MAINTENANCE OF GENETIC DIVERSITY IN *PARKIA SPECIOSA* IN LOGGED-OVER FORESTS

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LEE, C. T., WICKNESWARI, R., MAHANI, M. C. & ZAKRI, A. H. 2002. Maintenance of genetic diversity in *Parkia speciosa* in logged-over forests. This study examined the effects of a single logging event (taken place more than 40 years ago) on the genetic diversity of *Parkia speciosa* in a lowland dipterocarp forest. Random amplified polymorphic DNA (RAPD) markers were used to estimate the genetic diversity parameters, namely, Shannon's diversity index, mean number of alleles per locus, effective number of alleles per locus, Nei's gene diversity and percentage of polymorphic loci. A total of 27 to 33 adult *P. speciosa* trees from three adjacent forest management units in Pasoh Forest Reserve, i.e., the Unlogged Stand (control site), Regenerated Stand 1 (logged in 1951) and Regenerated Stand 2 (logged in 1955) were analysed. RAPD analyses using seven 10-mer arbitrary primers yielded a total of 51 consistent loci. The *t*-tests showed that the overall genetic diversity measures of *P. speciosa* from the two regenerated stands were not significantly different from the control site. With the assumption that these three "subpopulations" were genetically identical before logging, this may indicate that a single logging event under the Malayan Uniform System (MUS) of harvesting practice did not cause genetic erosion in *P. speciosa.*

Key words: Genetic diversity - logging - *Parkia speciosa* - RAPD - Popgene - sample size

LEE, C. T., WICKNESWARI, R., MAHANI, M. C. & ZAKRI, A. H. 2002. Kemapanan kepelbagaian genetik *Parkia speciosa* di hutan sudah kerja. Kajian ini menguji kesan pembalakan tunggal (berlaku lebih daripada 40 tahun yang lepas) ke atas kepelbagaian genetik *Parkia speciosa* di sebuah hutan pamah dipterokarpa. Penanda polimorfisme DNA teramplifikasi rawak (RAPD) telah digunakan untuk menganggarkan parameter kepelbagaian genetik, iaitu indeks kepelbagaian Shannon, purata bilangan alel bagi setiap lokus, bilangan alel berkesan, kepelbagaian gen Nei dan peratus lokus berpolimorfik. Sejumlah 27 hingga 33 pokok *P. speciosa* matang daripada tiga unit pengurusan hutan di Hutan Simpan Pasoh, yakni Dirian Tanpa Balak (tapak kawalan), Dirian Terpulih 1 (yang telah dibalak pada tahun 1951) dan Dirian Terpulih 2 (yang telah dibalak pada tahun 1955) telah dianalisis. Analisis RAPD menggunakan tujuh primer rawak 10-mer telah menghasilkan 51 lokus malar. Secara keseluruhan, ujian-*t* menunjukkan bahawa nilai kepelbagaian genetik *P. speciosa* daripada kedua-dua dirian terpulih tersebut tidak berbeza secara bererti berbanding dengan tapak kawalan. Dengan andaian bahawa "subpopulasi" tersebut adalah seiras dari segi genetik sebelum pembalakan, ini menunjukkan bahawa kejadian pembalakan tunggal di bawah amalan penebangan Sistem Seragam Malaya (MUS) tidak mengakibatkan hakisan kepelbagaian genetik dalam spesies *P. speciosa.*

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Introduction

Biodiversity includes genetic, species and ecosystem diversity. Though at the lowest hierarchy, genetic diversity is nonetheless very important as the ability of the populations to withstand or adapt the present and future environmental perturbation is very much dependent on the extent and pattern of genetic diversity of a particular species (Given 1994, Templeton 1995). Plant genetic resource erosion may also lead to the impoverishment of initial materials for plant breeding programmes and the loss of potentially useful genes. Hence, it is important to monitor the impact of logging on tropical rain forest genetic resources. Loss of genetic diversity following logging disturbance has been observed in white pine (Buchert *et al.* 1997), *Cycas siamensis* (Changtragoon 1997) and a few tropical rain forest tree species (Wickneswari *et al.* 1997) with varied severity. On the other hand, the genetic diversity of some pioneer species was enhanced in disturbed forests (Chaisurisri *et al.* 1997). For the non-harvested plant species, logging may also impact the genetic diversity indirectly, such as through incidental damage or death of individuals during logging operation and through disruption of the pollination system.

Genetic diversity assessment has been greatly facilitated by the development of molecular markers based on polymorphism found in proteins or DNA. Besides isozymes analysis, various DNA techniques have been used in plant breeding and population genetic studies, such as restriction fragment length polymorphism (RFLP) (Tanksley *et al.* 1989), random amplified polymorphic DNA (RAPD) (Welsh & McClelland 1990, Williams *et al.* 1990), sequence characterised amplified region (SCAR) (Paran & Michelmore 1993), amplified fragment length polymorphism (AFLP) (Vos *et al.* 1995) and simple sequence repeats (SSRs) or microsatellites (Chase *et al.* 1996). Selection of molecular markers often depends on their distinct advantages and disadvantages based on their levels of polymorphism, mode of inheritance and cost effectiveness (Forrest 1994, Ferguson *et al.* 1995) besides the availability of facilities and practicality.

Parkia speciosa (Leguminosae) is an important non-timber forest product in Malaysia, locally known as petai. It is normally not logged due to its low quality timber (Burkill 1966). This species occurs in Thailand, Sumatra, Peninsular Malaysia, Borneo, Java and the Philippines (Hopkins 1994). It is famous for its edible fruits, eaten raw or cooked. Harvesting of *P. speciosa* from the forests is an important source of income to the rural household with an estimated potential annual value of RM47.4 million in Peninsular Malaysia (Woon & Poh 1998). *Parkia speciosa* is postulated to have anti-diabetic properties (Gmelin *et al.* 1981) and its aqueous extract is able to reduce blood pressure in hypertensic rats (Suvachittanot & Peutpaiboon 1992).

In this study, impacts of logging on the genetic diversity of *P. speciosa* were investigated. Genetic diversity measures of *P. speciosa* from two logged-over forest management units (FMUs) and an unlogged forest of a continuous population were compared, with the assumption that they were genetically identical before logging. RAPD was used in the genetic diversity estimation because it is highly

polymorphic, applicable to a wide range of species and generally requires no purification of the target DNA. Results from this study were compared with an earlier study using isozyme markers (Wickneswari *et al.* 1997), whereby no significant differences were detected in the genetic diversity measures of *P. speciosa* between the logged-over and unlogged FMUs.

Materials and methods

Sampling sites and plant materials

Pasoh Forest Reserve is a mixed dipterocarp lowland forest, located about 8 km from Simpang Pertang in the state of Negeri Sembilan, Malaysia at 2° 59' N latitude and 102° 19' E longitude, at 80–120 m asl. This forest reserve of 2450 ha is surrounded on three sides by oil palm plantations and joined to a virgin hill dipterocarp forest on its north-eastern boundary (Lee 1995). It has FMUs that are unlogged and those logged under the Malayan Uniform System (MUS). In the MUS harvesting practice, all commercial trees more than 45 cm diameter at breast height (dbh) can be logged if regenerants of the species are sufficient. Three adjacent FMUs were selected for this study, (1) Unlogged Stand (US, Compartment 22 and a 50-ha demographic plot), (2) Regenerated Stand 1 logged in 1951 (RS1, Compartment 21) and (3) Regenerated Stand 2 logged in 1955 (RS2, Compartment 25) (Figure 1). A total of 27, 33 and 33 inner bark samples of adult *P. speciosa* (with dbh > 20 cm) were randomly collected from the US, RS1 and RS2 respectively.[1]

Estimation of relative disturbance levels

Basal area per unit area (m^2 ha^{-1}), tree density per unit area, basal area and number of trees by dbh classes and species richness (cumulative number of species) were estimated for the three FMUs to compare the disturbance levels for the RS1 and RS2 with US as the control. Dbh was classified into four groups, namely, 10–15 cm (poles), > 15–30 cm (small trees), > 30–45 cm (medium trees) and above 45 cm (large trees). These parameters were calculated based on the demographic data of several ecological plots in each FMUs. The demographic data of five randomly selected 20 × 20 m plots (census of year 1995) in the US and RS2 was acquired from the Biodiversity Division of the Forest Research Institute Malaysia (FRIM). The 50-ha plot was established by FRIM in collaboration with the National Science Foundation and the Smithsonian Tropical Research Institute, USA in 1985 whereas the ecological plots in Compartment 25 were established in collaboration with Oversea Development Administration (ODA), UK. With the help of the Biodiversity Division, FRIM, demographic data from five random 20 × 20 m plots in the RS1 were obtained in July 1997.

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[1]Originally 34 samples each were collected from RS1 and RS2, but after RAPD analysis, one sample each from these two FMUs was discarded from further analysis (explaination in results and discussion).

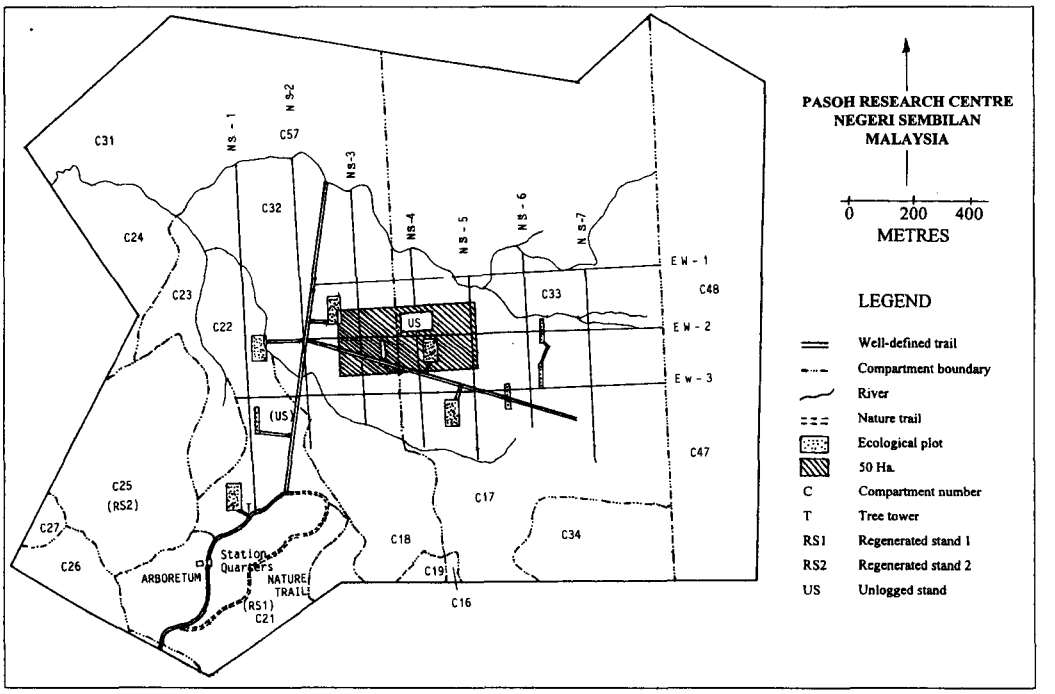

Figure 1 Pasoh Forest Research map showing the three forest management units. US - Unlogged Stand, RS1-Regenerated Stand 1, RS2 - Regenerated Stand 2

DNA extraction

Total DNA was extracted using a modified CTAB method of Murray and Thompson (1980). Approximately 5 g of inner bark tissue was ground in a homogeniser (Iwatani Co.). The frozen grindate was immediately transferred into a Nunc tube (Falcon) with 20 ml of CTAB extraction buffer (20 mM Na_2EDTA, 100 mM TrisHCl pH 8.0, 1.4 M NaCl, 1% PVP-40, 2% CTAB, 0.2% 2-mercaptoethanol) and was incubated at 60 °C for about 30 min to 1 hour. Following this, 20 ml of chloroform-isoamyl alcohol (24:1) was then added and mixed gently for 15 min. After centrifuging at 3000 rpm for 10 min, the aqueous layer was transferred to a new tube. Two-thirds volume of cold (- 20 °C) propan-2-ol was added and mixed gently to precipitate the nucleic acids. Precipitated DNA was dissolved in TE (10 mM TrisHCl pH 8.0, 1 mM Na_2EDTA). DNA concentration was estimated using 0.85% agarose gel electrophoresis in comparison with calf thymus DNA concentration markers (Boehringer Mannheim).

Random amplified polymorphic DNA

The polymerase chain reaction (PCR) mixture (20 µl) consisted of approximately 1 ng of DNA, 1.5 mM $MgCl_2$, 0.17 mM of each dNTP, 1 unit *Taq* polymerase (Perkin Elmer), 1 × PCR buffer and 0.25 µM primer (Operon Technologies). Each reaction was overlaid with mineral oil (Sigma) to prevent evaporation. All PCR

reactions were carried out in an Omnigene Thermal Cycler (Hybaid) with 40 cycles of 1 min at 92 °C, 3 min at 34 °C and 2 min at 72 °C, followed by a final extension step of 72 °C for 3 min using block control. All reactions were held at 29 °C prior to analysis. A total of 100 primers (Operon Kit A, B, C, H, J) were screened for polymorphism, specificity and consistency.

Amplification products were separated in a 2.0% agarose gel containing 0.5 µg/ml of ethidium bromide in 1 × TAE buffer, pH 8.0 (40 mM Tris-acetate, 1 mM Na_2EDTA). After electrophoresis, the gel was soaked in 1 µg/ml ethidium bromide solution with gentle agitation for 10 to 20 min and visualised under UV light. Polaroid 667 film was used for gel documentation. The fragment size of amplified bands were determined using a DNA sizing program on Microsoft Excel with reference to a 100 bp DNA marker (Promega).

Southern analysis

Southern analysis was performed in this study to determine if the RAPD products of similar molecular weights generated by a particular primer were homologous besides testing whether these bands were non-allelic. Only one random locus per primer was tested. Separated RAPD products in the agarose gel were blotted onto nylon membranes following the procedures described by Sambrook *et al.* (1989). RAPD bands for the use as probes were extracted from the agarose gel using the BIO 101 Geneclean Kit. Labelling of probes, hybridisation and immunological detection were carried out using a non-radioactive DNA labelling and detection kit (DIG System of Boehringer Mannheim) following the manufacturer's instructions.

Data scoring and analysis

Amplified bands were scored present (1) or absent (0) regardless of band intensities. Ambiguous data and unsuccessful amplifications were scored as missing data. Shannon's index of phenotypic diversity *(H)* was estimated with the formula $H = - \sum p_i \ln p_i$, where p_i is the frequency of phenotype i (King & Schaal 1989).

Genotypic diversity measures were estimated using a software for population genetics, Popgene Version 1.2. Each scored band was considered as an independent locus with only two alleles, namely, allele-1 and allele-0 with each RAPD marker being a diploid dominant marker. Null-allele (allele-0) frequencies were corrected for potential deviations from Hardy-Weinberg equilibrium based on the fixation indices (F_{is}) obtained from a similar study using isozymes (Wickneswari *et al.* 1997). However, as two misidentified samples were detected through RAPD analysis, isozymes data analysis excluding these samples was newly performed. The F_{is} values obtained for US, RS1 and RS2 were 0.091, - 0.154 and 0.106 respectively (results not published). The parameters computed from the RAPD data matrix included Nei's gene diversity index (H) (Nei 1973), mean number of alleles per locus (A), effective number of alleles per locus (A_e) and the percentage of polymorphic loci at 0.99 criterion (P).

Analysis of relationship between sample size and genetic diversity

Originally 30 samples were collected for the US but three were excluded because there was no DNA yield probably due to mishandling during sampling of inner bark tissue or DNA extraction. Due to the difference in sample sizes for the regenerated stands compared with the unlogged stand (33 and 27 respectively), the effect of sample size on the genetic diversity measures was analysed to avoid bias caused by sampling error. Genetic diversity parameters mentioned above were computed for different sample size classes (5, 10, 15, 20, ...) based on randomly chosen samples from the US. Graph of genetic diversity against number of samples was plotted.

Results and discussion

Relative disturbance levels

The estimated mean basal area per unit area for RS1 and RS2 were reduced by 13.5 and 40.7% respectively in comparison with the US (Figure 2a), with the large tree diameter class being most affected (Figure 2b). Assuming that these three FMUs were homogeneous before logging, this may infer that the logging intensity in the RS2 (logged in 1955) was relatively higher than in RS1 (1951). There was an increase in tree density for both the regenerated stands (28.7 % in RS1 and 18.3% in RS2) compared with the US (Figure 2c) with the poles and small tree diameter classes showing the largest increases (Figure 2d). There was no distinct difference in species number for the three FMUs (Figure 2e) although the species composition has changed.

Random amplified polymorphic DNAs and Southern analysis

The primers screened were evaluated based on the criteria of polymorphism, specificity and consistency. OPJ primers generally did not give consistent amplifications. More than 50% of the primers screened yielded considerable number of distinctive and polymorphic bands. Nevertheless, due to budget constraint, only seven primers which yielded highly repeatable, very distinctive amplified products were selected, namely, OPA04, OPB07, OPB12, OPC07, OPH07, OPH15 and OPJ18. A total of 51 reproducible RAPD products ranging from 400 bp to 1.4 kb were scored with an average of seven scorable bands per primer. More than 50% of the RAPD loci were polymorphic with 18 being non-polymorphic for all the three FMUs.

Williams *et al.* (1993) have demonstrated that RAPD amplifications are extremely sensitive to single-base changes in the primer-target site and that RAPD can be highly useful for phylogenetic analysis among closely related species. RAPD profiles generated by some primers consistently showed distinctively different banding patterns (Figure 3) for two samples (one from RS1 and one from RS2). These two samples were thus, inferred as misidentified species during sample collection and

subsequently excluded from data analysis. They were probably of closely related species occurring in the regenerated stands. In contrary, these misidentified samples were not detected during the previous isozymes analysis using a total of nine loci (with six being polymorphic). This is probably due to the limited number of loci assayed and also the lower polymorphism of isozyme markers.

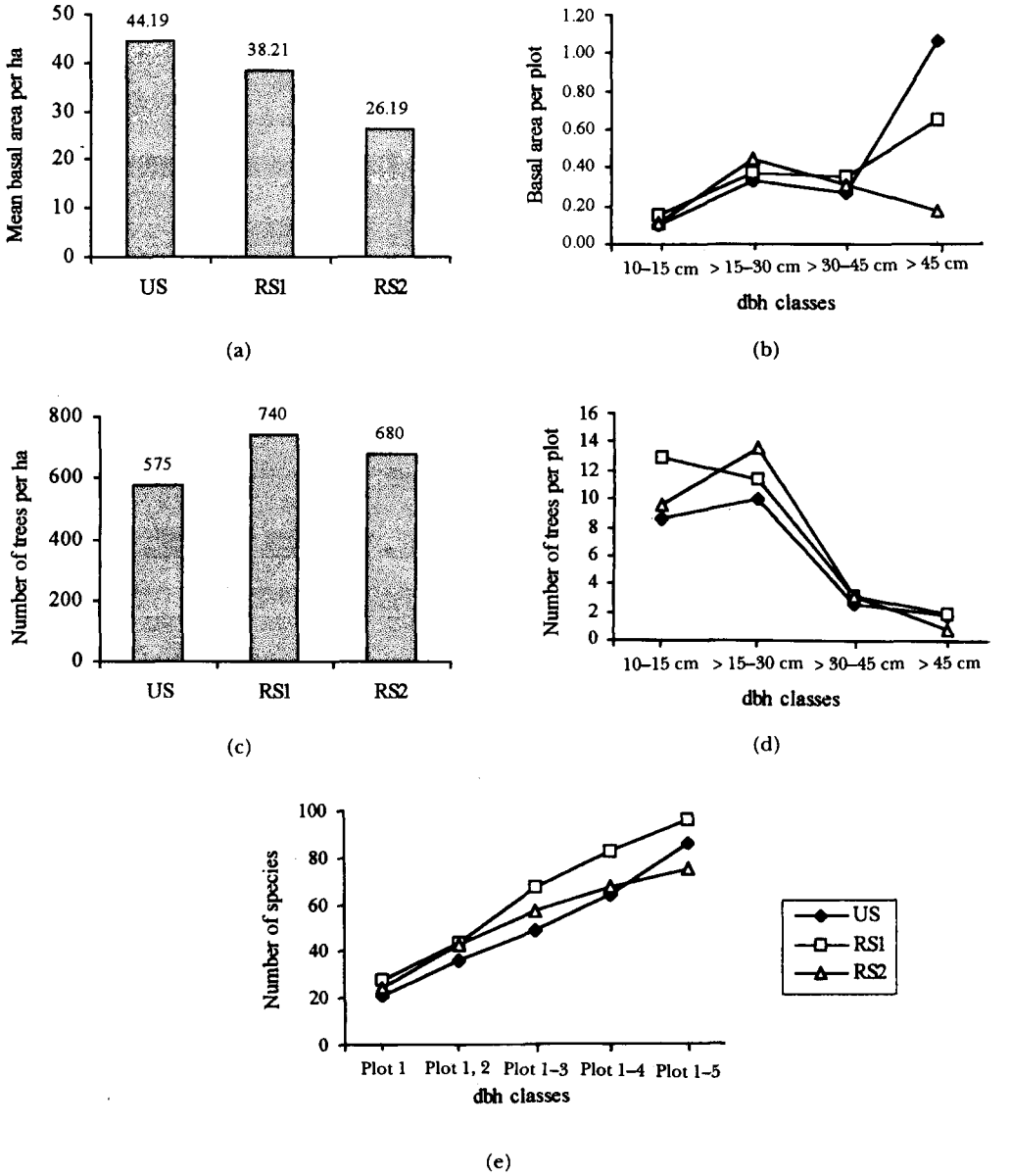

Figure 2 Illustrations reflecting the relative disturbance levels of Regenerated Stand 1 (RS1) and Regenerated Stand 2 (RS2) in comparison with Unlogged Stand (US): (a) mean basal area per unit area (m^2 per ha), (b) mean basal area per plot by diameter classes, (c) mean density (number of trees per ha), (d) mean density (number of trees per plot) by diameter classes, and (e) species richness

The objectives of Southern analysis were to test the homology and independence of RAPD bands with the same mobility, generated by the same primer. Non-related, co-migrating RAPD products have been detected in *Xanthomonas* strains (Smith *et al.* 1994) and *Brassica* species (Quiros *et al.* 1995). However, the problem is only critical in phylogenetic inferences among different species. Lee *et al.* (1996) in their population genetics study of *Shorea leprosula* (Dipterocarpaceae) in Peninsular Malaysia revealed allelic loci generated by primer OPB20 and these loci were discarded from further analysis. In this study, Southern analysis showed that all the loci tested (one from each primer) were independent/non-allelic and displayed homology across bands of the same size. Therefore, all the scorable bands generated by the seven primers were included for analysis. Figure 4 shows one of the tested bands hybridised to the rest of the bands of similar mobility generated by the primer OPC07.

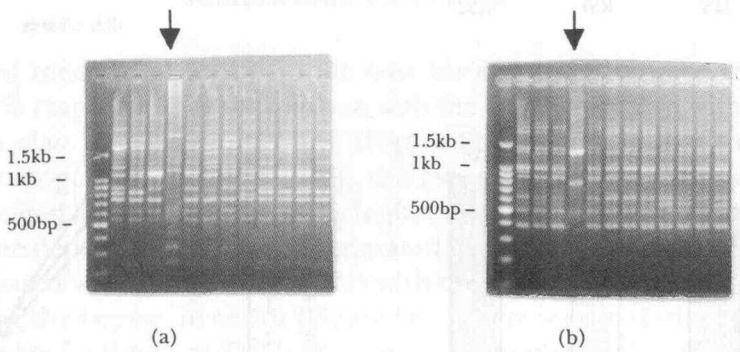

Figure 3 Some of the RAPD profiles generated by primers (a) OPB12 and (b) OPH15 showing the detection of a misidentified sample (arrow)

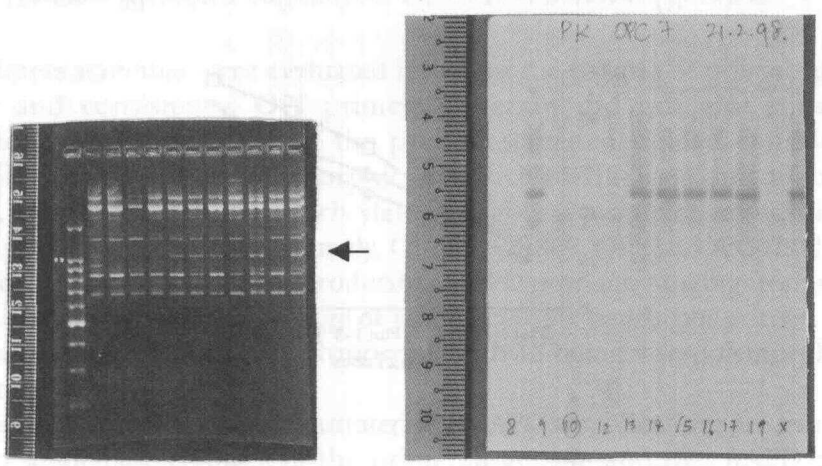

Figure 4 Southern analysis to test homology and non-allelism. (a) RAPD products generated by primer OPC07; band tested is indicated by the arrow, (b) labelled probe hybridised to the rest of the bands of similar mobility.

Measures of genetic diversity

Shannon's index of phenotypic diversity derived from RAPD data has been used as a parameter to quantify genetic diversity in *Glircidia* (Chalmers *et al.* 1992), cocoa, *Theobroma cacao,* (Russell *et al.* 1993), tea, *Camelia sinensis,* (Wachira *et al.* 1995) and Brazil nut, *Bertholletia excelsa,* (Kanashiro *et al.* 1997). Table 1 summarises the Shannon's diversity index by primers for *P. speciosa* from each FMU. Primers varied in their capacity to detect polymorphism within each FMU, of which OPB12 contributed the most polymorphism. Total Shannon's diversity index for the RS1 (7.086) was higher than the US (6.768) and the RS2 (6.449). However, the differences in the diversity indices between the regenerated stands and the US was not significant ($p > 0.05$). Cumulative Shannon's diversity indices were plotted against the number of primers for the three FMUs (Figure 5). From the graphs, it is clear that the estimates of phenotypic diversity of *P. speciosa* from the three FMUs in response to the number of primers used are very similar, indicating maintenance of genetic diversity about 50 years after logging. It is postulated that the same trend would be observed if more primers were to be used. Hence, unlike in cases of genetic diversity evaluation or genetic distance estimation, whereby many (100–150) RAPD markers are needed to generate robust phenograms or relatively repeatable cluster analysis (Ramser *et al.* 1996, Fanizza *et al.* 1999), the number of primers used in this study is enough.

RAPD markers are dominant markers. The method of estimating the null-allele frequencies corrected for deviations from Hardy-Weinberg equilibrium was proposed by Chong *et al.* (1994) based on the assumption that all molecular markers are selectively neutral or nearly neutral. Szmidt *et al.* (1996) provided empirical evidence to support this suggestion, however, higher deviations were found for RAPD loci than for allozyme loci. In this study, null-allele frequencies were corrected from Hardy-Weinberg disequilibrium with the available F_{is} values from isozymes analysis of the same samples (results not published).

Table 1 Shannon's diversity index by primers

Primer	Shannon's diversity index		
	US	RS1	RS2
OPA04	0.664	0.529	0.676
OPB07	0.988	0.866	0.683
OPB12	2.393	2.446	2.134
OPC07	0.634	0.791	0.934
OPH07	0.667	0.432	0.671
OPH15	0.812	1.392	0.907
OPJ18	0.610	0.630	0.444
Total	6.768	7.086	6.449
Mean	0.967	1.012	0.921

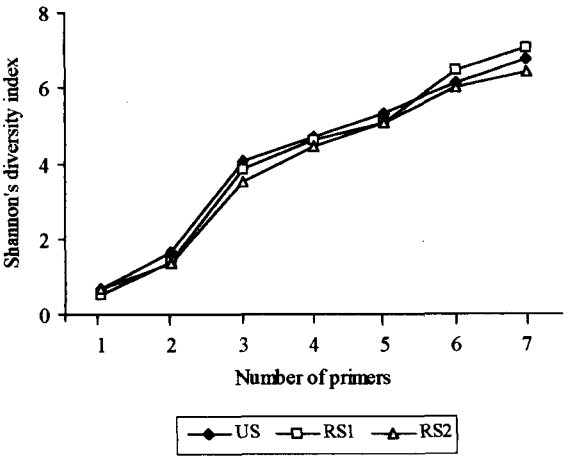

Figure 5 Comparison of Shannon's diversity indices (cumulative) of *Parkia speciosa* in the three forest management units

Besides Shannon's diversity index, mean number of alleles per locus, effective number of alleles per locus, Nei's gene diversity and percentage of polymorphic loci for *P. speciosa* in the three FMUs are presented in Table 2. Based on the results obtained from the US, *P. speciosa* in Pasoh Forest Research had a substantial level of genetic diversity (Shannon's diversity index per primer = 0.967 and 58.8% polymorphic loci). However, the level of genetic diversity revealed may not be applicable for other *P. speciosa* populations. Overall, RS1 exhibited slightly higher measures of genetic diversity compared with US. For example, the mean Shannon's diversity index per primer for RS1 was 1.012 compared with 0.967 in US. In contrast, *P. speciosa* from RS2 had a relatively lower level of genetic diversity, with 13.3% reduction in the percentage of polymorphic loci compared with US. Nevertheless, as a whole, *t*-tests showed no significant differences between the genetic diversity measures for the two regenerated stands and the control site, except for Nei's gene diversity (H). An increment of 11.3% was observed in H for RS1 while there was a 13.2% reduction for RS2. However, this was not conclusive as the standard deviation for H was too high (Table 2). This could be due to the heterogeneity of allele frequencies ranging from 0 to 1 ($H = 1 - \sum x_k^2$, where n = number of alleles at a locus and the frequency of the k^{th} allele = x_k in a population (Nei 1973)).

As the sample size for the US was 27 compared with 33 in both the regenerated stands, the effect of number of samples on genetic diversity was determined (Figure 6). The graph indicates that the effect of sample size is negligible for all parameters except for the percentage of polymorphic loci. Even so, there is a plateau after sample size of 25. Hence, the possibility of misleading results caused by uneven sample size is minimal. With the assumption that the three subpopulations (US, RS1 and RS2) were genetically identical before logging, the homogeneity of the subpopulations obtained from this finding indicated no loss of genetic diversity in *P. speciosa* despite logging. Likewise, the isozymes analysis also did not reveal

any significant difference between the regenerated stands and the control site (Table 3). In addition, the mean Nei's (1973) genetic distance between the three FMUs was only 0.011. In fact an appreciable increase in both observed and expected heterozygosity was observed in the regenerated stands.

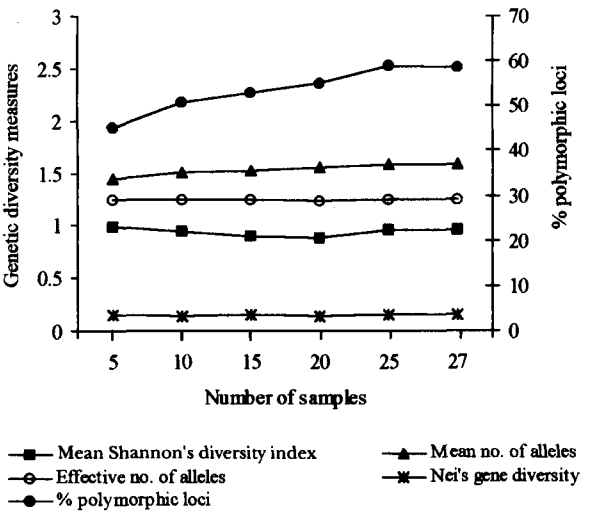

Figure 6 Effect of sample size on genetic diversity measures

Table 2 Estimates of genetic diversity measures for *Parkia speciosa* in the three forest management units (FMUs)

FMU	*H*	A	A_e	H	P
US	0.967	1.588 (0.497)	1.249 (0.338)	0.151 (0.505)	58.8
RS1	1.012	1.608 (0.493)	1.278 (0.347)	0.168 (0.188)*	60.8
RS2	0.921	1.510 (0.505)	1.214 (0.323)	0.131 (0.175)*	51.0

Figures in parentheses are standard deviations
Student's *t*-tests were performed for H, A, A_e and H; * indicates significant difference at $p < 0.05$.

H = mean Shannon's diversity index per primer
A = mean number of alleles per locus
A_e = effective number of alleles per locus
H = Nei's (1973) gene diversity
P = percentage of polymorphic loci

Table 3 Isozymes analysis for *Parkia speciosa* in the three forest management units (FMUs)

FMU	N	A	A_e	P	H_o	H_e
US	27.6	2.4 (0.6)	1.24	71.4	0.163 (0.065)	0.191 (0.071)
RS1	31.1	2.4 (0 5)	1.31	71.4	0.273 (0.096)	0.236 (0.081)
RS2	32.0	2.6(06)	1.47	71.4	0.286 (0.085)	0.320(0.085)

Figures in parentheses are standard deviations

N = mean sample size per locus
A = mean number of alleles per locus
A_e = effective number of alleles per locus
P = percentage of polymorphic loci
H_o = observed heterozygosity
H_e = expected heterozygosity

Logging (more than 40 years ago) most likely has a minimal impact on the existing gene pool of *P. speciosa* because it is often left uncut during logging operations (Whitmore 1972). Its low quality timber and non-durability (Burkill 1966) are the causes of it being an under-utilised timber species (Wong 1981). Moreover, maintenance of genetic diversity in *P. speciosa* can also be attributed to its good regeneration and increased seedling stocks in disturbed forests as it is a light demanding species (Wyatt-Smith 1963). Based on the demographic data, the tree density of *P. speciosa* in the regenerated stands was relatively higher compared with the unlogged stand. Woon and Poh (1998) also reported higher number of *Parkia* trees per ha in logged over forests and higher pod productivity of *P. speciosa* trees in regenerated forests as crown competition from the more dominant timber species is reduced.

Absence of genetic erosion in *P. speciosa* may also imply absence of inbreeding. Hence, this further suggests that the early logging activities in Peninsular Malaysia have minimal impact on the associated pollinators and seed disperser guilds of *P. speciosa*. *Parkia speciosa* is chiropterophilous, i.e., bat pollinated (Hopkins 1992). *Eonycteris spelaea* and *Cynopterus brachiotis* from the suborder of Megachiroptera have been reported as visitors to the hermaphrodite flowers of *P. speciosa* produced in globular heads (Baker & Harris 1957). In concordance, Zubaid (1993) reported that logging affects the insectivorous bats (microchiropterans) more than the frugivores and folivores (megachiropterans). Logging also does not affect selection in this species as these evolutionary processes influence the levels of genetic diversity besides random genetic drift and mutation (Namkoong *et al.* 1996).

As mentioned in the introduction, *P. speciosa* is an important non-timber forest product exploited for its fruit pods. However, the effect of fruit harvesting on the genetic diversity was not addressed in this study as the study site is a forest reserve whereby access for outsiders is restricted. Nevertheless, it has been reported that the pods do not all mature at the same time and a harvester will only climb a tree that has at least 100 mature pods (Woon 1995). Therefore, there are always some pods left on the tree, indicating sustainable harvesting. Gan and Weinland (1998) also noted that over-exploitation is not an issue. In contrast, Shaanker *et al.* (1996) reported negative impact of *Phyllanthus emblica* fruit harvesting on its seedling fitness and genetic diversity, probably because the fruits are more accessible as *P. emblica* is a relatively small understorey tree. Studies comparing the genetic diversity of regenerated seedlings of *P. speciosa* with the mature trees in a population where fruit harvesting is practised will help reveal any possible negative impacts of fruit harvesting on genetic variation in this species.

In conclusion, a single logging event under the Malayan Uniform System in a lowland dipterocarp forest in Peninsular Malaysia did not cause genetic erosion in *P. speciosa*. However, this result may not be valid for extrapolation to other non-logged species with different pollination system and other characteristics. One should be careful not to generalise that logging does not affect the genetic diversity of all non-logged species or all bat-pollinated species.

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Appendix 1 RAPD band sizes

Primer	Sequence (5'→ 3')	Band no.	Polymorphism[a]	Size (bp)
OPA04	AATCGGGCTG	1	-	996
		2	***	770
		3	***	675
		4	-	566
OPB07	GGTGACGCAG	1	-	1017
		2	-	626
		3	**	593
		4	***	575
		5	***	545
		6	-	468
OPB12	CCTTGACGCA	1	***	1122
		2	***	1050
		3	***	1005
		4	**	890
		5	***	861
		6	***	834
		7	***	814
		8	***	797
		9	**	774
		10	-	736
		11	***	651
		12	**	621
		13	-	554
		14	***	517
OPC07	GTCCCGACGA	1	-	1313
		2	***	989
		3	***	955
		4	***	881
		5	-	827
		6	-	742
		7	-	674
OPH07	CTGCATCGTG	1	***	1380
		2	-	937
		3	-	724
		4	***	496
OPH15	AATGGCGCAG	1	-	1054
		2	*	1017
		3	***	990
		4	***	950
		5	**	917
		6	***	867
		7	-	667
		8	***	612
		9	-	533
		10	-	394
OPJ18	TGGTCGCAGA	1	*	941
		2	-	903
		3	***	825
		4	**	654
		5	***	578
		6	*	542

[a]: - non-polymorphic loci
* polymorphic for one of the FMUs
** polymorphic for two FMUs
*** polymorphic for all three FMUs