GENETIC DIVERSITY OF *DRYOBALANOPS AROMATICA* GAERTN. F. (DIPTEROCARPACEAE) IN PENINSULAR MALAYSIA AND ITS PERTINENCE TO GENETIC CONSERVATION AND TREE IMPROVEMENT

Lee, S. L.,* K. C. Ang & M. Norwati

Forest Research Institute Malaysia, Kepong, 52109 Kuala Lumpur, Malaysia
*corresponding author

Received January 26, 2000; accepted July 25, 2000

ABSTRACT

The genetic diversity of *Dryobalanops aromatica* from Peninsular Malaysia was evaluated using allozyme markers based on ten natural and two planted populations. Genetic diversity parameters (average and effective number of alleles per polymorphic locus = 4.1 and 2.5 respectively, proportion of polymorphic loci = 77.1% and expected heterozygosity = 0.459) based on the ten natural populations, indicated that the species harbors higher levels of genetic diversity, if compared with others regionally distributed tropical long-lived tree species. High value of gene flow (6.69) and low value of population differentiation (0.036), implied of extensive gene flow in the past, among the ten natural populations. Cluster analysis of genetic distances among populations formed four distinctive genetic clusters, with populations from Terengganu and Pahang forming a common cluster, those from Johor splitting into two unique clusters and the only population from Selangor as the outlier. Cluster analysis also disclosed that the two planted populations were most closely related to Kanching, reasoning the population being the conceivable seed sources for the two planted populations. Genetically unique populations were identified by "jackknife" analysis. Four populations were identified to be genetically unique and were recommended for consideration for conservation purposes. With mean effective population size across the ten natural populations ranged from 21,264 to 2,126,381, effective breeding unit for the maintenance of current level of allozyme heterozygosity might be more than 700 ha.

Key words: *Dryobalanops aromatica*, Dipterocarpaceae, allozyme, genetic diversity, genetic conservation, tree improvement.

INTRODUCTION

Dryobalanops aromatica, locally known as Kapur, is an emergent canopy tree occurring in Sumatra, Riau Archipelago, Borneo and Peninsular Malaysia (SYMINGTON 1943). It occurs abundantly in the lowlands but does occur in the hills (up to 365 m altitude). In Peninsular Malaysia, *D. aromatica* is comparatively restricted in distribution. It is found mainly in two large areas on the east coast as long belts just inside the beach area: one area extends from the Pahang–Terengganu border in Baloh Forest Reserve northwards as far as the Sungai Marang in Terengganu and other area from just north of the Sungai Rompin in south Pahang to Panti Forest Reserve in south central Johor (WYATT-SMITH 1964). The distribution of Kapur within these areas is however discontinuous. A further small pocket is found at Kanching, near Rawang, on the west coast of the Peninsular. In gregarious stands, it may make up to 90 percent of the total volume of timber (FOXWORTHY 1927). The tree is easily distinguished by the purple brown, scaly bole, the aromatic cut, and aromatic-small ovate leaves. It flowers synchronously and has small white hermaphrodite flowers. In Peninsular Malaysia, the reported floral visitors are honey bees, *Apis dorsata* and *A. indica* var. *cerrana* (APPANAH 1981, ASHTON 1988). In natural and undisturbed forests, it is reported to be predominantly outcrossed (LEE 2000). It is known that in general, good seed years only occur once in two to five years (WYATT-SMITH 1964). The evidence for any climatic stimulus to flowering is indecisive, but there is an indication that fruiting is associated with dry weather of at least a month, some six-months before fruiting (SMITH 1957). The fruits have an ovate nut, large wing (about 5 cm long) and are dispersed by gravity, where most of the fruits fall under the crown of the mother tree. The seeds germinate immediately after falling, or even while still attached to the tree during wet weather. The timber is a medium hardwood (BARNARD 1954) and is moderately durable in tropical conditions. It is suitable for heavy construction, posts, beams, joints and railway sleepers.

Survival of a species in the long term depends on the sustenance of adequate genetic variability within and among populations to accommodate new selection pressures brought about by environmental changes (LEDIG 1988). Populations of a species may have different degrees of genetic variation according to their

breeding systems and life history traits (*e.g.*, HAMRICK *et al.* 1979, LOVELESS & HAMRICK 1984, HAMRICK & GODT 1989, LOVELESS 1992). In terms of management, high heterozygosity suggests that populations of each species may carry substantial genetic load. An alleviation of population size in those species could result in increased homozygosity and lower reproductive output. In recent years, our knowledge about genetic and reproductive modes has increased greatly, especially in the case of lowland tropical rain forest trees (BAWA *et al.* 1985a, b; HAMRICK & LOVELESS 1986; ASHTON 1988; HAMRICK & MURAWSKI 1991). However, this information is not being used as part of a management strategy and conservation program directed towards a particular ecosystem or a species. Rather, the goal of these studies is to gather basic data to explicate ecological and evolutionary processes in the tropics.

In this study, we employed allozyme electrophoresis to describe the genetic structure of *D. aromatica* in Peninsular Malaysia. Although *D. aromatica* can be found abundantly in Peninsular Malaysia, but with continued exploitation for its timber and conversion of forests to other land uses, it is unlikely for the species to remain abundant indefinitely and populations that are unique and adapted to specific environments can easily be lost, unless a proper conservation strategy is developed and practiced. Moreover, current selective logging practices where trees with good form and straight bole are preferable can easily cause dysgenic effects, resulting in the loss of the best genotypes. Hence, this study will assumes the task to: (1) determine the amount of genetic diversity displayed by *D. aromatica* in Peninsular Malaysia and describe how genetic diversity is distributed within and among populations, (2) estimate the extent of gene flow and genetic relationship among populations, and (3) identify unique populations and estimate effective population size for genetic conservation and forest management practices.

MATERIALS AND METHODS

Sampling sites

Ten natural populations and two planted populations of *D. aromatica* distributed throughout Peninsular Malaysia were used for this study. The locations and names of the sampled populations are presented in Table 1. In the Terengganu populations, *D. aromatica* is found on free draining, deep sandy loams of low fertility, apparently avoiding the heavier granite-derived soils (WYATT-SMITH 1964, reference here in). In the Johor populations, it is found on similar light soils and less commonly on heavier granite derived Rengam series soils. In the Kanching Forest Reserve, where the true status is uncertain (introduced or natural), it flourishes on a variety of soils ranging from sandy silts derived from decomposed vein-quartz, through light loams over shales, to heavy clay loams over granite. In plantation at FRIM and Tampin, it is growing healthily on granite-derived heavy clay loams (Rengam series). A transect-line sampling method as described by LEE *et al.* (2000) was utilized as a guide for the sampling activities. A total of 823 sapling samples were collected and the number of samples per population ranged between 48

Table 1. Locations and sample sizes of ten natural and two planted populations of *D. aromatica* from Peninsular Malaysia included in the study.

Population	Code	Sample size	Location	Latitude	Longitude
Natural					
Bukit Bauk	BUBA	70	Terengganu	4° 46′N	103° 20′E
Rasau Kerteh	RAKE	70	Terengganu	4° 30′N	103° 19′E
Bukit Sai	BUSA	70	Terengganu	4° 15′N	103° 20′E
Bukit jemalang	BUJE	62	Terengganu	4° 12′N	103° 20′E
Kanching*	KANC	73	Selangor	3° 18′N	103° 37′E
Lesong	LESO	70	Pahang	2° 42′N	103° 09′E
Gunong Arong	GUAR	48	Johor	2° 33′N	103° 47′E
Mersing	MERS	75	Johor	2° 21′N	103° 47′E
Lenggor	LGOR	69	Johor	2° 11′N	103° 41′E
Ulu Sedili	ULUS	56	Johor	2° 10′N	103° 45′E
Planted					
FRIM	FRIM	80	Selangor	3° 17′N	103° 38′E
Tampin	TAMP	80	N. Sembilan	2° 28′N	102° 15′E

* The true status is uncertain (introduced or natural). In this study, it was assumed as natural population.

to 80 (averaging 69). The collected samples were wrapped with tissue paper, moistened and maintained at low temperature (4°C) before being brought back to the laboratory for enzyme extraction.

Enzyme extraction and allozyme analyses

Inner bark tissues were homogenized in 200 ml extraction buffer, consists of 50 mM borate buffer (pH 8.0), 1% PVP-40, 2% BSA, 10 mM ascorbic acid, 6 mM DTT, 20 mM $Na_2S_2O_5$, 0.1% b-mercaptoethanol, 0.05 M DIECA, 0.5 M sucrose, 1% tween-80, 1% 20 M PEG, 0.5% 2-phenoxyethanol, 1% tergitol, 0.2% $MgCl_2$, 0.2% $CaCl_2$ and 5 mM EDTA. Electrophoresis was performed using horizontal starch gel. While no controlled crosses were performed, segregation patterns of polymorphic loci were confirmed from unpublished analyses of open pollinated progeny arrays LEE (2000). Eleven allozyme systems were selected for high enzymatic activity. Malate dehydrogenase (MDH), isocitrate dehy-drogenase (IDH), phosphogluconate dehydrogenase (PGD) and hexokinase (HEX) staining zones appeared polymorphic but were omitted for further discussion due to tight banding patterns and inconsistent dosage effects. The remaining allozyme systems were assayed on two gel and electrode buffer systems. Alcohol dehydrogenase (ADH) and leucine aminopeptidase (LAP) were resolved on a lithium borate buffer (ASHTON & BRADEN 1961); glucose phosphate isomerase (GPI), malic enzyme (ME), phosphoglucomutase (PGM), shikimate dehydrogenase (SDH) and uridine diphosphogluconate pyrophosphatase (UGP) were resolved on a morpholine citrate buffer system at pH 6.1 (CLAYTON & TRETIAK 1972). For enzyme systems with either more than one zone of activity or in zones of activity with more than one allozyme, the zones/loci were designated numerically (beginning with 1) and alleles were designated alphabetically (beginning with A), both in decreasing order of relative mobility.

Data analysis

Levels of genetic diversity were described in terms of total number of alleles (including polymorphic and monomorphic loci, A_T), average number of alleles per polymorphic locus (A_a), effective number of alleles per polymorphic locus (A_e) following the method of CROW and KIMURA (1970), proportion of polymorphic loci (95% criteria, P), observed heterozygosity (H_o) and NEI's (1978) expected heterozygosity (H_e). Total genetic diversity (H_T) at polymorphic loci, distribution of genetic diversity within population (H_S) and among populations ($D_{ST} = H_T - H_S$) together with coefficient of population differentiation ($G_{ST} = D_{ST} / H_T$) were examined using the G-statistic of NEI (1987) based on the ten natural populations. An indirect estimate of gene flow was calculated as $Nm = (1 - F_{ST}) / 4F_{ST}$ (WRIGHT 1931, SLATKIN 1987), where F_{ST} was considered equivalent to G_{ST} and Nm estimates the number of migrants per generation. To asses the relatedness among the populations investigated, NEI's (1978) genetic distance (D) was calculated for each pairwise combination of populations and cluster analysis *via* the unweighted pairwise groups with arithmetic averaging (UPGMA, SNEATH & SOKAL 1973). All of these parameters except the A_e and Nm were calculated with the assistance of BIOSYS–1 (SWOFFORD & SELANDER 1981) and FSTAT (GOUDET 2000) computer programs. To determine the effective population size (N_e) needed to maintain current level of allozyme heterozygosity, the heterozygosity formula of CROW and KIMURA (1970) for neutral alleles was calculated. It was estimated based on the theory that for a population under selective neutrality, heterozygosity at equilibrium is a function of N_e and the neutral mutation rate. The general relationship is $N_e = H_e / [4\mu\ (1 - H_e)]$, where H_e is the expected heterozygosity and μ is the neutral mutation rate. The genetic uniqueness of a specific population (a_i) was determined using "jackknife" analysis (SLATKIN 1985, JAQUISH & EL-KASSABY 1998) as follows: (1) removing population a_i data from the original data-set (*i.e.*, $-a_i$), (2) estimating the average D, G_{ST} and Nm for the new data-set and (3) comparing the estimates obtained from the original analysis to that of the ten new data-sets (*i.e.*, $-a_1, -a_2, \ldots\ldots, -a_{10}$).

RESULTS

Six out of the seven loci investigated were polymorphic in all the populations. A total of 32 alleles were observed at the six polymorphic loci (Table 2). The most frequent allele for some loci varied among populations, *e.g.*, *Pgm*, *Ugp–1* and *Me*. RAKE was the only population that harbored a private allele H at *Pgm* (Table 2). At the species level, the total number of alleles (A_T) and the average number of alleles per polymorphic locus (A_a) were 33 and 5.3 respectively. At the population level, the A_T ranged between 21 (KANC) and 29 (RAKE), with an average of 26 alleles (Table 3). Subsequently, the A_a was lowest in KANC (3.3) and highest in RAKE (4.7). The effective number of alleles per polymorphic locus (A_e) provides a measure of allelic evenness and is invariant to the average number of alleles only when all alleles are present in equal frequencies. The effective number of alleles per polymorphic locus varied from 2.3 (GUAR) to 2.7 (ULUS and RAKE), with a mean of 2.5 across populations. In general, the values of A_e were much lower than A_a in all the populations, indicating the existence of many rare alleles, with only one or two alleles predominating in

Table 2. Allele frequencies for polymorphic loci in ten natural and two planted populations (FRIM and TAMP) of *D. aromatica*.

Locus	Allele	BUBA	RAKE	BUSA	BIKE	KANC	LESO	GUAR	MERS	LGOR	ULUS	FRIM	TAMP
Gpi-1	A	0.008	0.000	0.000	0.000	0.243	0.058	0.000	0.000	0.047	0.000	0.200	0.262
	B	0.550	0.418	0.368	0.302	0.382	0.449	0.615	0.545	0.406	0.480	0.438	0.195
	C	0.017	0.030	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000
	D	0.008	0.097	0.000	0.069	0.000	0.072	0.064	0.037	0.063	0.049	0.025	0.000
	E	0.000	0.015	0.018	0.000	0.000	0.007	0.000	0.015	0.000	0.010	0.000	0.000
	F	0.217	0.201	0.307	0.259	0.208	0.203	0.077	0.254	0.281	0.206	0.069	0.293
	G	0.058	0.037	0.079	0.086	0.090	0.051	0.141	0.037	0.070	0.059	0.144	0.006
	H	0.033	0.007	0.079	0.000	0.021	0.014	0.000	0.007	0.023	0.039	0.013	0.000
	I	0.108	0.179	0.105	0.241	0.056	0.145	0.103	0.104	0.109	0.127	0.081	0.244
	J	0.000	0.015	0.044	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000
Pgm	A	0.008	0.069	0.047	0.000	0.000	0.015	0.088	0.041	0.000	0.029	0.000	0.000
	B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.039	0.000	0.000
	C	0.373	0.315	0.172	0.380	0.493	0.381	0.176	0.616	0.553	0.245	0.481	0.419
	D	0.016	0.092	0.070	0.190	0.063	0.045	0.074	0.103	0.083	0.167	0.162	0.175
	E	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.049	0.000	0.000
	F	0.389	0.300	0.570	0.340	0.174	0.448	0.574	0.185	0.303	0.382	0.136	0.150
	G	0.214	0.192	0.141	0.090	0.271	0.112	0.088	0.048	0.053	0.088	0.221	0.256
	H	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ugp-1	A	0.047	0.037	0.007	0.000	0.000	0.000	0.000	0.146	0.032	0.077	0.006	0.000
	B	0.195	0.209	0.232	0.229	0.507	0.221	0.474	0.201	0.230	0.317	0.513	0.375
	C	0.477	0.552	0.457	0.551	0.486	0.441	0.382	0.194	0.341	0.337	0.462	0.625
	D	0.141	0.142	0.239	0.093	0.007	0.213	0.066	0.313	0.294	0.212	0.019	0.000
	F	0.063	0.060	0.058	0.085	0.000	0.103	0.079	0.139	0.087	0.058	0.000	0.000
	G	0.078	0.000	0.007	0.042	0.000	0.022	0.000	0.007	0.016	0.000	0.000	0.000
Lap	A	0.467	0.613	0.688	0.602	0.556	0.745	0.543	0.592	0.585	0.588	0.660	0.512
	B	0.533	0.387	0.312	0.398	0.444	0.255	0.457	0.408	0.415	0.412	0.340	0.488
Me	A	0.420	0.391	0.476	0.519	0.545	0.451	0.447	0.554	0.320	0.528	0.600	0.568
	B	0.339	0.430	0.405	0.352	0.194	0.287	0.263	0.185	0.234	0.104	0.158	0.136
	C	0.241	0.180	0.119	0.130	0.261	0.262	0.289	0.262	0.445	0.368	0.242	0.296
Sdh	A	0.000	0.000	0.008	0.008	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.012
	B	0.993	0.985	0.929	0.984	0.884	0.935	0.962	0.993	0.941	0.991	0.969	0.988
	C	0.007	0.015	0.063	0.008	0.116	0.065	0.038	0.007	0.051	0.009	0.031	0.000

some populations. The genetic diversity of *D. aromatica* was relatively high. At the species level, the mean expected heterozygosity (H_e) was 0.477. At the population level, the H_e across the ten natural populations was 0.459, with GUAR showed the lowest (0.440) and LGOR the highest (0.475). The two planted populations (FRIM and TAMP) in general exhibited lower value of H_e (0.431 and 0.434 respectively), in comparisons with natural populations, although FRIM exhibited high levels of A_T (25) and A_a (3.8). The observed heterozygosity (H_o) within population ranged from 0.367 (GUAR) to 0.430 (KANC), with a mean of 0.398 (Table 3). Overall, H_o is generally lower than H_e across the ten natural populations, postulating an excess of homozygotes. Effective population size (N_e) estimates based on expected heterozygosity and two neutral mutation rates (μ), ranged from 19,643 (GUAR) to 22,619 (LGOR) with $\mu = 10^{-5}$ and subsequently 1,964, 286 to 2,261,905 with $\mu = 10^{-7}$ (Table 3). Mean N_e across the ten natural populations ranged from 21,264 to 2,126,381.

Total genetic diversity (H_T) and within population genetic diversity (H_S) generating overall loci averages of 0.555 and 0.535 respectively (Table 4, ALL–10). The mean proportion of total diversity among populations (G_{ST}) was 0.036, indicating that about 3.6 percent of the encountered genetic diversity was due to interpopulational gene frequency differences. Hence, majority of genetic diversity (96.4%) resided within population. The level of gene flow among the ten

Table 3. Summary of allozyme diversity and effective population size for ten natural and two planted populations of *D. aromatica* in Peninsular Malaysia. Values in parentheses are standard deviation.

Population	A_T	A_a	A_e	P	H_o	H_e	$Ne\ \mu = 10^{-5}$	$Ne\ \mu = 10^{-7}$
Natural								
BUBA	27	4.3 (1.0)	2.5	71.4	0.414 (0.111)	0.456(0.118)	20.956	2.095.588
RAKE	29	4.7 (1.2)	2.7	71.4	0.427 (0.116)	0.469 (0.123)	22.081	2.208.098
BUSA	27	4.3 (0.8)	2.5	85.7	0.426 (0.116)	0.460 (0.109)	21.296	2.129.630
BUJE	25	4.0 (0.7)	2.6	71.4	0.383 (0.104)	0.461 (0.120)	21.382	2.138.219
KANC	21	3.3 (0.6)	2.4	85.7	0.430 (0.102)	0.459 (0.100)	21.211	2.121.072
LESO	26	4.2 (0.9)	2.6	85.7	0.388 (0.099)	0.462 (0.113)	21.468	2.146.840
GUAR	22	3.5 (0.6)	2.3	71.4	0.367 (0.093)	0.440 (0.106)	19.643	1.964.286
MERS	27	4.3 (0.9)	2.5	71.4	0.388 (0.104)	0.441 (0.117)	19.723	1.972.272
LGOR	27	4.3 (0.8)	2.6	85.7	0.373 (0.099)	0.475 (0.114)	22.619	2.225.898
ULUS	28	4.5 (1.1)	2.7	71.4	0.388 (0.112)	0.471 (0.125)	22.259	2.126.381
Popul. mean	26	4.1	2.5	77.1	0.398	0.459	21.264	2.126.381
Species level	33	5.3(1.3)	2.7	83.3	0.400(0.103)	0.477(0.117)	–	–
Planted								
FRIM	25	3.8(0.9)	2.3	71.4	0.351(0.089)	0.431(0.110)	–	–
TAMP	19	3.0(0.5)	2.4	71.4	0.439(0.126)	0.434(0.115)	–	–

A_T = Total number of alleles (including polymorphic and monomorphic loci).
A_a = Average number of alleles per polymorphic locus.
A_e = Effective number of alleles per polymorphic locus.
P = Proportion of polymorphic loci (95% criterion).
H_o = Observed heterozygosity.
H_e = Expected heterozygosity.
Ne = Effective population size needed to maintain current level of allozyme heterozygosities. Estimates are calculated from Crow and Kimura's (1970) equilibrium heterozygosity formula for neutral alleles. Two mutation rates (μ) are given as a range. Values for species level and planted populations (FRIM and TAMP) were not calculated.

population. The level of gene flow among the ten natural populations was high (6.69 migrants per genera tion), indicating the presence of substantial gene flow among populations (Table 4, ALL–10).

Cluster analysis of genetic distance (*D*) among populations formed four distinctive genetic clusters, with populations from Terengganu (BUBA, BUJE, RAKE, and BUSA) and Pahang (LESO) forming a common cluster, those from Johor splitting into two unique clusters (ULUS–GUAR and MERS–LGOR respectively), and the only population from Selangor (KANC) as the outlier (Figure 1). Cluster analysis also disclosed that the two-planted populations were closely related to KANC, reasoning the population being the conceivable seed sources for the two planted populations (Figure 1).

The genetic uniqueness of each population was concluded by calculating the G_{ST} statistic, average genetic distance (*D*) and gene flow (*Nm*) for the remaining populations after the elimination of this specific population from the data. Low among populations genetic diversity (G_{ST}) values of 0.030, 0.030, 0.032, 0.033 were noticed for the analysis of –KANC, –MERS, –GUAR and –BUSA populations respectively (Table 4). These values were lower than that obtained from the analysis of the ten populations altogether (0.036), as well as for the remaining six analyses (Table 4). In a like manner, the genetic distance analysis produced low averages of *D* for the –KANC (0.034), –MERS (0.035), –GUAR (0.036) and –BUSA (0.037) populations as compared to the average of the ten populations (0.039) and the remaining six analyses (Table 4). The analyses of gene flow also produced results that resembled those obtained from the G_{ST} and *D*. While *Nm* for the ten populations was 6.69 individual/generation, estimates for –KANC, –MERS, –GUAR and –BUSA were 8.08, 8.08, 7.56 and 7.33 respectively (Table 4). This result may indicate that the inclusion of these four populations in the analysis resulted in a reduction of the actual estimate of gene flow.

Table 4. Genetic diversity statistics (NEI 1987), average genetic distance (*D*) and gene flow (*Nm*) in *D. aromatica* populations from Peninsular Malaysia after the removal of one population at a time. –BUBA represents an analysis that contained all populations after the removal of the BUBA population. ALL-10 represents an analysis of the ten natural populations.

Population	H_T	H_S	G_{ST}	*D*	*Nm*
– BUBA	0.557	0.537	0.036	0.0408	6.69
– RAKE	0.556	0.536	0.036	0.0407	6.69
– BUSA	0.555	0.537	0.033	0.0374	7.33
– BUJE	0.556	0.537	0.035	0.0398	6.89
– KANC	0.554	0.537	0.030	0.0344	8.08
– LESO	0.557	0.537	0.036	0.0413	6.69
– GUAR	0.557	0.539	0.032	0.0364	7.56
– MERS	0.556	0.539	0.030	0.0354	8.08
– LGOR	0.554	0.535	0.034	0.0386	7.10
– ULUS	0.555	0.535	0.036	0.0405	6.69
ALL-10	0.555	0.535	0.036	0.0385	6.69

H_T = Total genetic diversity.
H_S = Within population genetic diversity.
G_{ST} = Proportion of total diversity partitioned among populations

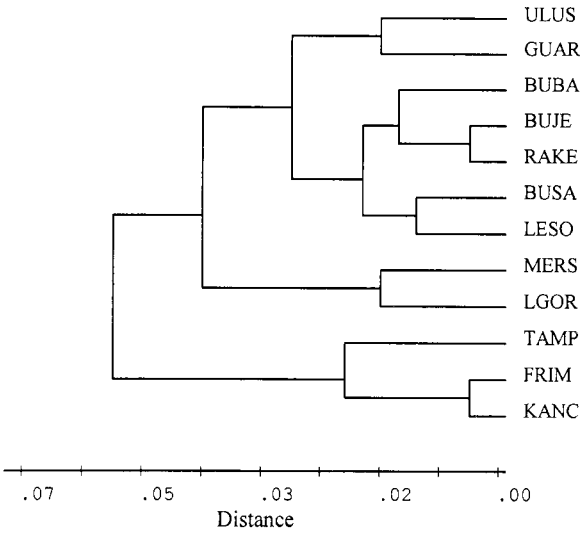

Figure 1. Dendrogram for UPGMA cluster analysis based on NEI's (1978) genetic distance between the ten natural and two planted populations of *D. aromatica* in Peninsular Malaysia.

DISCUSSION

This present study showed that *D. aromatica* from Peninsular Malaysia harbors higher levels of genetic diversity if compared with others regionally distributed tropical long-lived tree species (A_a = 1.51, A_e = 1.16, *P* = 39.8 and H_e = 0.125, HAMRICK *et al.* 1992). Allozyme variation of three natural populations of *D. aromatica* from Brunei has been reported by SHIRAISHI *et al.* (1994). They found out that *D. aromatica* maintained high level of genetic diversity (H_e = 0.481). However, estimation of their study was based on a single polymorphic locus, validity of the results obtained is skeptical and should be considered with due precautions. The studies by MURAWSKI and BAWA (1994) and LEE *et al.* (2000) on the genus *Stemonoporus* and *Shorea* respectively, also from the same family (Dipterocarpaceae), provide some basis for comparisons. MURAWSKI and BAWA (1994), in their study of an endemic Sri Lankan dipterocarp (based on four natural populations and nine allozyme loci), reported that *Stemonoporus oblongifolius* maintained high level of within population genetic diversity (A_e = 1.67, *P* = 91.7 and H_e = 0.282). The study of LEE *et al.* (2000) based on eight natural populations distributed throughout Malaysia and nine allozyme loci also encountered exceptionally high level of genetic diversity on *Shorea leprosula* (A_a = 2.6, A_e = 1.79 and H_e = 0.369). Ultimately, levels of genetic diversity observed on *D. aromatica* in this study were the highest among the tropical tree species that have been reported (HAMRICK & LOVELESS 1986; HAMRICK & MURAWSKI 1991; LOVELESS 1992; HALL *et al.* 1994a, b, 1996; SHEELY & MEAGHER 1996; DOLIGEZ & JOLY 1997). High values of genetic diversity can be attributed to the species' evolutionary history and life-history traits such as predominantly outcrossed, long-life span, regional geographical range and high fecundities. It might also be affected to some degree by the low number of loci used in this study, although there was no bias in the selection of monomorphic and polymorphic loci.

The relatively low G_{ST} value (3.6%) obtained is indicative of extensive gene flow among the ten natural populations studied. This is slightly lower than the result reported by LEE *et al.* (2000) on *Shorea leprosula* (8.5%), and much lower that the result reported by MURAWSKI and BAWA (1994) on *Stemonoporus oblongifolius* (16.3%), which was based on four populations separated by maximum distance of only 10 km. However, low values of G_{ST}, comparable to the present study have been reported for other tropical trees (PÉREZ-NASSER *et al.* 1993, ALVAREZ-BUYLLA & GARAY 1994, CHASE *et al.* 1995). High gene flow is known to lessen the level of genetic differentiation among populations (GREGORIUS & NAMKOONG 1983, NAMKOONG & GREGORIUS 1985). Estimates of *Nm* less than one indicate relatively little gene flow and one or greater suggest high levels of gene flow (SLATKIN 1985, SLATKIN & BARTON 1989). Indirect estimates of *Nm* represent historical average levels of gene flow and may not represent present day levels (LOVELESS 1992).

Thus, the indirect estimate of *Nm* of 6.69 indicates the presence of substantial gene flow among populations in the past, which might responsible for the maintenance of low genetic differentiation among populations in *D. aromatica*.

One of the major sampling considerations for conservation of abundant species such as *D. aromatica* is geographical placement of units. For highly outcrossing species with low genetic differentiation among populations, large reserves would better maintain the important component of within population variability. If a few of these were strategically placed on the basis of patterns of genetic variation, much of the variability among populations could also be captured (MILLAR & LIBBY 1991). The low G_{ST} and *D* and high *Nm* estimates obtained for –KANC, –MERS, –GUAR and –BUSA analyses testify greater genetic similarity among the remaining populations. Grounded on the ten analyses conducted (Table 4), it could be concluded that KANC, MERS, GUAR and BUSA withhold some unique genetic temperament. The clustering of the populations in the dendrogram is also supportive of this conclusion (Figure 1). The KANC, MERS, GUAR and BUSA were respectively as the representative population for the four main clusters. Hence, at least for the allozyme systems considered, these four unique populations should secure additional attention for conservation purposes.

The other major question for designing new units in the genetic management system is, how big should individual areas be? The answer depends on whether the goal is to conserve a population size that retains the current levels of diversity or to let the population decrease to some permissible minimum number of individuals that hopefully will still warrant long-term survival of the population. The genetic factors influencing within-stand dynamics including inbreeding, genetic drift, immigration and selection and are influenced by the reproductive system of the species. For many outbreeding species, where genetic diversity within populations is generally high, inbreeding caused by reduced population size can produce a severe decline in health and vigor (MILLAR & LIBBY 1991). These species will need relatively large populations to oppose the long-term decline of heterozygosity as related individuals mate. Formula for calculating effective population sizes required to maintain heterozygosity have been developed for neutral alleles (CROW & KIMURA 1970), and these have been applied to allozyme data. Calculation of this size depends on the relation of effective population size to mutation rate and current levels of heterozygosity. Based on allozyme data for *D. aromatica*, the mean effective population sizes estimated ranged from 21,264 to 2,126,381. By using the similar approach and same values of mutation rates, MILLAR unpublished data quoted in MILLAR and LIBBY (1991) reported that the effective population sizes for Ponderosa pine and Douglas fir ranged from 2,230 to 910,640. From the enumeration surveys data of the big trees in Kapur forest by WYATT-SMITH (1964), the mean density of *D. aromatica* tree (more than 25 cm diameter at breast height) in Peninsular Malaysia was simplified into approximately 30 trees per ha. Applying this rough estimate to the mean effective population size might suggest that for the maintaining of current level of allozyme heterozygosity, the effective breeding unit for the species might be more than 700 ha.

Dryobalanops aromatica is not backed by any genetic improvement program in Peninsular Malaysia. The present results showed that *D. aromatica* in Peninsular Malaysia maintained a high level of genetic diversity. As the species produced valuable timber, disruption of some of these existing populations in future is unavoidable. The high levels of genetic diversity promise a 'massive gene pool" for selections and manipulations. Thus, it is still not too late for tree breeders to initiate a tree improvement program that can further improve the species for forest plantation. As all the present planting programs are relying on natural-stand seed collections, the information on mating system as reported by LEE (2000) and genetic uniqueness of some of its populations in the present study, could be used to instruct the attempt in establishing several seed production areas. In use, some of the seed production areas should be established in the four genetically unique populations. This approach will grant an opportunity to affiliate conservation and utilization efforts in a constructive way. In addition, the establishment of seed production areas in genetically unique populations will safeguard their elimination from short-term harvesting plans.

The information generated by the present study was solely based on genetic markers. Further study should be carried out to supplement the information with the traditional efforts that rely upon the use of adaptive and quantitative attributes in understanding the extent and nature of the genetic variation. Common-garden tests on inheritance of morphological and ecophysiological traits although are slow to yield information, and usually do not allow actual genotypic data to be estimated, they do, however, provide information about traits that more directly related to adaptation.

ACKNOWLEDGEMENTS

We wish to acknowledge the Forest Department of Peninsular Malaysia for granting permission to access the forest reserves. Mariam Din, Sharifah Talib and Ghazali Jaafar provided assistance with the lab work.

REFERENCES

ALVAREZ-BUYLLA, E. R. & GARAY, A. A. 1994: Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer tree species. *Evolution* **48**:437–453.

APPANAH, S. 1981: Pollination in Malaysia primary forests. *Malays. For.* **44**:37–42.

ASHTON, G. C. & BRADEN, A. W. H. 1961: Serum b-globulin polymorphism in mice. *Austral. J. Biol. Sci.* **14**:248 –254.

ASHTON, P. S. 1988: Dipterocarp biology as a window to the understanding of tropical forest structure. *Annu. Rev. Ecol. Syst.* **19**:347 –370.

BARNARD, R. C. 1954: A Manual of Malayan Silviculture for Inland Lowland Forest. Research Pamphlet No. 14. Forest Research Institute Kepong, Kuala Lumpur.

BAWA, K. S., PARRY, D. R. & BEACH, J. H. 1985a: Reproductive biology of tropical lowland rain forest trees: I. Sexual systems and incompatibility mechanisms. *Am. J. Bot.* **72**:331–345.

BAWA, K. S., BULLOCK, S. H., PERRY, D. R., COVILLE, R. E. & GRAYUM, M. H. 1985b: Reproductive biology of tropical lowland rain forest trees: II. Pollination systems. *Am. J. Bot.* **72**:346–356.

CHASE, M. R., BOSHIER, D. H. & BAWA, K. S. 1995: Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree. 1. Genetic variation in natural populations. *Am. J. Bot.* **82**:468–475.

CLAYTON, J. W. & TRETIAK, D. N. 1972: Amine-citrate buffers for pH control in starch gel electrophoresis. *J. Fish. Res. Board Can.* **29**:1169–1172.

CROW, J. F. & KIMURA, M. 1970: An Introduction to Population Genetics Theory. Harper & Row Publishers, New York.

Doligez, A. & Joly, H. I. 1997: Mating systems of *Carapa procera* (Meliaceae) in the French Guiana tropical forest. *Am. J. Bot.* **84**:461–470.

FOXWORTHY, F. W. 1927: Commercial Timber Trees of the Malay Peninsula. Mal. For. Rec. No.3. Forest Research Institute Kepong, Kuala Lumpur.

GREGORIUS, H.–R & NAMKOONG, G. 1983: Conditions for protective polymorphicm in subdivided plant populations. *Theor. Popul. Biol.* **24**:253 –267.

GOUDET, J. 2000: FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.1). Available from http://www.unil.ch/izea/softwares/ fstat.html. Updated from J. GOUDET (1995). FSTAT (vers. 1.2): a computer program to calculate F-statistics. *J. Hered.* **86**:485–486.

HALL, P., CHASE, M. R. & BAWA, K. S. 1994a: Low genetic variation but high population differentiation in a common tropical forest tree species. *Conserv. Biol.* **8**:471 –482.

HALL, P., ORRELL, L. C. & BAWA, K. S. 1994b: Genetic diversity and mating system in a tropical tree, *Carapa guianensis* (Meliaceae). *Am. J. Bot.* **81**:1104–1111.

HALL, P., WALKER, S. & BAWA, K. S. 1996: Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. *Conserv. Biol.* **10**: 757–768.

HAMRICK, J. L. & GODT, M. J. W. 1989: Allozyme diversity in plant species. *In*: Plant Population Genetics, Breeding and Genetic Resources. (eds. A. H. D. Brown, M. J. Clegg, A. L. Kahler and B. S Weir). pp. 43–63. Sinauer Associate, Sunderland, Massachusetts.

HAMRICK, J. L. & LOVELESS, M. D. 1986: Isozyme variation in tropical trees: procedures and preliminary results. *Biotropica* **18**:201–207.

HAMRICK, J. L. & MURAWSKI, D. A. 1991: Levels of allozyme diversity in populations of uncommon neotropical tree species. *J. Trop. Ecol.* **7**:395 –399.

HAMRICK, J. L., GODT, M. J. W. & SHERMAN-BROYLES, S. L. 1992: Factor influencing levels of genetic diversity in woody plant species. *New For.* **6**:95–124.

HAMRICK, J. L., LINHART, Y. B. & MITTON, J. B. 1979: Relationship between life history characteristic and electrophoretically detectable genetic variation in plants. *Annu. Rev. Ecol. Syst.* **10**:173–200.

JAQUISH, B. & EL-KASSABY, Y. A. 1998: Genetic variation of western larch in British Columbia and its conservation. *J. Hered.* **89**:248–253.

LEDIG, F. T. 1988: The conservation of diversity in forest trees. Why and how should gene be conserved? *Bioscience* **38**:471–479.

LEE, S. L. 2000: Mating system parameters of *Dryobalanops aromatica* Gaertn. F. (Dipterocarpaceae) in three different forest types and a seed orchard. *Heredity* (in press).

LEE, S. L., WICKNESWARI, R., MAHANI, M. C. & ZAKRI, A. H. 2000: Genetic diversity of *Shorea leprosula* Miq. (Dipterocarpaceae) in Malaysia: Implications for conservation of genetic resources and tree improvement. *Biotropica* **32**:213–224.

LOVELESS, M. D. 1992: Isozyme variation in tropical trees: patterns of genetic organization. *New For.* **6**:67–94.

LOVELESS, M. D. & HAMRICK, J. L. 1984: Ecological determinants of genetic structure in plant populations. *Annu. Rev. Ecol. Syst.* **15**:65–95.

MILLAR, C. I. & LIBBY, W. J. 1991: Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. *In*: Genetics and Conservation of Rare Plants. (eds. D. A. Falk and K. E. Holsinger). pp. 149 –170. Oxford University Press, Oxford.

MURAWSKI, D. A. & BAWA, K. S. 1994: Genetic structure and mating system of *Stemonoporus oblongifolius* (Dipterocarpaceae) in Sri Lanka. *Am. J. Bot.* **81**:155–160.

NAMKOONG, G & GREGORIUS, H.–R. 1985: Conditions for protective polymorphism in subdivided plant populations. 2. Seed versus pollen migration. *Am. Nat.* **125**: 521–534.

NEI, M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**:583–590.

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

PÉREZ-NASSER, N., EGUIARTE, L. E. & PIÑERO, D. 1993: Mating system and genetic structure of the distylous tropical tree *Psychotria faxlucens* (Rubiaceae). *Am. J. Bot.* **80**:45–52.

SHEELY, D. L. & MEAGHER, T. R. 1996: Genetic diversity in Micronesian island populations of the tropical tree *Campnosperma brevipetiolata* (Anacardiaceae). *Am. J. Bot.* **83**: 1571–1579.

SHIRAISHI, S., LATIFF, N. H., MIYATAKE, S., OCHIAI, Y. & FURUKOSHI, T. 1994: Population genetical studies on *Dryobalanops* (Kapur) species. *Bull. For. & For. Prod.*

Res. Inst. **366**:107–114.

SLATKIN, M. 1985: Rare alleles as indicators of gene flow. *Evolution* **39**:53–65.

SLATKIN, M. 1987: Gene flow and the geographic structure of natural populations. *Science* **236**:787–792.

SLATKIN, M. & BARTON, N. H. 1989: A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**:1349–1368.

SMITH, J. S. 1957: Report on the Forest Administration. Forest Department, Federation of Malaya, pg. 13.

SNEATH, P. H. A. & SOKAL, R. R. 1973: Numerical Taxonomy. W. H. Freeman Press, San Francisco.

SWOFFORD, D. L. & SELANDER, R. B. 1981: BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered* **72**:281–283.

SYMINGTON, C. F. 1943: Forester's Manual of Dipterocarps. Mal. For. Rec. No. 16. Forest Research Institute Kepong, Kuala Lumpur (Reprinted with plates and historical introduction, University of Malaya Press, Kuala Lumpur [1994]).

WRIGHT, S. 1931: Evolution in Mendelian populations. *Genetics* **16**: 97–159.

WYATT-SMITH, J. 1964: Manual of Malayan Silviculture for Inland Forests. Mal. For. Rec. No.23. Forest Research Institute Kepong, Kuala Lumpur.