American Journal of Botany 89(3): 447–459. 2002.

# POPULATION GENETICS OF *INTSIA PALEMBANICA* (LEGUMINOSAE) AND GENETIC CONSERVATION OF VIRGIN JUNGLE RESERVES IN PENINSULAR MALAYSIA[1]

SOON-LEONG LEE,[2] KEVIN K.-S. NG, LENG-GUAN SAW, ADNAN NORWATI, M. H. SITI SALWANA, CHAI-TING LEE, AND MUHAMMAD NORWATI

Forest Research Institute Malaysia, Kepong, 52109 Kuala Lumpur, Malaysia

A field survey of Virgin Jungle Reserve (VJR) compartments in Peninsular Malaysia allowed us to identify six populations of *Intsia palembanica* for this study. These were Pasoh Forest Reserve (FR) (Pasoh), Sungai Lalang FR (Lalang), Bukit Lagong FR (Lagong), Bubu FR (Bubu), Bukit Kinta FR (Kinta), and Bukit Perangin FR (Perangin). About 40 adult individuals were sampled in each population. In addition, progeny arrays were collected from nine mother plants at Lagong for a mating system study. A total of nine allozymes, encoded by 14 putative gene loci, were consistently resolved in *I. palembanica*. The mating system study showed that the species exhibited a mixed-mating system, with multilocus outcrossing rate of 0.766. The levels of diversity were comparably high (mean number of alleles per polymorphic locus = 2.4, mean effective number of alleles per polymorphic locus = 1.64, and mean expected heterozygosity ($H_e$) = 0.242), and the majority of the diversity was partitioned within population ($G_{ST}$ = 0.040 and $F_{ST}$ = 0.048). Significant levels of inbreeding were detected in Bubu and Perangin. Probability tests of recent effective population size reduction using the Infinite Allele Model showed the occurrence of genetic bottlenecks on Lalang and Kinta. Two genetically unique populations (Pasoh and Perangin) were inferred using jackknife analysis. By using the neutral mutation rates, effective population size ($N_e$) to maintain the $H_e$ was 80–800 000 individuals. A simulation study based on pooled samples, however, circumscribed the $N_e$ to 200 and 210 individuals. Implications of the study for managing the species and the VJRs are discussed.

**Key words:** allozyme diversity; effective population size; *Intsia palembanica*; Leguminosae; mating system; population genetic structure; tropical tree species; Virgin Jungle Reserves.

Genetically sound conservation requires a robust understanding of the processes by which species display genetic variation in local populations and the patterns of this variation among populations throughout the ranges of these species (Falk and Holsinger, 1991; Hamrick and Godt, 1996a). The levels of genetic diversity reflect the genetic resources necessary for short-term ecological adaptation and for long-term evolutionary change; species must have available a pool of genetic diversity if they are to survive environmental pressures exceeding the limits of developmental plasticity (Lande and Shannon, 1996). Because environmental changes are unpredictable, it is critical that sufficient genetic diversity be secured to permit the species to continuously evolve in response to environmental changes (Müller-Starck and Gregorius, 1986).

Range-wide survey of genetic diversity, based on genetic markers, have now been performed on thousands of species of plants, including some of the tropical tree species (e.g., Pérez-Nasser, Eguiarte, and Piñero, 1993; Alvarez-Buylla and Garay, 1994; Chase, Boshier, and Bawa, 1995; Lee, Ang, and Norwati, 2000; Lee et al., 2000a). One of the goals of conservation genetics is to use this information to develop conservation policies for given species and in particular to identify areas for in situ conservation (Riggs, 1990; Millar and Libby, 1991). A similar problem has been discussed at length in the ecological literature that identifies areas deserving conservation priority on the basis of maximum species richness (e.g., Van-Wright, Humphries, and William, 1991).

Recent reviews on plant allozymes have shown that abundant and widely distributed plants tend to contain more allozyme variation than species with a narrow distribution (Hamrick and Godt, 1989; Hamrick, Godt, and Sherman-Broyles, 1992). However, several studies reported that geographical range is not always a good predictor of the genetic structure of species (Lewis and Crawford, 1995; Young and Brown, 1996; Gitzendanner and Soltis, 2000; Mateu-Andrés and Segarra-Moragues, 2000). The objective for conservation of genetic diversity within a widespread species differs from that of a rare species. For rare species, the final race against extinction is being fought. In contrast, for widespread species, where species existence is not threatened, it is the long-term evolutionary fitness of the species that is being protected. There are two major sampling considerations for conservation of widespread species; geographical placement of units and size of individual units (Millar and Libby, 1991). Many factors determine the final numbers, and these are categorized as genetic, demographic, environmental, and catastrophic (Shaffer, 1981).

In the zoological literatures, there have been many debates in the conservation community over the relative merits of a few large vs. many small conservation areas. The history of this debate has been reviewed by Simberloff and Abele (1982). Most of these arguments have stemmed from ecological considerations of island biogeography, in which the equilibrium theory dictates that single large refuges are generally preferable to groups of small ones. In the plant literatures, Hamrick

[1] Manuscript received 20 March 2001; revision accepted 7 September 2001.

The authors thank the Forest Department of Peninsular Malaysia for granting permission to access the Forest Reserves; Mariam Din, Sharifah Talib, Ghazali Jaafar, and Yahya Mahani for technical assistance; and Ayau Kanir, Ramli Ponyoh, Baya Busu, Angan Atan, Mustapa Data, and Mohd. Lan Musa for their excellent field assistance. This work was supported in part by the Export Levy Fund under the Project A179 QIZZ-Optimum size of Virgin Jungle Reserves and IRPA Grant 01-04-01-0133.

[2] Author for reprint requests (e-mail: leesl@frim.gov.my).

TABLE 1. Details of *Intsia palembanica* populations included in the study. Sample collections were carried out in Virgin Jungle Reserve (VJR) compartments (Pasoh, Bubu, Kinta, and Perangin) or compartments adjacent to VJR compartments (Lalang and Lagong) within a forest reserve (FR). Compartments are located in the center of an FR or on the edge of an FR adjoining farmland or plantations.

| Forest reserve | Population code | State | Compartment number | Size of compartment (ha) | Compartment altitude (m asl) | Location of compartment in FR | Size of FR (ha) |
|---|---|---|---|---|---|---|---|
| Pasoh | Pasoh | Negeri Sembilan | 51 | 108 | 152–534 | Central | 13 910 |
| Sungai Lalang | Lalang | Selangor | 24 | 82 | 152–429 | Edge | 17 722 |
| Bukit Lagong | Lagong | Selangor | 15 | 161 | 279–575 | Central | 4 499 |
| Bubu | Bubu | Perak | 6,7 | 567 | 213–960 | Edge | 38 095 |
| Bukit Kinta | Kinta | Perak | 161 | 123 | 122–533 | Edge | 68 566 |
| Bukit Perangin | Perangin | Kedah | 24 | 866 | 152–483 | Edge | 13 097 |

et al. (1991) stated that to capture 95% of the genetic diversity in a species with a degree of population differentiation of 0.6, six populations should be sampled, assuming that each population has equal levels of genetic diversity. To preserve genotype frequencies at their natural levels and to insure the capture of rare alleles, they also recommended that 50 individuals from each population be sampled.

The Virgin Jungle Reserves (VJRs) of Peninsular Malaysia were established as a network of small protected patches of natural forest largely located within commercially productive forest (Wyatt-Smith, 1950). The usefulness and importance of the VJR system in the conservation of forest patches has received positive comment in reviews conducted by the Food and Agriculture Organization (FAO, 1984) and a comprehensive study by Laidlaw (1994). Wyatt-Smith (1963) suggested an absolute minimum size of 81 ha in an existing VJR system. While silviculturists (e.g., Borhan and Cheah, 1986) have considered that an area of 81 ha is too small for VJRs, others (e.g., Kochummen, La Frankie, and Manokaran, 1990) have pointed out the effectiveness of small forest patches in capturing good representations of a region's flora. Based on the size area proposed by Wyatt-Smith (1963), an arbitrary area of 150 ha for VJRs, including a buffer strip of 20 m width, was adopted in the formulation of criteria, indicators, activities, and management specifications for sustainable forest management at the national level (Thang, 1998). The feasibility of an arbitrary area of 150 ha for the VJRs to serve these roles is unknown and has never been investigated. A scientifically sound approach is critically needed to mitigate this issue. Laidlaw (1994), in the study of locality effects of the VJRs with reference to boundary of the forest reserve, showed that disturbed VJRs are more likely to be found on the edge of forest reserves, whereas undisturbed VJRs are more likely to be found in the center of forest reserves. It might be of interest to compare, in term of genetics, the VJRs located on the edges of forest reserves with those in the center.

In the present study, we investigate allozyme variation in *Intsia palembanica*, an important and widespread timber species in Malaysia. Because the species is known to be more valuable than others, when subjected to a greater degree of exploitation, it is expected that the species will be more vulnerable to become endangered. Locally known as Merbau, it occurs from the Andaman Islands, Thailand, and Malesia eastwards to western New Guinea in inland lowland forest up to 1000 m above sea level (asl). In Peninsular Malaysia, it occurs generally throughout the country, except Perlis, Langkawi, and Penang, along river valleys, in low hills near streams, and in moist sites on alluvial soil (Wyatt-Smith, 1953). At the Pasoh FR, the density of *I. palembanica* above 30 cm diameter at breast height (dbh) in the 50-ha plot is ~1.3 trees/ha (Manokaran et al., 1992). The mature trees of *I. palembanica* are large; they can reach 145 cm in dbh and 55 m in height. The bole is slightly sinuous, the buttresses are steep, and the crown is wide and domed, with big ascending limbs (Appanah and Weinland, 1993). The bark is smooth, with shallow depressions. It flowers and fruits regularly, and the flowers are pollinated mainly by bees, *Apis* sp. and *Trigona* sp. (S. Appanah, FAO, personal communication). The fruit is a thick, woody pod, and the seeds are heavy, not arillate, and can remain dormant for several years (Strugnell, 1937). It is deciduous, shedding all its leaves for a few days in a year. The growth is good while cotyledons are present, but then slows down. It does not form a straight stem in the earliest stages of development, its branches tending to be pulled down by climbers. *Intsia palembanica* is a slow-growing species; growth data from sample plots recorded an annual diameter increment of ~0.6 cm only. However, due to its fine timber and storable seed, the species has been used in planting trials (Appanah and Weinland, 1993). It is one of the main commercial heavy hardwood species, with fine growth rings and deep color. The timber is used for interior finishing, panelling, strip and parquet flooring, superior joinery, cabinet-making, musical instruments, decorative and novelty items, veneers, and power transmission poles.

This paper reports the results of an electrophoretic survey to examine the mating system, genetic diversity, and population genetic structure of *I. palembanica*, based on VJRs in Peninsular Malaysia. The objectives were (1) to describe the distribution of genetic variation within and among populations, (2) to characterize the mating system, (3) to identify genetically unique populations for conservation, (4) to test the locality effects of the VJRs with reference to boundary of the Forest Reserve on *I. palembanica*, and (5) to estimate effective population sizes of *I. palembanica* for the maintenance of genetic diversity.

## MATERIALS AND METHODS

***Population sampling***—The field survey of VJR compartments in Peninsular Malaysia allowed us to identify six natural populations of *I. palembanica* for this study (Table 1; Fig. 1). Two populations (Pasoh and Lagong) are located in the center of the forest reserve, whereas four (Lalang, Bubu, Kinta, and Perangin) are located on the edge of the forest reserve adjoining farmland or plantation. Approximately 40 adult individuals were sampled in each population, using the transect-line sampling method, as explained by Lee et al. (2000a). Branch samples were collected and young leaves were removed for further use in gel electrophoresis. In addition, for the mating system study, open-pollinated seeds (16–25) were collected from nine progeny families, which were chosen at random within an area of 30 ha in Lagong. The species produces heavy seeds in a thick, woody pod; seed dispersal appears to be mainly by gravity and seeds fall around individual parent plants. If the mother

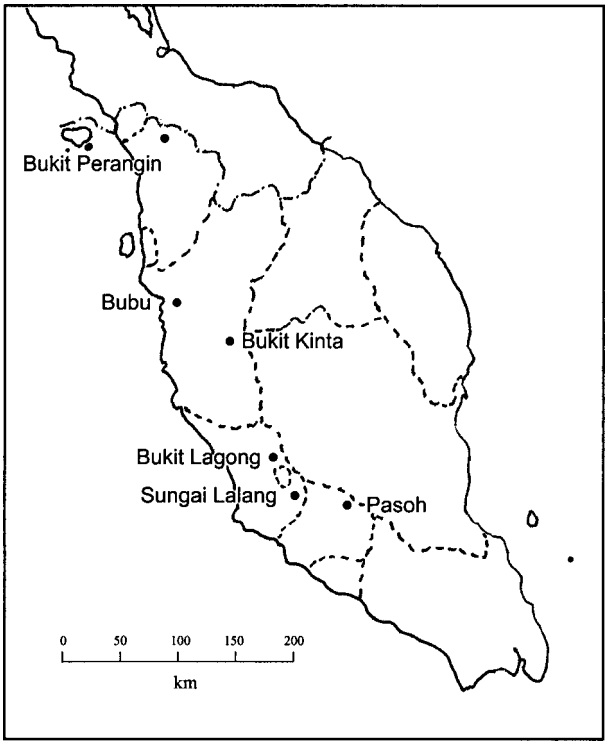


Fig. 1. Location of collection sites in Peninsular Malaysia.

tree was isolated from neighboring mother trees, seeds were collected from the ground below the maternal tree. When mother trees were near each other, seeds were harvested from a representative sample of branches within the crown by a specially trained tree climber.

***Electrophoresis***—Fresh pieces of young, actively growing leaf tissues or seed embryos were ground in cold extraction buffer, as described by Lee et al. (2000b). The extract was absorbed onto 1.2 × 1.5 mm filter paper wicks (chromatography paper Whatman no. 3) and loaded directly into 12% (w/v) starch gel. Standard methods for starch gel electrophoresis and enzyme activity staining were employed in this study (Wendel and Weeden, 1989). The enzyme analysis was carried out using three systems of electrode and gel buffers as described by Lee et al. (2000b). The morpholine citrate (MC) buffer was used to assay glucose phosphate isomerase (GPI), phosphoglucomutase (PGM), menadione reductase (MR), phosphogluconate dehydrogenase (PGD), and uridine diphosphogluconate pyrophosphatase (UGP); the tris citrate (TC) buffer was used for aspartate aminotransferase (AAT), peroxidase (PER), and acid phosphatase (ACP); and the histidine (H) buffer was used for malic enzyme (ME). The starch gels were run at 70 mA for 5–6 h on MC system, 80 mA for 5–6 h on TC, and 45 mA for 4–5 h on H. Isozymes and allozymes were inferred by observing segregation of bands among individuals in the populations sampled and from segregation patterns in the open-pollinated progeny arrays. Assignment of genotypes was then conducted in accordance with the known enzyme substructure (Wendel and Weeden, 1989). The locus specifying the most anodally migrating isozyme was designated as 1, the next 2, and so on. Similarly, the most anodal allozyme of a gene (allele) was labeled A, etc.

***Statistical analyses***—Allelic frequencies were obtained for each locus in each population. Based on these data, the following levels of genetic diversity within each population were estimated: average number of alleles per polymorphic locus ($A_a$), percentage of polymorphic loci ($P_o$; 95% criteria), effective number of alleles per polymorphic locus ($A_e$; Crow and Kimura, 1970), observed heterozygosity ($H_o$), and Nei's (1978) expected heterozygosity ($H_e$). All of these parameters, except $A_e$, were calculated with the program BIOSYS-1 (Swofford and Selander, 1981).

Differentiation among populations was quantified using Nei's genetic diversity statistics (Nei, 1973, 1977); total genetic diversity at polymorphic loci ($H_T$) was partitioned into a within-population component ($H_S$) and among-population component ($D_{ST}$), so that $H_T = H_S + D_{ST}$. Among-population variation was compared to total genetic variation to give $G_{ST} = D_{ST}/H_T$. The $G_{ST}$ values were calculated for each polymorphic locus and then averaged over all loci. Relatedness among populations was quantified using Nei's genetic identities for pairwise comparison of divergence between populations (Nei, 1978), and cluster analysis on genetic identities via the unweighted pairwise groups with arithmetic averaging (UPGMA, Sneath and Sokal, 1973). The relationship between geographical distances among populations and levels of genetic differentiation were determined with Mantel test to compare matrices and by using Rousset's (1997) isolation by distance procedure. Multilocus estimates of the effective number of migrants ($N_m$) between populations were calculated using the private allele method of Slatkin (1985) and were corrected for sample size as given in Barton and Slatkin (1986), with the program GENEPOP (version 3.2a; Raymond and Rousset, 2000).

In addition, Wright's $F$ statistics (1951) were calculated to measure the deviation from Hardy-Weinberg equilibrium at each polymorphic locus in each population. The fixation indices, $F_{IS}$ (inbreeding within individual in population; inbreeding coefficient) and $F_{ST}$ (inbreeding due to population subdivision, an indicator of the degree of differentiation among populations), were calculated based on Weir and Cockerham's (1984) estimators $f$ and $\theta$, respectively, using the program FSTAT (version 2.9.1; Goudet, 2000). Significant positive or negative $F_{IS}$ was tested using 6600 randomization (default parameter in FSTAT) for each locus and across loci for each population. The standard error of $F_{ST}$ was calculated using unbiased jackknife analysis, and the probability of the $F_{ST} > 0$ was determined using bootstrap analysis with a 95% confidence interval.

Cornuet and Luikart (1996) developed a test based on the assumption that when populations experience a recent reduction in effective population size, they generally have higher heterozygosity ($H_e$) than predicted by their allelic diversity ($H_{eq}$) because allelic diversity is reduced faster than heterozygosity. Heterozygosity was estimated using expected heterozygosity (Nei, 1978). Allelic diversity was calculated from the number of alleles observed and the sample size of individuals, assuming neutrality and mutation drift equilibrium (Cornuet and Luikart, 1996). Both parameters were calculated for each polymorphic locus and then averaged over all loci. The significance of genetic diversity excess ($H_e > H_{eq}$) was tested based on 5000 replications using Wilcoxon sign-rank test (Luikart et al., 1998) in the BOTTLENECK program (Piry, Luikart, and Cornuet, 1998).

The contribution of each population to species levels of genetic diversity and genetic uniqueness of a specific population ($a_i$) was determined using ''jackknife'' analysis (Slatkin, 1985; Jaquish and El-Kassaby, 1998) as follows: (1) removing population $a_i$ data from the original data set (i.e., $-a_i$), (2) estimating the average of $G_{ST}$, $F_{ST}$, and $I$ for the new data set, and (3) comparing the estimates obtained from the original analysis to that of the six new data sets (i.e., $-a_1$, $-a_2$, $-a_3$, $-a_4$, $-a_5$, and $-a_6$).

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 $\mu$ is the neutral mutation rate. In addition, the low values of $G_{ST}$ and $F_{ST}$ (as described in the results) might suggest that the populations studied were probably part of one continuous population in the recent past. Hence, the allozyme data from all the studied populations were pooled (total number of samples is 241) for simulation analysis. To determine the $N_e$ required for maintaining $H_e$ and the total number of alleles ($A_T$), 230 of the 241 samples were sampled without replacement 50 times using a computerized algorithm. The $A_T$ and $H_e$ were calculated. The $A_T$ and $H_e$ were also estimated for sample sizes of 220 to 10, with a 10-sample reduction interval. The mean genetic

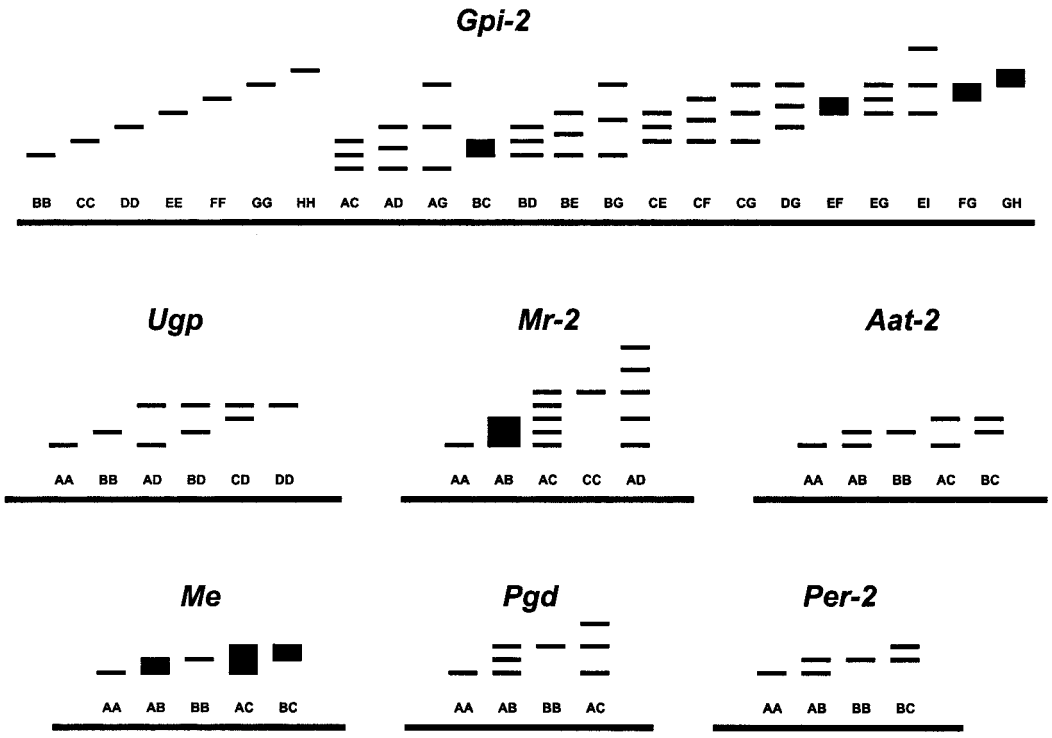


Fig. 2. Schematic representation of banding patterns observed for polymorphic loci with more than two alleles in six natural populations of *Intsia palembanica* in Peninsular Malaysia.

diversity parameters with standard errors were plotted against sample sizes to reveal trends.

Linkage among loci may effect the results of mating system analysis based on a mixed-mating model (Ritland and Jain, 1981). Linkage disequilibrium analysis was performed using contingency tables for all pairs of polymorphic loci in the Lagong population and a probability test (Fisher exact test) was performed for each table using the Markov chain method (Rousset and Raymond, 1995) with the program GENEPOP (version 3.2a; Raymond and Rousset, 2000). Mating system parameters were determined using the multilocus mating system program (MLTR) developed by Ritland (1996), based on the procedure of Ritland and Jain (1981) for multilocus outcrossing rate ($t_m$), average single locus outcrossing rate ($t_s$), pollen and ovule allele frequencies, and the variances of the above quantities using the bootstrap method where the progeny array (within families) is the unit of resampling (250 bootstrap replicates). Maternal genotypes were inferred from progeny data by the method of Brown and Allard (1970), as described by Ritland (1996) in the estimation program. The assumptions made in formulating maximum likelihood estimates of mating system parameters are summarized in Lee et al. (2000c). To test the assumption that allele frequencies in the outcross pollen pool are distributed uniformly over the population of maternal trees, a chi-square test was performed, where $\chi^2 = N \times G'_{ST} (A - 1)$, where $N$ is the total number of pollen gametes, $G'_{ST}$ is the proportion of among-tree variance in pollen allele frequencies relative to the total variance in pollen allele frequencies, and $A$ is the number of alleles at a locus (James et al., 1998).

## RESULTS

***Allelic variation***—A total of nine allozymes encoded by 14 putative gene loci (*Gpi-1*, *Gpi-2*, *Pgm-1*, *Pgm-2*, *Ugp*, *Mr-1*, *Mr-2*, *Aat-1*, *Aat-2*, *Me*, *Pgd*, *Per-1*, *Per-2*, and *Acp*) were consistently resolved in *Intsia palembanica*. Of these, three loci (*Gpi-1*, *Pgm-2*, and *Aat-1*) were monomorphic throughout the entire study, *Per-1* and *Acp* were monomorphic in all but one population (Pasoh and Lagong, respectively), while *Pgd* was polymorphic in three populations (Pasoh, Bubu, and Perangin). The schematic representation of polymorphic loci with more than two alleles is given in Fig. 2. The number of allozymes and banding phenotypes within loci were comparable to those of other diploid plants (Weeden and Wendel, 1989). A complete list of allele frequencies for all the polymorphic loci in each population is shown in Table 2. The 241 individuals, representing six natural populations of *I. palembanica*, produced a total of 37 alleles with 11 polymorphic allozyme loci. The most frequent allele for some loci varied among populations (*Gpi-1*, *Ugp*, *Aat-2*, *Me*, and *Per-2*). A total of six private alleles were detected: three (*Gpi-2A*, *Gpi-2I*, and *Per-2C*) in Lalang, two (*Pgd-B* and *Per-1B*) in Pasoh, and one (*Acp-B*) in Lagong. Most of these alleles, which have a restricted distribution, occur at a low frequency.

***Levels of genetic diversity***—The average number of alleles per polymorphic locus ($A_a$) ranged from 2.1 (Kinta) to 2.7 (Pasoh), with a mean of 2.4 (Table 3). Values of $A_e$, the effective number of alleles per polymorphic loci, were consistently lower than $A_a$, averaging 1.64, and ranging from 1.57 (Kinta) to 1.74 (Lalang). This may indicate the presence of rare alleles. The mean percentage of polymorphic loci ($P_o$), evaluated at the 95% confidence level, was ~56%. For each population, over all loci, the observed heterozygosity ($H_o$) was generally higher than Hardy-Weinberg expectation ($H_e$) (except for the Bubu and Perangin); the $H_o$ ranged from 0.188 (Bubu) to 0.270 (Pasoh), with a mean of 0.239, while the $H_e$ ranged from 0.222 (Bubu) to 0.265 (Pasoh), with a mean of 0.242.

***Fixation index and bottleneck***—The fixation indices ($F_{IS}$), calculated for all polymorphic loci in each population (Table

TABLE 2. Allele frequencies for polymorphic loci in six natural populations of *Intsia palembanica*.

| Locus | Allele | Population: Pasoh | Lalang | Lagong | Bubu | Kinta | Perangin | Species |
|---|---|---|---|---|---|---|---|---|
| *Gpi-2* | A | 0.000 | 0.050 | 0.000 | 0.000 | 0.000 | 0.000 | 0.008 |
| | B | 0.038 | 0.087 | 0.013 | 0.000 | 0.063 | 0.000 | 0.034 |
| | C | 0.295 | 0.175 | 0.397 | 0.244 | 0.338 | 0.400 | 0.308 |
| | D | 0.038 | 0.025 | 0.051 | 0.077 | 0.000 | 0.000 | 0.032 |
| | E | 0.179 | 0.213 | 0.154 | 0.205 | 0.050 | 0.325 | 0.188 |
| | F | 0.051 | 0.000 | 0.026 | 0.026 | 0.000 | 0.000 | 0.017 |
| | G | 0.385 | 0.438 | 0.359 | 0.449 | 0.525 | 0.275 | 0.405 |
| | H | 0.013 | 0.000 | 0.000 | 0.000 | 0.025 | 0.000 | 0.006 |
| | I | 0.000 | 0.013 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 |
| *Pgm-I* | A | 0.776 | 0.732 | 0.863 | 0.842 | 0.703 | 0.875 | 0.799 |
| | B | 0.224 | 0.268 | 0.138 | 0.158 | 0.297 | 0.125 | 0.201 |
| *Ugp* | A | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.013 | 0.006 |
| | B | 0.423 | 0.548 | 0.350 | 0.231 | 0.449 | 0.213 | 0.370 |
| | C | 0.000 | 0.000 | 0.000 | 0.013 | 0.000 | 0.063 | 0.013 |
| | D | 0.577 | 0.452 | 0.625 | 0.756 | 0.551 | 0.712 | 0.611 |
| *Mr-2* | A | 0.782 | 0.841 | 0.936 | 0.897 | 0.925 | 0.962 | 0.891 |
| | B | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.013 | 0.006 |
| | C | 0.167 | 0.146 | 0.064 | 0.077 | 0.075 | 0.025 | 0.092 |
| | D | 0.026 | 0.012 | 0.000 | 0.026 | 0.000 | 0.000 | 0.011 |
| *Aat-1* | A | 0.051 | 0.105 | 0.103 | 0.000 | 0.050 | 0.013 | 0.054 |
| | B | 0.949 | 0.895 | 0.897 | 1.000 | 0.950 | 0.987 | 0.946 |
| *Aat-2* | A | 0.692 | 0.539 | 0.450 | 0.368 | 0.475 | 0.526 | 0.511 |
| | B | 0.269 | 0.461 | 0.525 | 0.632 | 0.525 | 0.474 | 0.478 |
| | C | 0.038 | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.011 |
| *Me* | A | 0.167 | 0.575 | 0.359 | 0.500 | 0.628 | 0.635 | 0.476 |
| | B | 0.833 | 0.363 | 0.641 | 0.473 | 0.359 | 0.351 | 0.504 |
| | C | 0.000 | 0.063 | 0.000 | 0.027 | 0.013 | 0.014 | 0.019 |
| *Pgd* | A | 0.769 | 1.000 | 1.000 | 0.962 | 1.000 | 0.756 | 0.914 |
| | B | 0.103 | 0.000 | 0.000 | 0.038 | 0.000 | 0.244 | 0.064 |
| | C | 0.128 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.021 |
| *Per-1* | A | 0.974 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.996 |
| | B | 0.026 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 |
| *Per-2* | A | 0.569 | 0.463 | 0.436 | 0.393 | 0.365 | 0.431 | 0.444 |
| | B | 0.431 | 0.500 | 0.564 | 0.607 | 0.635 | 0.569 | 0.549 |
| | C | 0.000 | 0.038 | 0.000 | 0.000 | 0.000 | 0.000 | 0.007 |
| *Acp* | A | 1.000 | 1.000 | 0.974 | 1.000 | 1.000 | 1.000 | 0.996 |
| | B | 0.000 | 0.000 | 0.026 | 0.000 | 0.000 | 0.000 | 0.004 |

4), showed significant, positive or negative, deviation from Hardy-Weinberg equilibrium in three loci (*Gpi-2, Mr-2,* and *Aat-2*) at Bubu, two loci (*Me* and *Per-2*) at Lalang, and one locus each at Lagong and Perangin (*Per-2* and *Pgd*, respectively). At the population level, an excess of homozygotes ($F_{IS} > 0$), or significant levels of inbreeding, were observed in Bubu (0.155; $P < 0.001$) and Perangin (0.117; $P < 0.05$), which may postulate that the plant practices some selfing and/or biparental mating in these two populations. Negative $F_{IS}$ was detected in the four remaining populations, but these were not significantly different from zero. The calculation of expected equilibrium heterozygosity ($H_{eq}$) depends on the model of mutation used to analyze the loci being studied; these are the infinite allele model (IAM; Kimura and Crow, 1964), stepwise mutation model (SMM; Ohta and Kimura, 1973), or two-phase model (TPM; Di Rienzo et al., 1994). A bottleneck has only been demonstrated for loci evolved under the IAM (Maruyama and Fuerst, 1985). For allozyme loci, heterozygosity equilibrium and deficiency for a nonbottlenecked population were detected using the IAM (Luikart and Cornuet, 1998). In this study, a probability test of recent effective population size reduction based on the IAM using polymorphic loci detected bottlenecks on Lalang, Bubu, and Kinta (Table 4). A similar analysis, using polymorphic loci in the Hardy-Weinberg equilibrium, however, detected bottlenecks only in Lalang and Kinta (Table 4).

***Differentiation and relatedness among populations***—Most of the total genetic diversity ($H_T = 0.321$) was partitioned within population ($H_S = 0.309$; $D_{ST} = 0.013$). The proportion of genetic variation distributed among populations ($G_{ST}$) was estimated as 0.040, thus only 4.0% of the genetic variability was distributed among populations (Table 5). The mean $F_{ST}$ (0.048) estimate was slightly higher than $G_{ST}$ and was significantly greater than zero ($F_{ST}$ within 95% confidence interval = 0.024–0.081; 95% confidence interval did not overlap with zero; Table 5). Nei's (1978) genetic identities ($I$) among pairs of populations for all loci were high and varied between 0.961 (Pasoh × Perangin) and 0.997 (Lalang × Kinta), with a mean 0.982 (Table 5; Fig. 3). At $I = 0.985$, cluster analysis among populations formed three genetic clusters. Lalang, Kinta, Lagong, and Bubu formed a common cluster, and Pasoh and Perangin were the two outliers (Fig. 3). However, the genetic distances between the populations are very small. Geographical distances between populations varied from 42 to 420 km. A Mantel test to compare the matrices of genetic differentiation and geographical distances showed that there was no relationship between them ($r = -0.015$, $P = 0.903$). This may indicate that the majority of the populations are genetically very similar. Over all populations and loci, the number of migrants ($N_m$) using the private alleles method according to Barton and Slatkin (1986) was 2.04, which is >1, indicating high levels of gene flow. It should be noted, however, that this value

TABLE 3. Summary of allozyme diversity[a] and effective population size necessary to maintain current level of allozyme heterozygosity[b] for six natural populations of *Intsia palembanica* in Peninsular Malaysia. Values in parentheses are standard deviations.

| Population | $A_a$ | $A_e$ | $P_o$ | $H_o$ | $H_e$ | $N_e$: $\mu = 10^{-3}$ | $N_e$: $\mu = 10^{-7}$ |
|---|---|---|---|---|---|---|---|
| Pasoh | 2.7 (0.5) | 1.70 | 64.3 | 0.270 (0.065) | 0.265 (0.064) | 90 | 900 000 |
| Lalang | 2.5 (0.5) | 1.74 | 57.1 | 0.269 (0.078) | 0.263 (0.071) | 89 | 890 000 |
| Lagong | 2.4 (0.4) | 1.62 | 57.1 | 0.251 (0.073) | 0.234 (0.067) | 76 | 760 000 |
| Bubu | 2.3 (0.4) | 1.58 | 50.0 | 0.188 (0.059) | 0.222 (0.066) | 71 | 710 000 |
| Kinta | 2.1 (0.3) | 1.57 | 57.1 | 0.247 (0.070) | 0.231 (0.066) | 75 | 750 000 |
| Perangin | 2.4 (0.3) | 1.60 | 50.0 | 0.208 (0.062) | 0.235 (0.066) | 77 | 770 000 |
| Mean | 2.4 (0.2) | 1.64 | 55.9 | 0.239 (0.034) | 0.242 (0.018) | 80 | 800 000 |

[a] Average number of alleles per polymorphic locus ($A_a$), effective number of alleles per polymorphic locus ($A_e$), percent polymorphic loci ($P_o$, 95% criterion), observed heterozygosity ($H_o$), and Nei's (1978) expected heterozygosity ($H_e$).

[b] Effective population size ($N_e$). Estimates are calculated from Crow and Kimura's (1970) equilibrium heterozygosity formula for neutral alleles. Two mutation rates ($\mu$) are given as a range.

TABLE 4. Fixation index ($F_{is}$),[a] expected heterozygosity ($H_e$),[b] and expected equilibrium heterozygosity ($H_{eq}$)[c] for six natural populations of *Intsia palembanica* in Peninsular Malaysia. Significance of gene diversity excess ($H_e > H_{eq}$), an indication of recent effective population size reductions (bottlenecks), was tested using Wilcoxon signed ranks test (Luikart et al., 1998) based on 5000 replications. * $P < 0.05$; ** $P < 0.01$.

| Locus | Pasoh | Lalang | Lagong | Bubu | Kinta | Perangin |
|---|---|---|---|---|---|---|
| ***F*IS** | | | | | | |
| *Gpi-2* | 0.096 | −0.096 | −0.071 | 0.196* | 0.060 | 0.140 |
| *Pgm-1* | 0.028 | −0.106 | 0.064 | 0.024 | −0.151 | 0.326 |
| *Ugp* | −0.090 | 0.051 | −0.016 | −0.014 | −0.075 | 0.054 |
| *Mr-2* | −0.201 | 0.020 | −0.056 | 0.465* | −0.068 | −0.017 |
| *Aat-1* | −0.041 | −0.104 | −0.101 | MONO[d] | −0.040 | 0.000 |
| *Aat-2* | 0.209 | 0.218 | 0.101 | 0.567** | −0.090 | 0.241 |
| *Me* | −0.188 | 0.309* | −0.101 | 0.139 | −0.118 | 0.156 |
| *Pgd* | 0.206 | MONO | MONO | −0.027 | MONO | 0.385* |
| *Per-1* | −0.013 | MONO | MONO | MONO | MONO | MONO |
| *Per-2* | −0.290 | −0.487** | −0.344* | −0.180 | −0.094 | −0.290 |
| *Acp* | MONO | MONO | −0.013 | MONO | MONO | MONO |
| All | −0.017 | −0.023 | −0.071 | 0.155** | −0.070 | 0.117* |
| ***H*e** | | | | | | |
| *Gpi-2* | 0.737 | 0.731 | 0.695 | 0.700 | 0.611 | 0.667 |
| *Pgm-1* | 0.352 | 0.397 | 0.240 | 0.269 | 0.424 | 0.222 |
| *Ugp* | 0.495 | 0.501 | 0.492 | 0.379 | 0.501 | 0.449 |
| *Mr-2* | 0.364 | 0.274 | 0.122 | 0.190 | 0.141 | 0.074 |
| *Aat-1* | 0.099 | 0.191 | 0.186 | MONO | 0.096 | 0.026 |
| *Aat-2* | 0.453 | 0.504 | 0.528 | 0.472 | 0.505 | 0.505 |
| *Me* | 0.281 | 0.541 | 0.466 | 0.533 | 0.483 | 0.479 |
| *Pgd* | 0.386 | MONO | MONO | 0.075 | MONO | 0.373 |
| *Per-1* | 0.051 | MONO | MONO | MONO | MONO | MONO |
| *Per-2* | 0.497 | 0.541 | 0.498 | 0.486 | 0.470 | 0.497 |
| *Acp* | MONO | MONO | 0.051 | MONO | MONO | MONO |
| Mean 1[e] | 0.372 | 0.460 | 0.364 | 0.388 | 0.404 | 0.366 |
| Mean 2[f] | 0.372 | 0.433 | 0.348 | 0.348 | 0.404 | 0.365 |
| ***H*eq** | | | | | | |
| *Gpi-2* | 0.650 | 0.638 | 0.589 | 0.527 | 0.524 | 0.335 |
| *Pgm-1* | 0.204 | 0.198 | 0.191 | 0.203 | 0.198 | 0.199 |
| *Ugp* | 0.191 | 0.193 | 0.354 | 0.338 | 0.195 | 0.437 |
| *Mr-2* | 0.442 | 0.337 | 0.197 | 0.339 | 0.201 | 0.342 |
| *Aat-1* | 0.200 | 0.203 | 0.198 | MONO | 0.196 | 0.197 |
| *Aat-2* | 0.334 | 0.200 | 0.343 | 0.203 | 0.199 | 0.196 |
| *Me* | 0.191 | 0.331 | 0.196 | 0.335 | 0.340 | 0.333 |
| *Pgd* | 0.335 | MONO | MONO | 0.186 | MONO | 0.198 |
| *Per-1* | 0.195 | MONO | MONO | MONO | MONO | MONO |
| *Per-2* | 0.196 | 337 | 0.195 | 0.123 | 0.199 | 0.198 |
| *Acp* | MONO | MONO | 0.198 | MONO | MONO | MONO |
| Mean 1[e] | 0.295 | 0.304** | 0.271 | 0.294* | 0.255* | 0.272 |
| Mean 2[f] | 0.295 | 0.293* | 0.281 | 0.257 | 0.255* | 0.281 |

[a] $F_{IS}$ was estimated according to Weir and Cockerham (1984). Significant positive or negative $F_{IS}$ was tested using 6600 randomization.
[b] $H_e$ was estimated according to Nei's (1978) based on polymorphic loci.
[c] $H_{eq}$ was estimated from the number of alleles observed and the sample size of individuals, under the infinite allele model, assuming neutrality and mutation drift equilibrium (Cornuet and Luikart, 1996).
[d] MONO = monomorphic loci.
[e] Mean across all the polymorphic loci.
[f] Mean across polymorphic loci in Hardy-Weinberg equilibrium.

of $N_m$ represents historical average levels of gene flow and may not represent present-day levels.

***Genetic uniqueness of a specific population***—The genetic uniqueness of each population was concluded by calculating the $G_{ST}$, $F_{ST}$, and $I$ for the remaining populations after the elimination of this specific population from the data. As shown in Table 5, low genetic diversities among populations ($G_{ST}$ and $F_{ST}$) were observed for the analysis without Pasoh (0.026 and 0.033, respectively) and without Perangin (0.037 and 0.045, respectively). These values were lower than that obtained from the analysis of the six populations altogether (0.040 and 0.048, respectively), as well as for the remaining four analyses (Table 5). Similarly, the genetic identity analysis produced higher averages of $I$ without Pasoh (0.987) and without Perangin (0.985) in comparisons with the average of the six populations (0.982) and the remaining four analyses (Table 5).

***Effective population sizes***—Direct estimates of mutation rate are not easily obtained, and they are only available for model organisms. However, the theory of molecular evolution predicts that at neutral sites of the genome, the rate of nucleotide substitution between two species will equal the mutation rate. Nei (1975) suggested that the average mutation rate ($\mu$) of codon substitution detectable by allozyme electrophoresis was $10^{-7}$. However, this low estimate of mutation rates is somewhat surprising, given the high levels of variation that have been observed in many trees (Hamrick and Godt, 1996b).

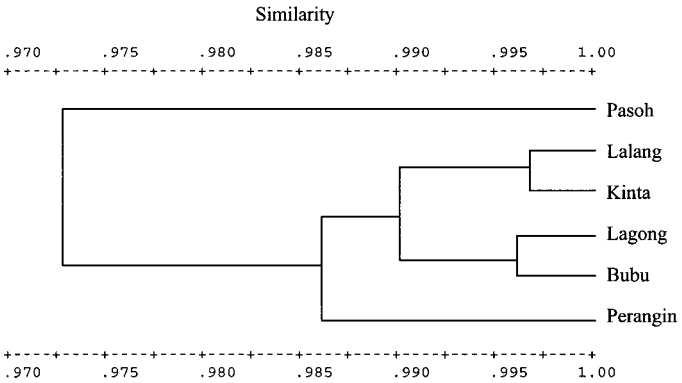


Fig. 3. Unweighted pairwise groups with arithmetic averaging (UPGMA) dendogram based on mean genetic identity values for six natural populations of *Intsia palembanica* in Peninsular Malaysia. Mean genetic similarity = 0.982, in the range of 0.961–0.997.

Recent evidence has shown that generational mutation rates in long-lived woody plants are higher (e.g., $10^{-3}$) than those in other organisms (Klekowski and Godfrey, 1989; Lowenfeld and Klekowski, 1992). Thus, estimates of effective population sizes needed to maintain the current level of allozyme heterozygosity ($N_e$), based on the formula of Crow and Kimura (1970), were calculated using these two mutation rates as a range. The $N_e$ ranged from 71 (Bubu) to 90 (Pasoh) when $\mu = 10^{-3}$ and subsequently 710 000–900 000 when $\mu = 10^{-7}$ (Table 3). Mean across the six populations ranged from 80 to 800 000. Changes in total number of allele ($A_T$) and expected heterozygosity ($H_e$) with changes in the number of samples is shown in Fig. 4. The basic relationship between $A_T$ with sample size and between $H_e$ with sample size was logarithmic; $A_T$

TABLE 5. Nei's (1987) genetic diversity statistic ($H_S$, $H_T$, $D_{ST}$ and $G_{ST}$), Wright's (1951) $F_{ST}$ value, and mean genetic identity ($I$) in *Intsia palembanica* populations from Peninsular Malaysia. −Pasoh represents an analysis that contained all populations after the removal of the Pasoh population.

| | $H_T$ | $H_S$ | $D_{ST}$ | $G_{ST}$ | $F_{ST}$ | $I$ |
|---|---|---|---|---|---|---|
| **Locus** | | | | | | |
| *Gpi-2* | 0.705 | 0.691 | 0.014 | 0.020 | 0.025 | — |
| *Pgm-1* | 0.323 | 0.317 | 0.005 | 0.016 | 0.020 | — |
| *Ugp* | 0.490 | 0.469 | 0.020 | 0.041 | 0.051 | — |
| *Mr-2* | 0.198 | 0.194 | 0.004 | 0.022 | 0.027 | — |
| *Aat-2* | 0.511 | 0.496 | 0.016 | 0.031 | 0.036 | — |
| *Aat-1* | 0.102 | 0.100 | 0.002 | 0.022 | 0.023 | — |
| *Me* | 0.519 | 0.464 | 0.055 | 0.106 | 0.128 | — |
| *Pgd* | 0.159 | 0.140 | 0.020 | 0.125 | 0.135 | — |
| *Per-1* | 0.501 | 0.496 | 0.005 | 0.010 | 0.012 | — |
| *Per-2* | 0.009 | 0.008 | 0.000 | 0.010 | 0.023 | — |
| *Acp* | 0.009 | 0.008 | 0.000 | 0.011 | 0.025 | — |
| Overall | 0.321 | 0.309 | 0.013 | 0.040 | 0.048[a] | 0.982 |
| **Population** | | | | | | |
| −Pasoh | 0.310 | 0.302 | 0.008 | 0.026 | 0.033 | 0.987 |
| −Lalang | 0.315 | 0.302 | 0.013 | 0.041 | 0.051 | 0.981 |
| −Lagong | 0.324 | 0.310 | 0.014 | 0.043 | 0.056 | 0.979 |
| −Bubu | 0.326 | 0.313 | 0.013 | 0.040 | 0.053 | 0.980 |
| −Kinta | 0.324 | 0.310 | 0.014 | 0.043 | 0.051 | 0.981 |
| −Perangin | 0.321 | 0.309 | 0.012 | 0.037 | 0.045 | 0.985 |

[a] The standard error of $F_{ST} = 0.016$. The probability of the $F_{ST} > 0$ was determined using bootstrap analysis. $F_{ST}$ within 95% confidence interval = 0.024–0.081.

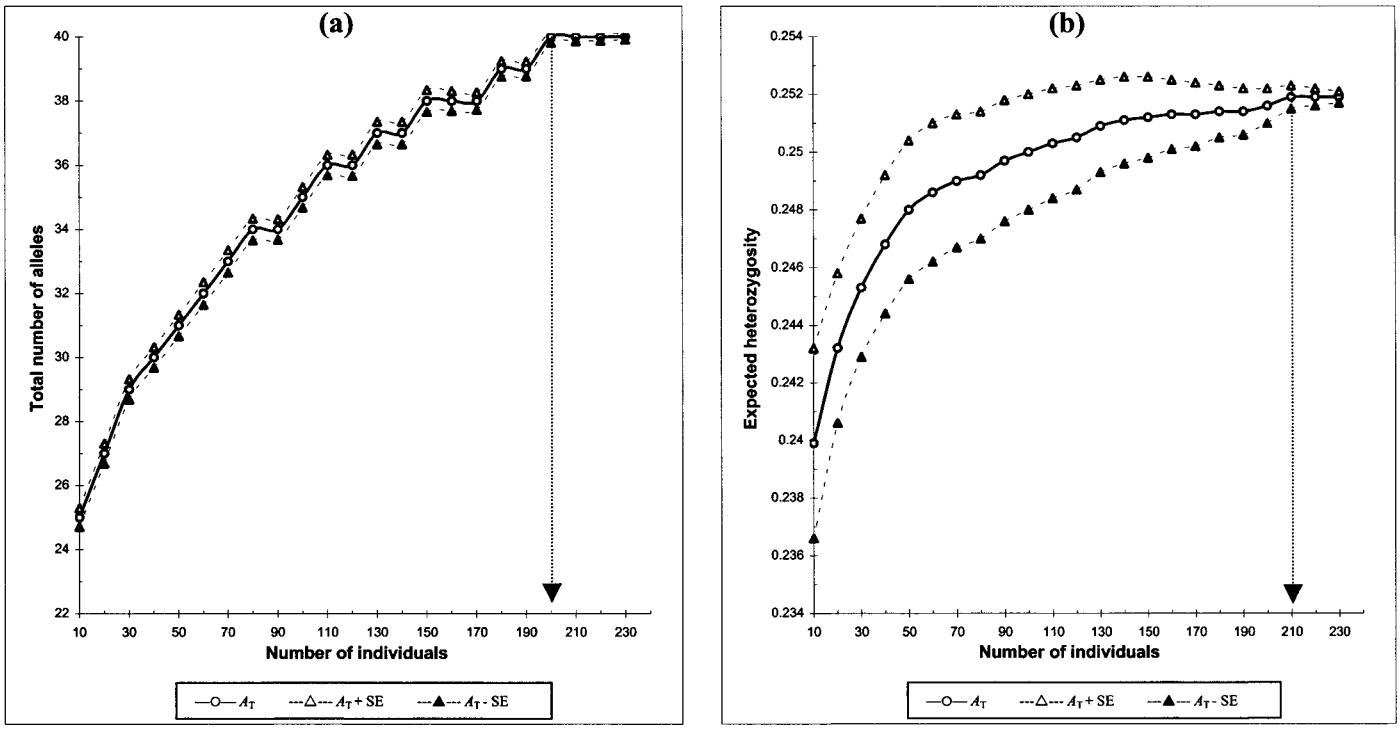


Fig. 4. Changes in genetic diversity with changes in the number of individuals of *Intsia palembanica*. (a) Total number of alleles ($A_T$) and (b) expected heterozygosity ($H_e$). All values were based on a mean of 50 resamplings with standard errors (SE). The pointed arrows indicate the optimum population size.

TABLE 6. Single locus outcrossing rate ($t_s$), multilocus outcrossing rate $t_m$, and pollen pool allele frequencies for *Intsia palembanica* from Bukit Lagong Forest Reserve. A $\chi^2$ test was performed to assess homogeneity of the pollen pool reaching each mother tree. Values in parentheses are standard deviations * $P < 0.01$.

| Locus | $t_s$ | Pollen pool allele frequency | | | | $\chi^2$ |
|---|---|---|---|---|---|---|
| | | *A* | *B* | *C* | *D* | |
| *Gpi-2* | 0.607 (0.079) | 0.014 | 0.389 | 0.349 | 0.249 | 254.93* |
| *Pgm-1* | 0.896 (0.340) | 0.892 | 0.102 | 0.007 | — | 32.51* |
| *Ugp* | 0.716 (0.274) | 0.311 | 0.689 | — | — | 176.87* |
| *Mr-2* | 0.547 (0.328) | 0.822 | 0.178 | — | — | 92.22* |
| *Aat-2* | 0.401 (0.278) | 0.602 | 0.398 | — | — | 93.52* |
| $t_s$ (mean) | 0.711 (0.087) | | | | | |
| $t_m$ | 0.766 (0.091) | | | | | |
| $t_m - t_s$ | 0.054 (0.025) | | | | | |

and $H_e$ at first increased with sample size and then reached a plateau. The $A_T$ and $H_e$ levels plateau at around 200 and 210 individuals, respectively (Fig. 4).

***Mating system parameters***—The *Me* and *Acp* loci showed good activity in leaf tissues, but were not expressed in embryos. The *Gpi-1, Pgm-2, Aat-1, Pgd,* and *Per-1* loci were monomorphic in the Lagong population. A probability test of linkage disequilibrium in the Lagong population detected significant linkage between two pairs of loci: *Pgm-1* and *Me* ($P = 0.041$) and *Pgm-1* and *Per-2* ($P = 0.005$). Hence, only *Gpi-2, Pgm-1, Ugp, Mr-2,* and *Aat-2* were used in the mating system study. *Intsia palembanica* at Lagong has a mixed-mating system with ~23% of the seeds produced from selfing (Table 6). The mean single locus outcrossing rate ($t_s$) for the five loci was 0.711, while the multilocus outcrossing rate ($t_m$) was 0.766. Genetic substructuring, in terms of biparental inbreeding (detectable by the difference between multilocus and single locus outcrossing rates), was not significant ($t_m - t_s = 0.054$). The null hypothesis of homogeneity of the pollen pool gene frequency over maternal parents was rejected for all the loci (Table 6), indicating that the maternal tree did not receive pollen randomly from all synchronously flowering trees. Differences among mating system parameters for different loci were observed in the range of 0.401 (*Aat-2*) to 0.896 (*Pgm-1*). The most reasonable explanation of this phenomenon seems to be the spatial and temporal variation among trees in flowering phenology and fecundity, which causes heterogeneity of the pollen pool. The consequence of this phenomenon is not readily measurable, but as shown by Ritland and Jain (1981), it has a minor effect on the multilocus estimate of population outcrossing rate.

## DISCUSSION

***Outcrossing rate and population structure***—*Intsia palembanica* at Lagong exhibited a mixed-mating system ($t_m = 0.766$); this value was comparable to the report on outcrossing rates for some tropical tree populations (O'Malley et al., 1988; Murawski and Hamrick, 1991, 1992a, b; Kitamura et al., 1994; Murawski and Bawa, 1994; Murawski, Dayanandan, and Bawa, 1994; Murawski, Gunatilleke, and Bawa, 1994; Doligez and Joly, 1997; Lee, 2000; Lee et al., 2000c). The present study also showed that *I. palembanica* harbors higher levels of genetic diversity than other regionally distributed tropical long-lived tree species ($A_a = 1.51$, $A_e = 1.16$, $P = 40\%$, and $H_e = 0.125$; Hamrick, Godt, and Sherman-Broyles, 1992). The $G_{ST}$ value (0.040) is lower than the mean for regionally distributed tropical long-lived tree species ($G_{ST} = 0.119$), long-lived outcrossing animal-pollinated tree species ($G_{ST} = 0.099$), long-lived tree species with seed dispersed by gravity ($G_{ST} = 0.131$), and long-lived tree species that reproduce sexually ($G_{ST} = 0.086$; Hamrick, Godt, and Sherman-Broyles, 1992).

The genetic diversity maintained within and among populations is a function of historical events and recent evolutionary processes. Because very little is usually known of a species' evolutionary and ecological history, explanations for the levels and patterns of genetic diversity found within and among populations rely primarily on inference. High levels of genetic diversity for *I. palembanica* might be attributed to the species' life history and ecological traits, such as its common, long-life, wide ranges, and its mixed-mating system. Low values of population differentiation ($G_{ST} = 0.017$–$0.085$), comparable to the present study ($G_{ST} = 0.040$), have been reported for other tropical trees (Pérez-Nasser, Eguiarte, and Piñero, 1993; Alvarez-Buylla and Garay, 1994; Chase, Boshier, and Bawa, 1995; Lee, Ang, and Norwati, 2000; Lee et al., 2000a).

Seed dispersal mechanisms were shown to be significantly correlated with $G_{ST}$ (abiotically dispersed species showed more than twice as much population differentiation as biotically dispersed species; Loveless, 1992), and this was somewhat surprising when we considered the fact that no primary special seed dispersal mechanisms were apparent in the species. The species produces heavy seeds in a thick, woody pod; seed appears to be mainly dispersed by gravity and fall around individual parent plants. However, we cannot rule out the possibility of secondary seed movement by water stream, as the species occurs along river valleys and in low hills near streams, or by animals, as the edible seed is preferred by monkeys and squirrels. In addition, the species is pollinated by energetic pollinators, mainly by bees. These factors suggest that pollen and seed dispersal in this species occur over long distances. However, the observed high rate of historical gene flow among populations ($N_m = 2.04$), also suggests that the species is not at a genetic equilibrium under the present levels of gene flow, with populations derived from each other in the recent past. Rapid and recent fragmentation of a big population should result in populations being more similar genetically than if populations were isolated for longer periods of time.

One of the major sampling considerations for conservation of wide-ranging species such as *I. palembanica* is geographical placement of units. If a few of these were strategically placed on the basis of patterns of genetic variation, much of the variability among population could also be captured (Millar and Libby, 1991). Hamrick (1993) suggested that for tropical tree species, if 80% of the total genetic diversity resides within a population, five strategically placed populations should capture 99% of their total genetic diversity. However, because *I. palembanica* is an outcrossed species and variation in adaptive traits is not known, more than five populations should be conserved. The low $G_{ST}$ and $F_{ST}$ and high *I* obtained without Pasoh and without Perangin analyses may indicate that these two populations harbor some unique genetic characters and they should receive additional attention for conservation purposes. Although the allozyme alleles probably do not confer adaptation, attempts also should be made to conserve populations with private alleles, such as *Gpi-2A, Gpi-2I,* and *Per-2C* in Lalang; *Pgd-B* and *Per-1B* in Pasoh; and *Acp-B* in Lagong.

A comprehensive review by Hamrick, Godt, and Sherman-Broyles (1992) of woody plant species showed that life history

and ecological traits explain a significant proportion (34%) of the variation among species for the genetic parameters measured. Woody species with large geographical ranges, outcrossing breeding systems, and wind or animal-ingested seed dispersal mechanisms have more genetic diversity within species and populations but less variation among populations than woody species with other combinations of traits. Some authors (e.g., Holsinger and Gottlieb, 1991; Frankel, Brown, and Burdon, 1995) have argued that there are no guidelines about the levels of genetic diversity in plants; it is in general not possible to relate variability at neutral marker loci directly to population viability (Holsinger, 1996). This is affirmed by observations of high levels of genetic diversity on narrow ranges or rare species (e.g., Lewis and Crawford, 1995; Young and Brown, 1996; Mateu-Andrés and Segarra-Moragues, 2000). A recent review by Gitzendanner and Soltis (2000) on rare and widespread plant congeners also showed no significant difference between rare and widespread species in terms of how genetic variation is partitioned within and among populations. However, for predominantly outcrossed or mixed-mating plant species, such as *I. palembanica* and other tropical tree species (Pérez-Nasser, Eguiarte, and Piñero, 1993; Alvarez-Buylla and Garay, 1994; Chase, Boshier, and Bawa, 1995; Lee, Ang, and Norwati, 2000; Lee et al., 2000a), a high level of genetic diversity and a low level of population differentiation were consistently observed. This is somewhat supported in that life history and ecological traits, in particular the mating system, can be used as guidelines to infer the levels of genetic diversity and how the level of genetic diversity is partitioning within and among populations in plants. A more recent review by Hamrick and Godt (1996b) also showed that the outcrossing vs. selfing mating system is the most powerful single determinant of the level of variation in trees. The average level of variation (measured as expected heterozygosity) at marker loci for selfing plants is 0.12 and is 0.16 for outcrossers. The level of differentiation of population (measured as $F_{ST}$) for outcrossers is on average ~0.1 and 0.5 for selfers (Hamrick and Godt, 1996b).

Genetically sound conservation requires a robust understanding of the processes by which species organize genetic variation in local populations and the patterns of this variation among populations. Tropical forests are rich in plant species diversity. An area of 50 ha in the Pasoh Forest Reserve was reported to contain 814 different tree species (Kochummen, 1997). For the majority of these species, we will never obtain adequate knowledge of the genetic structure. It is suggested that for the genetic conservation of widespread and common tree species in VJRs, we might want to group the species according to mating system. Genetic information generated for a species then can be adapted to species that have similar types of mating systems. High levels of genetic diversity require big effective population size for capturing genetic diversity, and subsequently, effective population size is proportionate to the areas to be conserved. Hence, conservation of outcrossing species will require the largest area, and this in turn will favor the conservation of other species with mixed-mating systems, selfing, or apomixis.

***Bottleneck and fixation index***—Identification of recently bottlenecked populations is important because such populations may not yet have had time to adapt to the problems often caused by small population size and therefore may have a high risk of extinction. The more recent a bottleneck, the greater the probability that the deleterious effects of a bottleneck can be avoided or minimized by mitigative management procedures, such as habitat enhancement or introduction of immigrants (Luikart et al., 1998). Probability test of recent effective population size reduction using polymorphic loci detected bottlenecks on Lalang, Bubu, and Kinta. Luikart et al. (1998), while restating the assumptions of Cornuet and Luikart (1996), suggested that inclusion of loci deviating from Hardy-Weinberg equilibrium might cause violations. With a similar test, but using polymorphic loci in Hardy-Weinberg equilibrium, genetic bottlenecks were detected only on Lalang (mean $H_e$ = 0.433; mean $H_{eq}$ = 0.293; $P < 0.05$) and Kinta (mean $H_e$ = 0.404; $H_{eq}$ = 0.255; $P < 0.05$). This may suggest that inclusion of loci not in Hardy-Weinberg equilibrium could cause nonbottlenecked populations to appear to have been recently bottlenecked.

A genetic bottleneck can also be detected by the changes in allele frequencies (Nei, Maruyama, and Chakraborty, 1975); recently bottlenecked populations are more likely to have lost their rare alleles than their common alleles. One way of measuring the change in allele frequencies is by examining the ratio of effective to observed number of alleles. If all alleles in a population were present at the same frequencies, this number would be one. The presence of rare alleles or variance in allele frequencies reduces this ratio. The ratios for Lalang and Kinta are 0.70 and 0.75, respectively, and these values are higher than Pasoh (0.63), Lagong (0.68), Bubu (0.69), and Perangin (0.67), suggesting that the allele frequency distributions in Lalang and Kinta are more even, with fewer rare alleles or lower variance in allele frequencies. Both of these patterns would be expected in a population following a bottleneck (Nei, Maruyama, and Chakraborty, 1975)

One of the main genetic effects of inbreeding within populations is to increase levels of homozygosity relative to those expected under conditions of random mating (Wright, 1931; Brown, 1979; Hamrick, Linhart, and Mitton, 1979). Thus, comparisons of genotype frequencies within populations with genotype frequencies expected under Hardy-Weinberg equilibrium conditions (i.e., large populations with no genetic drift, random mating, no selection on alleles, and no inflow of alleles into the population via mutations or gene flow) are often used to detect historical levels of inbreeding in natural populations.

Positive significance of fixation indices ($F_{IS}$), an indication of excess of homozygotes, was observed in Bubu ($F_{IS}$ = 0.155; $P < 0.01$) and Perangin ($F_{IS}$ = 0.117; $P < 0.05$). Populations that have experienced a long period of bottleneck should have high rates of inbreeding, low levels of genetic variation, and fixation of mildly deleterious alleles, which might reduce evolutionary potential and increase the probability of population extinction (summarized by Luikart et al., 1998). In this study, however, high levels of inbreeding in Bubu and Perangin may not be caused by long periods of severe reduction of population sizes because population bottlenecks were not detected in these two populations. This high level of inbreeding might be due to other historical disturbances that could not be deduced from the present study, or the Bubu and Perangin populations may be genetically substructured; sampling across subpopulation patches that differ in gene frequency would probably generate high values of the positive fixation index, due to biparental inbreeding. Numerous studies have shown that most tree species have reduced viability and growth after selfing or other close inbreeding

(reviewed in Williams and Savolainen, 1996). This feature must be considered in the conservation of VJRs because in natural populations, inbreeding may lower the viability of populations (Saccheri et al., 1998).

As summarized by Luikart et al. (1998), populations that have experienced a long period of bottleneck should have high rates of inbreeding and low levels of genetic variation. However, these were not observed in the two bottlenecked populations. The lack of significant levels of inbreeding ($-0.023$ and $-0.070$, respectively) and high levels of observed genetic diversity (0.269 and 0.247, respectively) in Lalang and Kinta might be due to the short time frame of the population bottleneck compared to the generation time of the species. *Intsia palembanica* may grow for 30–35 yr before flowering. Because the severe decline of the Lalang and Kinta populations can only occur within 60–100 yr, it seems unlikely that the populations have experienced a sufficient number of generations at such a small population size that significant genetic variation would be lost. Species with large, outcrossing populations and high levels of variation might actually be the most threatened by reduction in population size due to logging activities and fragmentation (Tilman et al., 1994). If actions are not taken, and the two bottlenecked populations are allowed to exist in small population sizes for a long period of time, they are expected to exhibit lower levels of genetic diversity due to inbreeding. Inbreeding causes the loss of heterozygosity with no change in allele frequencies, because continuous mating between relatives will purge the deleterious recessive alleles and expose them as homozygotes to the environment. Small populations with low levels of genetic diversity might still able to sustain short- to medium-term fitness, but in the long term, they may be unable to respond to environmental changes.

Laidlaw (1994), in the study of locality effects of the VJRs with reference to boundary of the forest reserves, showed that disturbed VJRs are more likely to be found on the edge of forest reserves, whereas undisturbed VJRs are more likely to be found in the center of forest reserves. In addition, her results also indicated that distortions within certain sectors of the mammal community and the vegetation can occur in the more accessible VJRs on the edge of reserves. This analysis agrees with the present study, in which high levels of inbreeding and bottlenecks were detected in VJRs that are located on the edge of forest reserves (Lalang, Bubu, Kinta, and Perangin). Thus, we suggest that establishment of the newly proposed VJRs should consider compartments that are located in the center of the forest reserves.

***Effective population size***—In terms of conservation, the concept of minimum viable population size implies that a population in a given habitat cannot persist if the number of individuals is reduced below a certain threshold (ITTO, 2000). Generally, three broad approaches could be taken to determine the minimum viable population sizes for targeted species: (1) the effective population size could be estimated on the basis of ability to withstand loss of genetic variability due to drift, which is apparent in small populations; (2) the effective population size could be estimated on the basis of the mating system and the minimum number of reproductive individuals required to prevent inbreeding or reduced fitness of regenerants; and (3) the effective population size could be estimated based on calculating the population size that will minimize the sampling loss of alleles occurring at low frequencies (ITTO, 2000). Wright (1931) defined the effective population size as the size of an ideal population whose genetic composition is influenced by random processes in the same way as the real population. Various genetic and ecological approaches have been proposed to estimate the effective population size (reviewed by Nunney and Elam, 1994).

One method for quantitative analysis of effective population size of widespread species that calls for conserving populations that are large enough to maintain current levels of genetic diversity is derived from the equilibrium equation for neutral mutations (Crow and Kimura, 1970). Calculation of effective population size using the equilibrium equation for neutral mutation is very much dependent on mutation rates. The effective population sizes that are required to maintain expected allozyme heterozygosities in equilibrium for most forest trees are large. For instance, in *Pinus ponderosa* and *Pseudotsuga menziesii* populations, when conservative mutation rates are used ($10^{-7}$) in the equilibrium formula, the effective population sizes necessary to maintain the expected allozyme heterozygosities ranged from 223 300 to 910 600 (Millar and Libby, 1991). At appropriate stand densities, these numbers would correspond to areas far larger than are currently protected or potentially can be protected. Recently, evidence has shown that generational mutation rates in long-lived woody plants are higher (e.g., $10^{-3}$) than those in other organisms (Klekowski and Godfrey, 1989; Lowenfeld and Klekowski, 1992). By using mutation rates in the range of $10^{-3}$–$10^{-7}$, the mean effective population sizes for *I. palembanica* were estimated as 80–800 000 individuals. Several criticisms of this approach have been raised; neutral mutations may not be the variants of concern to conservation. The rates of advantageous mutation are not known, but are probably much lower (Millar and Westfall, 1992). Lande and Barrowclough (1987) have shown that estimates of variability based on allozymes are much lower than the estimates based on quantitative traits, even if neutral mutation rates prevail. Another criticism questions the value of maintaining current levels of heterozygosity. Most populations are in nonequilibrium states (Namkoong, 1986), and current levels of population heterozygosity may have little to do with population stability or vitality (Lande, 1988).

The low $G_{ST}$ and $F_{ST}$ among populations and the high rate of historical gene flow among populations might suggest that the populations studied were probably part of one continuous populations in the recent past. To further circumscribe the effective population size, the allozyme data from all the studied populations were pooled for simulation analysis. At sample sizes of $\sim$200 and 210 individuals, the total number of alleles ($A_T$) and expected heterozygosity ($H_e$), respectively, reach asymptotic levels, indicating no alleles will be lost at the sample size $>$200. Reduction of $H_e$ can be avoided if 210 individuals or more were maintained. From the survey data of the big trees in the Pasoh Forest Reserve (Manokaran et al., 1992), the mean density of *I. palembanica* $>$30 cm dbh is $\sim$1.3 trees/ha. Applying this estimate to the effective population sizes based on pooled data may suggest that for the maintenance of allozyme genetic diversity, the effective population size for the species, in term of area size, should be at least 154–162 ha. Hamrick and Murawski (1990) suggested that the effective breeding unit area for a common tropical tree species could be in the order of 25–50 ha (estimation based on pollen movement within and among populations). With a similar method, Konuma et al. (2000) reported that the breeding unit area for five reproductive trees of *Neobalanocarpus heimii* was 86.3

ha. However, for figs (*Ficus* sp.), a tropical keystone plant resource, the breeding unit areas were reported to be 10 600–63 200 ha (Nason, Herre, and Hamrick, 1998). Koski, (1996) in his guideline for the in situ gene conservation of wind-pollinated temperate conifers, without stating a general formula, stated that the target area should be >100 ha, and in areas where the species in question is uncommon, smaller areas (<100 ha) may sometimes be acceptable.

In the zoological literature, Franklin (1980) and Soulé (1980) have proposed the 50/500 rule. According to this rule, a population size of 50 individuals is considered the minimum size necessary to avoid inbreeding depression (based on the experience of animal breeders, who generally accept a 1% increase in the inbreeding coefficient, which increases by $1/[2N]$ each generation), and a population of 500 individuals is deemed sufficient to avoid the risk of genetic drift (derived empirically using evidence from experiments on fruit flies). The universal validity of the 50/500 rule has been challenged frequently, particularly as a general prescription for managing natural populations. In the general consensus, there is no universally applicable population size below which a serious reduction in genetic diversity will occur or above which a population is safe from genetic erosion (Gray, 1996). The concept of genetically effective population size refers to the risk of extinction from genetic causes; to this we also must add the risks imposed by environmental stochasticity, which are often harder to estimate (Stewart and Hutchings, 1996). Our estimate was solely based on genetics. Moreover, the simulation analysis will undoubtedly provide different estimates of effective population sizes between species and between populations, depending on levels of genetic diversity and population density. Hence, this estimate should be considered as exploratory, preliminary information on the management of the species and the VJRs.

## LITERATURE CITED


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

APPANAH, S., AND G. WEINLAND. 1993. Planting quality timber trees in Peninsular Malaysia: a review. Malayan Forest Records No. 38, Forest Research Institute Malaysia, Kepong, Kuala Lumpur, Malaysia.

BARTON, N. H., AND M. SLATKIN. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* 56: 409–415.

BORHAN, M., AND L. C. CHEAH. 1986. The Virgin Jungle Reserves of Peninsular Malaysia: a need for a positive management strategy. *In* H. Yusuf, A. Kamis, M. Nik Muhamed, and M. Shukri [eds.], Proceedings of regional workshop on impact of man's activities on tropical upland forest ecosystems, 81–90. Faculty of Forestry, Universiti Pertanian Malaysia, Selangor, Malaysia.

BROWN, A. H. D. 1979. Enzyme polymorphism in plant populations. *Theoretical Population Biology* 15: 1–42.

BROWN, A. H. D., AND R. W. ALLARD. 1970. Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. *Genetics* 66: 133–145.

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

CORNUET, J. M., AND G. LUIKART. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144: 2001–2014.

CROW, J. F., AND M. KIMURA. 1970. An introduction to population genetics theory. Harper and Row, New York, New York, USA.

DI RIENZO, A., A. C. PETERSON, J. C. GARZA, A. M. VALDES, M. SLATKIN, AND M. B. FREIMER. 1994. Mutational processes of simple sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences, USA* 91: 3166–3170.

DOLIGEZ, A., AND H. I. JOLY. 1997. Mating systems of *Carapa procera* (Meliaceae) in the French Guiana tropical forest. *American Journal of Botany* 84: 461–470.

FALK, D. A., AND K. E. HOLSINGER. 1991. Genetics and conservation of rare plants. Oxford University Press, New York, New York, USA.

FAO. 1984. A guide to in situ conservation of genetic resources of tropical woody species Food and Agriculture Organization (FAO), Rome, Italy.

FRANKEL, O. H., A. H. D. BROWN, AND J. J. BURDON. 1995. The conservation of plant biodiversity. Cambridge University Press, Cambridge, UK.

FRANKLIN, I. A. 1980. Evolutionary change in small population. *In* M. E. Soulé and B. A. Wilcox [eds.], Conservation biology: an evolutionary-ecological perspective, 135–150. Sinauer Associates, Sunderland, Massachusetts, USA.

GITZENDANNER, M., AND P. S. SOLTIS. 2000. Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* 87: 783–792.

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 (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86: 485–486.

GRAY, A. J. 1996. The genetic basis of conservation. *In* I. F. Spellerberg [ed.], Conservation biology, 107–121. Longman, Singapore.

HAMRICK, J. L. 1993. Genetic diversity and conservation in tropical forest. *In* R. M. Drysdale, S. E. T. John, and A. C. Yapa [eds.], Proceedings of the ASEAN-Canada symposium on genetic conservation and production of tropical tree seed, 1–9. ASEAN-Canada Forest Tree Seed Center, Muaklek, Saraburi, Thailand.

HAMRICK, J. L., AND M. J. W. GODT. 1989. Allozyme diversity in plant species. *In* A. H. D. Brown, M. J. Clegg, A. L. Kahler, and B. S. Weir [eds.], Plant population genetics, breeding and genetic resources, 43–63. Sinauer Associates, Sunderland, Massachusetts, USA.

HAMRICK, J. L., AND M. J. W. GODT. 1996a. Conservation genetics of endemic plant species. *In* J. C. Avise and J. L. Hamrick [eds.], Conservation genetics: case histories from nature, 281–304. Chapman and Hall, London, UK.

HAMRICK, J. L., AND M. J. W. GODT. 1996b. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions: Biological Sciences (The Royal Society)* 351: 1291–1298.

HAMRICK, J. L., M. J. W. GODT, D. A. MURAWSKI, AND M. D. LOVELESS. 1991. Correlation between species traits and allozyme diversity: implications for conservation biology. *In* D. A. Falk and K. E. Holsinger [eds.], Genetics and conservation of rare plants, 75–83. Oxford University Press, New York, New York, USA.

HAMRICK, J. L., Y. B. LINHART, AND J. B. MITTON. 1979. Relationship between life history characteristic and electrophoretically detectable genetic variation in plants. *Annual Review of Ecology and Systematics* 10: 173–200.

HAMRICK, J. L., AND D. A. MURAWSKI. 1990. The breeding structure of tropical tree populations. *Plant Species Biology* 5: 157–165.

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

HOLSINGER, K. E. 1996. The scope and limits of conservation genetics. *Evolution* 50: 2558–2561.

HOLSINGER, K. E., AND L. D. GOTTLIEB. 1991. Conservation of rare and endangered plants: principles and prospects. Oxford University Press, New York, New York, USA.

ITTO. 2000. Technical guidelines for the establishment and management of in situ conservation stands of tropical timber species. International Tropical Timber Organization, Yokohama, Japan.

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

JAMES, T., S. VEGA, P. ALDRICH, AND J. L. HAMRICK. 1998. Mating system of three tropical dry forest tree species. *Biotropica* 30: 587–594.

KIMURA, M., AND J. F. CROW. 1964. The number of alleles that can be maintained in a finite population. *Genetics* 49: 725–738.

KITAMURA, K., M. Y. ABDUL RAHMAN, Y. OCHIAI, AND H. YOSHIMARU. 1994. Estimation of the outcrossing rate on *Dryobalanops aromatica*

Gaertn. f. in primary and secondary forest in Brunei, Borneo, Southeast Asia. *Plant Species Biology* 9: 37–41.

KLEKOWSKI, E. J., JR., AND P. J. GODFREY. 1989. Aging and mutation in plants. *Nature* 340: 389–391.

KOCHUMMEN, K. M. 1997. Tree flora of Pasoh forest. Malayan Forest Records No. 44. Forest Research Institute Malaysia, Kepong, Kuala Lumpur, Malaysia.

KOCHUMMEN, K. M., J. V. LA FRANKIE, AND N. MANOKARAN. 1990. Floristic composition of Pasoh Forest Reserve, a lowland rain forest in Peninsular Malaysia. *Journal of Tropical Forest Science* 3: 1–13.

KONUMA, A., Y. TSUMURA, C. T. LEE, S. L. LEE, AND T. OKUDA. 2000. Estimation of gene flow in the tropical-rainforest tree *Neobalanocarpus heimii* (Dipterocarpaceae), inferred from paternity analysis. *Molecular Ecology* 9: 1843–1852.

KOSKI, V. 1996. Management guidelines for in situ conservation of wind pollinated temperate conifers. *Forest Genetic Resources* 24: 1–7.

LAIDLAW, R. K. 1994. The Virgin Jungle Reserves of Peninsular Malaysia: the ecology and dynamics of small protected areas in managed forest. Ph.D. dissertation, University of Cambridge, Cambridge, UK.

LANDE, R. C. 1988. Genetics and demography in biological conservation. *Science* 241: 1455–1460.

LANDE, R. C., AND G. BARROWCLOUGH. 1987. Effective population size, genetic variation, and their use in population management. *In* M. E. Soulé [ed.], Viable populations for conservation, 87–124. Cambridge University Press, Cambridge, UK.

LANDE, R. C., AND S. SHANNON. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* 50: 434–437.

LEE, S. L. 2000. Mating system parameters of *Dryobalanops aromatica* Gaertn. f. (Dipterocarpaceae) in three different forest types and a seed orchard. *Heredity* 85: 338–345.

LEE, S. L., K. C. ANG, AND M. NORWATI. 2000. Genetic diversity of *Dryobalanops aromatica* Gaertn. f. (Dipterocarpaceae) in Peninsular Malaysia and its pertinence to genetic conservation and tree improvement. *Forest Genetics* 7: 209–217.

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

LEE, S. L., R. WICKNESWARI, M. C. MAHANI, AND A. H. ZAKRI. 2000b. Inheritance of allozyme variants in *Shorea leprosula* Miq. (Dipterocarpaceae). *Journal of Tropical Forest Science* 12: 124–138.

LEE, S. L., R. WICKNESWARI, M. C. MAHANI, AND A. H. ZAKRI. 2000c. Mating system parameters in a tropical tree species, *Shorea leprosula* Miq. (Dipterocarpaceae) from Malaysia Lowland Dipterocarp Forest. *Biotropica* 32: 429–438.

LEWIS, P. O., AND D. J. CRAWFORD. 1995. Pleistocene refugium endemics exhibit greater allozyme diversity than widespread congeners in the genus *Polygonella* (polygonaceae). *American Journal of Botany* 82: 141–149.

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

LOWENFELD, R., AND E. J. KLEKOWSKI, JR. 1992. Mangrove genetics. 1. Mating system and mutation rates of *Rhizophora mangle* in Florida and San Salvador Island, Bahamas. *International Journal of Plant Sciences* 153: 394–399.

LUIKART, G., F. W. ALLENDORF, J.-M. CORNUET, AND W. B. SHERWIN. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity* 89: 238–247.

LUIKART, G., AND J.-M. CORNUET. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology* 12: 228–237.

MANOKARAN, N., J. V. LA FRANKIE, K. M. KOCHUMMEN, E. S. QUAH, J. E. KLAHN, P. S. ASHTON, AND S. P. HUBBELL. 1992. Stand table and distribution of species in the 50-ha research plot at Pasoh Forest Reserve. Forest Research Institute Malaysia, Kuala Lumpur, Malaysia.

MARUYAMA, T., AND P. A. FUERST. 1985. Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics* 111: 675–689.

MATEU-ANDRÉS, I., AND J. G. SEGARRA-MORAGUES. 2000. Population subdivision and genetic diversity in two narrow endemics of *Antirrhinum* L. *Molecular Ecology* 9: 2981–2987.

MILLAR, C. I., AND W. J. LIBBY. 1991. Strategies for conserving clinal ecotypic, and disjunct population diversity in widespread species. *In* D. A. Falk and K. E. Holsinger [eds.], Genetic and conservation of rare plants, 149–170. Oxford University Press, New York, New York, USA.

MILLAR, C. I., AND R. D. WESTFALL. 1992. Allozyme markers in forest genetic conservation. *In* W. T. Adam, S. H. Strauss, D. L. Copes, and A. R. Griffin [eds.], Proceedings of the international symposium on population genetics of forest tress, 347–371. Kluwer Academic, Dordrecht, The Netherlands.

MÜLLER-STARCK, G., AND H.-R. GREGORIUS. 1986. Monitoring genetic variation in forest tree populations. Proceedings of the 18th International Union of Forestry Research Organization (IUFRO) world congress, division 2, vol. II, forest plants and forest protection, 589–599. Yugoslav IUFRO World Congress, Vienna, Yugoslavia.

MURAWSKI, D. A., AND K. S. BAWA. 1994. Genetic structure and mating system of *Stemonoporus oblongifolius* (Dipterocarpaceae) in Sri Lanka. *American Journal of Botany* 81: 155–160.

MURAWSKI, D. A., B. DAYANANDAN, AND K. S. BAWA. 1994. Outcrossing rates of two endemic *Shorea* species from Sri Lankan tropical rain forests. *Biotropica* 26: 23–29.

MURAWSKI, D. A., I. A. U. N. GUNATILLEKE, AND K. S. BAWA. 1994. The effect of selective logging on inbreeding in *Shorea megistophylla* (Dipterocarpaceae) from Sri Lanka. *Conservation Biology* 8: 997–1002.

MURAWSKI, D. A., AND J. L. HAMRICK. 1991. The effect of density of flowering individuals on the mating systems of nine tropical tree species. *Heredity* 67: 167–174.

MURAWSKI, D. A., AND J. L. HAMRICK. 1992a. Mating system and phenology of *Ceiba pentandra* (Bombacaceae) in Central Panama. *Journal of Heredity* 83: 401–404.

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

NAMKOONG, G. 1986. Genetics and the forests of the future. *Unasylva* 38: 2–18.

NASON, J. D., E. A. HERRE, AND J. L. HAMRICK. 1998. The breeding structure of a tropical keystone plant resource. *Nature* 391: 685–687.

NEI, M. 1973. Analysis of genetic diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321–3323.

NEI, M. 1975. Molecular population genetics and evolution. North-Holland Publishing, Amsterdam, The Netherlands.

NEI, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* 41: 225–233.

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

NEI, M., T. MARUYAMA, AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in population. *Evolution* 29: 1–10.

NUNNEY, L., AND D. R. ELAM. 1994. Estimating the effective population size of conserved populations. *Conservation Biology* 8: 175–184.

OHTA, T., AND M. KIMURA. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research* 22: 201–204.

O'MALLEY, D. M., D. P. BUCKLEY, G. T. PRANCE, AND K. S. BAWA. 1988. Genetics of Brazil nut (*Bertholletia excelsa* Humb & Bonpl.: Lecythidaceae). 2. Mating system. *Theoretical and Applied Genetics* 76: 929–932.

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

PIRY, S., G. LUIKART, AND J.-M. CORNUET. 1998. BOTTLENECK (version 1.2.02), a program for detecting recent population size reduction from allele frequency data. Available from: http://www.ensam.inra.fr/URLB/bottleneck/bottleneck.html. Based on J. M. Cornuet and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144: 2001–2014.

RAYMOND, M., AND F. ROUSSET. 2000. GENEPOP (version 3.2a). Available from: ftp://ftp.cefe.cnrs-mop.fr/pub/PC/MSDOS/GENEPOP/. Updated from M. Raymond and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 83: 239.

RIGGS, L. A. 1990. Conserving genetic resources on-site in forest ecosystem. *Forest Ecology and Management* 35: 45–68.

RITLAND, K. 1996. Multilocus mating system program MLTR (version 1.1). Available from: http://genetics.forestry.ubc.ca/ritland/programs.html. Up-

dated from K. Ritland. 1990. A series of FORTRAN computer programs for estimating plant mating systems.*Journal of Heredity* 81: 235–237.

RITLAND, K., AND S. K. JAIN. 1981. A model for the estimation of outcrossing rate and gene frequencies based on independent loci. *Heredity* 47: 37–54.

ROUSSET, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219–1228.

ROUSSET, F., AND M. RAYMOND. 1995. Testing heterozygote excess and deficiency. *Genetics* 140: 1413–1419.

SACCHERI, I., M. KUUSSAARI, M. KANKARE, P. VIKMAN, W. FORTELIUS, AND I. HANSKI. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392: 491–494.

SHAFFER, M. L. 1981. Minimum population sizes for species conservation. *Bioscience* 31: 131–134.

SIMBERLOFF, D. S., AND L. G. ABELE. 1982. Refuge design and island biogeographic theory: effects of fragmentation. *American Naturalist* 120: 41–50.

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

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy. W. H. Freeman, San Francisco, California, USA.

SOULÉ, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. *In* M. E. Soulé and B. A. Wilcox [eds.], Conservation biology: an evolutionary-ecological perspective, 111–124. Sinauer Associates, Sunderland, Massachusetts, USA.

STEWART, A. J. A., AND M. J. HUTCHINGS. 1996. Conservation of population. *In* I. F. Spellerberg [ed.], Conservation biology, 122–140. Longman, Singapore.

STRUGNELL, E. J. 1937. Delayed germination of merbau. *Malayan Forester* VI: 271.

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

THANG, H. C. 1998. Formulation and implementation of criteria and indicators for sustainable forest management in Malaysia. *In* S. Appanah, M. Samsudin, H. C. Thang, and P. Ismail [eds.], Forest management certification workshop proceedings, 29–93. Forest Research Institute Malaysia, Kuala Lumpur, Malaysia.

TILMAN, D., R. M. MAY, C. L. LEHMAN, AND M. A. NOWAK. 1994. Habitat destruction and extinction debt. *Nature* 371: 65–66.

VAN-WRIGHT, R. I., C. J. HUMPHRIES, AND P. H. WILLIAM. 1991. What to protect? Systematics and the agony of choice. *Biological Conservation* 55: 235–254.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.

WENDEL, J. F., AND N. F. WEEDEN. 1989. Visualization and interpretation of plant isozymes. *In* D. E. Soltis and P. S. Soltis [eds.], Isozyme in plant biology, 5–45. Dioscorides, Portland, Oregon, USA.

WILLIAM, C. G., AND O. SAVOLAINEN. 1996. Inbreeding depression in conifers: implications for breeding strategy. *Forest Science* 42: 102–117.

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

WRIGHT, S. 1951. The genetic structure of population. *Annals of Eugenetics* 15: 323–354.

WYATT-SMITH, J. 1950. Virgin jungle reserves. *Malayan Forester* 13: 92–94.

WYATT-SMITH, J. 1953. Manual of Malayan timber trees: Leguminosae. FRI Kepong Research Pamphlet No. 2. Forest Research Institute Malaysia, Kuala Lumpur, Malaysia.

WYATT-SMITH, J. 1963. Manual of Malayan Silviculture for Inland Forests. Malayan Forest Records No. 23. Forest Research Institute Malaysia, Kuala Lumpur, Malaysia.

YOUNG, A. G., AND H. D. BROWN. 1996. Comparative population genetic structure of the rare woodland shrub *Daviesia suaveolens* and its common congener *D. mimosoides*. *Conservation Biology* 10: 1220–1228.

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