processes of mutation, natural selection, and random drift, singly or in combination. The extent of differentiation can be quantified by an analysis of the gene diversity represented as heterozygosity (H). The heterozygosity of the total population (H_T) is partitioned into the heterozygosity within (H_S) and between (D_{ST}) populations (NEI, 1973). The relative magnitude of genetic differentiation among populations (G_{ST}) is measured as the proportion of D_{ST} to H_T, that is, $G_{ST} = D_{ST} / H_T$.

The heterozygosity of the total population (H_T) calculated from the mean allele frequencies of all populations was 0.493, whereas the heterozygosity within populations (H_S), that is, the mean value of the heterozygosity in each population, was 0.481. The partition attributed to among populations (D_{ST}) was 0.012. The proportion (G_{ST}) of between-population heterozygosity (D_{ST}) to the total-population heterozygosity (H_T) was 0.024. This indicated that 2.4 percent of the total gene diversity was attributable to gene differences among populations, and the remaining 97.6 percent was maintained within populations. This value may change with the variability of the locus used as a marker, and a more reliable value may be obtained generally by using more loci.

2.2.3 Genetic relationship among populations

The genetic distances of the three natural populations were estimated according to the procedures of NEI (1972) (Table 2). The genetic distance between Andulau and Lamunin was very small (0.004). The remaining two pairs, however, had large genetic distances (0.052 and 0.046). The three populations were grouped into two; one was Andulau and Lamunin, and the other was Temburong. It is suggested that *D. aromatica* in Temburong differed genetically from those in Andulau and Lamunin.

Table 2. Pair-wise genetic distance measurements between populations

Population	Andulau	Lamunin	Temburong
Andulau	-	0.997	0.955
Lamunin	0.004	-	0.950
Temburong	0.052	0.046	-

Above the diagonal; genetic identity
Below the diagonal; genetic distance

3 Inter-species comparison of allele frequencies among *Dryobalanops* species

Four species of *Dryobalanops* are distributed in Brunei Darussalam (ASHTON 1964). To clear the phylogenetic relationship in *Dryobalanops*, the isozyme technique was utilized as a molecular marker, and *D. aromatica*, *D. lanceolata* (Kapur Paji), and *D. rappa* (Kapur Paya) were investigated.

3.1 Materials and Methods

Three species in *Dryobalanops*, *D. aromatica*, *D. lanceolata*, and *D. rappa*, were used for this study. In addition to the samples mentioned in chapter 2, twenty-four trees each from *D. lanceolata*, and *D. rappa* were chosen at random and their leaf blades were collected.

The procedures mentioned in chapter 2 were also used for the extraction of enzymes from leaf blades, gel electrophoresis, and the detection of LAP activities.

3.2 Results and Discussion

3.2.1 Isozyme variations

Of the three species studied, one stand was chosen in each of *D. lanceolata*, and *D. rappa* for a sampling site, and three stands were selected in *D. aromatica* (Fig. 1).

In addition to the seven phenotypes recognized in *D. aromatica*, five phenotypes (2, 3, 7, 11, 12, in Fig. 2) could be newly-detected in the LAP isozyme. One new allele, (Lap^e) at *Lap* locus was found, and five alleles, Lap^a, Lap^b, Lap^c, Lap^d, and Lap^e, were observed in all. This gene marker was utilized to investigate the genetic variation of the three species in *Dryobalanops*, and to compare their genetic composition.

3.2.2 Inter-species comparison of allele frequencies

The allele frequencies of each species at *Lap* locus were estimated from observed genotype frequencies (Table 3). In *D. aromatica*, the arithmetical mean of the three stands of each allele was used as the allele frequency estimate.

The allele frequency estimates of *Lap* differed among species. The frequencies of Lap^b were 0.67 in *D. aromatica*, 0.58 in *D. lanceolata*, and large values were given in these species. In *D. rappa*, however, the frequency was 0.02 and Lap^b was a very rare allele. The frequencies of Lap^d were 0.21 in *D. aromatica*, 0.25 in *D. lanceolata*, whereas it was 0.75 in *D. rappa*. Lap^a and Lap^c alleles could not be detected in *D. rappa*, and Lap^e allele could not be observed in *D. aromatica*.

The heterozygosity of the total population (H_T) in all species was 0.645, whereas the heterozygosity within species (H_S) was 0.488. The partition attributed to among species (D_{ST}) was 0.157. The proportion (G_{ST}) of between-species heterozygosity (D_{ST}) to the total heterozygosity (H_T) was 0.244. This indicated that one quarter of the total gene diversity was attributable to gene differences among species, and the remaining three quarters was maintained within species.

Genetic distances among species were also estimated according to NEI's procedures (1972) (Table 4). The genetic distance between *D. aromatica* and *D. lanceolata* was small (0.012). The remaining two pairs, however, had very large genetic distances (1.189 and 0.903). The result arrives at the agreement that *D. aromatica* and *D. lanceolata* were close, and *D. lanceolata* different from other two species morphologically (ASHTON, 1964).

Table 3. Estimated allele frequencies at *Lap* in *D. aromatica*, *D. lanceolata*, *D. rappa*

Species	Allele frequency				
	Lap^a	Lap^b	Lap^c	Lap^d	Lap^e
D. aromatica	0.02	0.67	0.10	0.21	0.00
D. lanceolata	0.09	0.58	0.06	0.25	0.02
D. rappa	0.00	0.02	0.00	0.75	0.23

Table 4. Pair wise genetic distance measurements between species in *Dryobalanops*

Species	*D. aromatica*	*D. lanceolata*	*D. rappa*
D. aromatica	-	0.99	0.31
D. lanceolata	0.01	-	0.41
D. rappa	1.19	0.90	-

Above the diagonal ; genetic identity
Below the diagonal ; genetic distance

4 Genetical structure of seedlings in the *D. aromatica* population

Wildlings have been widely utilized in reforestation in Brunei Darussalam. There are, however the possibility of including a lot of self pollinating seedlings in them, and these seedlings may cause inbreeding depression in growth after planting.

Using the isozyme technique, the degree of inbreeding was investigated in *D. aromatica* based on the relationship between allele frequency and genotype frequency in *Lap* locus.

4.1 Material and methods

The permanent experimental plot (100m × 100m) at Compartment 7 (K7) of Andulau hill F. R. was selected as a study site. Twenty mature trees are distributed within the plot, and they produced a lot of seedlings under them.

Sixty seedlings were chosen at random, and their leaf blades were collected, and brought immediately to the Research Centre in Sungai Liang. Samples were analyzed according to the procedures mentioned in chapter 2.

4.2 Results and discussion

Three phenotypes (4, 6, 10 in Fig. 2) corresponding to Lap^b/Lap^b, Lap^b/Lap^d, Lap^d/Lap^d were observed. The frequency in each genotype is shown in Table 5. The frequency estimates of two allele (Lap^b and Lap^d) calculated from the observed genotype frequencies are 0.767 and 0.233, respectively.

Table 5. Genotype frequencies of seedlings in the *D. aromatica* forest (K7)

Genotype	observed value	Expected value	χ^2
Lap^b/Lap^b	36	35.3	
Lap^b/Lap^d	20	21.4	
Lap^d/Lap^d	4	3.3	
	60	60.0	0.28

In the population regenerated by random mating among parents, the relationship between allele frequencies and genotype frequencies follows the Hardy-Weinberg law, that is,

$(f_b Lap^b + f_d Lap^d)^2 = f_b^2(Lap^b/Lap^b) + 2f_b f_d(Lap^b/Lap^d) + f_d^2(Lap^d/Lap^d)$,

where, f_b, f_d are the frequencies of Lap^b, Lap^d alleles.

The expected frequencies calculated according to the above formula were fit well to the observed value. Since insects are the agents of pollination in *Dryobalanops*, it is assumed that high self pollination occurs in general. The result, however, indicates that most of the surviving seedlings have originated from the seeds produced by random mating.

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Dryobalanops 属数種の集団遺伝学的研究

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摘　　要

ロイシンアミノペプチダーゼ(*Lap*)・アイソザイムの*Lap*遺伝子を遺伝的指標として用い，ブルネイ国熱帯降雨林の主要樹種であるフタバガキ科（*Dipterocarpaceae*）*Dryobalanops*属の数種について，遺伝的変異，種間の類縁関係，交配様式について調査した。

まず，*Dryobalanops*属のなかで最も広く分布する*D. aromatica*（カプールプリンギ）について，ブルネイ全土から3か所の集団を選び，各集団間の対立遺伝子組成を調査した。その結果，ブルネイ本土の2集団は似た遺伝子組成を示したが，この2集団とテンブロン（Temburong）地域の1集団間では差異が認められた。

さらに，*Dryobalanops*属の*D. aromatica*, *D. lanceolata*（カプールパジ），*D. rappa*（カプールパヤ）の3樹種について同様に対立遺伝子組成を調べた結果，*D. aromatica*と*D. lanceolata*の遺伝子組成は類似していたのに対し，この2樹種と*D. rappa*間では大きな差異が認められた。この結果は，これまでの形態分類学的知見と一致した。

また，*D. aromatica*の天然更新稚樹集団を対象として遺伝子型頻度と対立遺伝子頻度を調べた。その結果，両者間にハーディ・ワインベルク平衡が認められ，稚樹群を形成する個体の大多数は他殖種子に由来していることが示唆された。

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