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Proceeding

Determination of genetic relatedness of selected individual trees of *Shorea leprosula* Miq. and *Dipterocarpus cornutus* Dyer in forest seed production areas

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ABSTRACT The genetic relatedness of selected mother trees of *Shorea leprosula* (24 trees) and *Dipterocarpus cornutus* (10 trees) was investigated using four simple sequence repeats (SSRs) loci in two seed production areas, a 1 ha seed stand of *S. leprosula* and a 0.9 ha seed stand of *D. cornutus* in Compartment 17, Labis Forest Reserve, Segamat, Johor. A total of 24 and 32 saplings in the vicinity of selected mother trees of *S. leprosula* and *D. cornutus*, respectively was collected for parentage analysis. Based on SSR polymorphisms, four mother trees of *S. leprosula* (i.e. SM1, SM9, SM15 and SM21) and three mother trees of *D. cornutus* (i.e. DM2 or DM3, DM5 and DM8), are not closely related and therefore could be used as potential seed sources for an advanced breeding program. The mean genetic identity of the *S. leprosula* and *D. cornutus* mother trees was low (0.471 and 0.557, respectively). Low spatial genetic structure within the population of mother trees was detected in *S. leprosula* and *D. cornutus*. This implies that extensive gene flow occurred in these species within the seed production areas. This is validated in the present study for *D. cornutus* where only about 13.3% of alleles detected in saplings seemed to have originated from adult trees outside the forest seed production area (SPA).

Key words: Genetic relatedness, *Shorea leprosula*, *Dipterocarpus cornutus*, SSRs, forest seed production areas

INTRODUCTION

Understanding the genetic structure of forest tree species is a prerequisite for the appropriate utilisation of forest genetic resources, either for genetic improvement for plantation establishment or for management or conservation of natural communities. Although the population structure evolution results from complex interactions of selection, drift and gene flow, tree species with high rates of gene flow should have relatively more genetic variation distributed within and less among populations than species with more limited gene movement (Hamrick & Nason, 2000). Theoretical studies suggest that restricted gene flow reduces effective population size and causes inbreeding depression (Slatkin, 1985). Therefore, restricted gene flow can become a threat to the viability of populations of outcrossing plants.

Shorea leprosula Miq., commonly known as meranti tembaga, belongs to Dipterocarpaceae, the main timber family in the forests of Southeast Asia. In Peninsular Malaysia, *S. leprosula* is one of the most common emergent species in lowland dipterocarp forests, lower hill slopes and valleys in hill dipterocarp forests (Symington, 1943). Flowering in *S. leprosula*, like other dipterocarp species in *Mutica* section, is sporadic throughout the year and gregarious at intervals of 2 – 4 years (Soerianegara & Lemmens, 1993). *Shorea* flowers are hermaphroditic, pollinated by insects such as thrips and small beetles (Chan & Appanah, 1980; Chan, 1980) and produce single seeded fruits; more than half of the mature seeds land within 20 m of the parent tree (Burgess, 1969). However, if the mother trees are located on hill slopes or ridges, occasionally wind gust can carry the fruit 800 m away (Kochummen & Ng 1977).

Dipterocarpus cornutus Dyer, locally known as keruing gombang, belongs to Dipterocarpaceae. This species is widely distributed from Kedah to Singapore, usually on low-lying, flat or undulating land (Symington, 1943). Besides being an important source of heavy hardwood timber, this species is also one of the main oil producing (oleoresin of *Dipterocarpus*) species in Peninsular Malaysia.

Simple sequence repeats (SSRs) or microsatellite DNA markers are tandemly repeated DNA sequences with core motif repeat lengths of six bases or less, *e.g.* $(CA)_n$ or $(AGC)_n$ (Bruford & Wayne, 1993; Wang *et al*., 1994; Hancock, 1999). In forestry, SSRs have been applied to the study of genetic variation, gene flow, paternity analysis and mating systems because the markers detect high levels of genetic polymorphism (Chase *et al*., 1996; Dow & Ashley, 1996; Lefort *et al*., 1998; Konuma *et al*., 2000; Obayashi *et al*., 2002). For instance, Lefort *et al*., (1998) used nine SSR loci to determine the genetic relatedness

among elite oak (*Quercus robus* L.) trees and found that five selected trees were not closely related and could therefore be suitable seed source for an advanced breeding program.

In this study, we determined the genetic relatedness of selected mother trees of *S. leprosula* and *D. cornutus* in two SPAs in Compartment 17, Labis Forest Reserve using SSR analysis. We also estimated the magnitude of parentage genotype contribution in *S. leprosula* and *D. cornutus* in the SPAs.

MATERIALS AND METHODS

Field site

Two seed production areas (SPAs), a 1 hectare seed stand of *S. leprosula* (Figure 1A) and a 0.9 hectare seed stand of *D. cornutus* (Figure 1B) established in Compartment 17, Labis Forest Reserve, Segamat, Johor, Malaysia were used as the study sites.

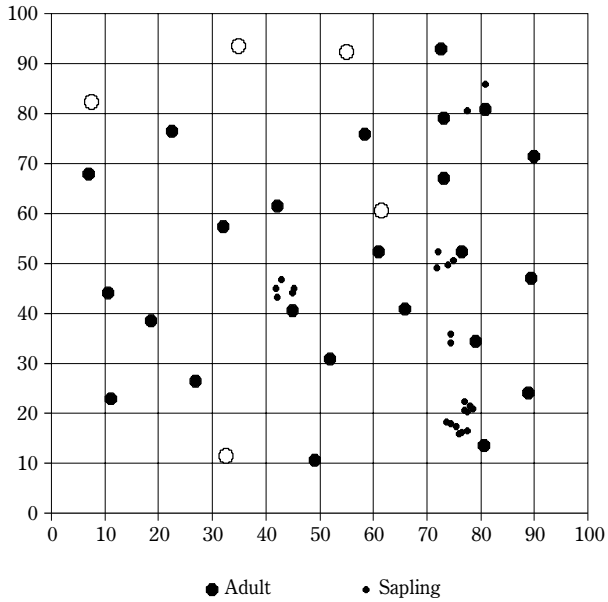

Fig. 1A. Spatial distribution of adult trees (●) and saplings (•) of *S. leprosula* in a 1 hectare seed production area of *S. leprosula* in Compartment 17, Labis Forest Reserve. The unfilled circles (○) indicate adult trees that were not selected in this study.

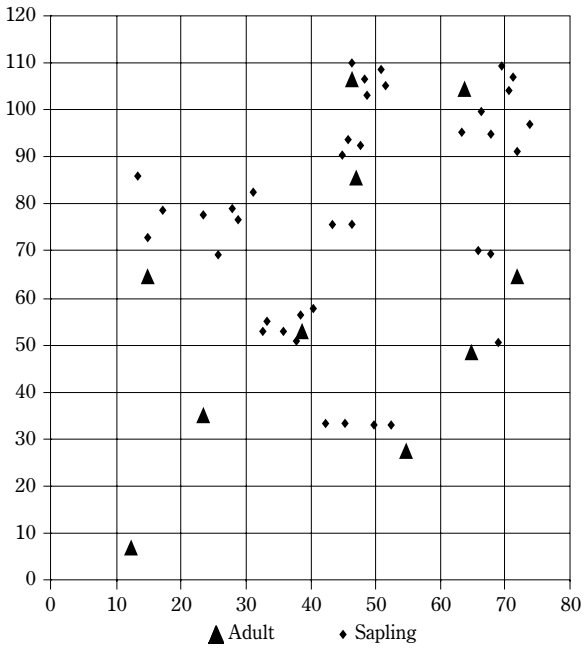

Fig. 1B. Spatial distribution of adult trees (▲) and saplings (◆) of *D. cornutus* in a 0.9 hectare seed production area of *D. cornutus* in Compartment 17, Labis Forest Reserve.

Sampling

Inner bark was collected from all the mother trees in both SPAs, totaling 24 trees of *S. leprosula* and 10 trees of *D. cornutus*. During sample collection, the spatial distribution of each tree was mapped. In addition, 24 and 32 saplings inside the seed stand were collected in the SPAs of *S. leprosula* and *D. cornutus*, respectively. The saplings were assumed to be offsprings of the nearest reproductive trees inside the respective SPA in the forest reserve.

DNA extraction and SSR genotyping

Total genomic DNA was isolated from the inner bark of mother trees and fresh leaves of saplings, using a modified CTAB method (Murray & Thompson, 1980; Doyle & Doyle, 1990). The genotype of each sample was determined using four pairs of SSR primers that were developed in *S. curtisii* (Ujino *et al*., 1998). We tested six primer pairs and chose the four that yielded good and consistent amplification products. The details of the SSR loci used for *S. leprosula* and *D. cornutus* are listed in Table 1.

Table 1. Selected SSR loci for *S. leprosula* and *D. cornutus*, core sequence, allele number and optimum primer annealing temperatures used in this study.

Locus	Core sequence*	Number of alleles observed		Annealing Temp. (℃)
		Saplings	Adults	
S. leprosula				
Shc02	$(CT)_2CA(CT)_nGC(AT)_2$	3	3	55.2
Shc03	$(CT)_n$	3	5	56.8
Shc09	$(CT)_n$	7	7	51.6
Shc11	$(CT)_m(A/T)T(CT)_n$	4	5	52.5
	Total	17	20	
	Average	4.25	5.0	
D. cornutus				
Shc02	$(CT)_2CA(CT)_nGC(AT)_2$	3	2	55.2
Shc03	$(CT)_n$	3	3	56.8
Shc08	$(CT)_n$	5	4	52.5
Shc11	$(CT)_m(A/T)T(CT)_n$	2	2	52.5
	Total	13	11	
	Average	3.25	2.75	

* n and m show the repeated nucleotides (Ujino *et al*. 1998)

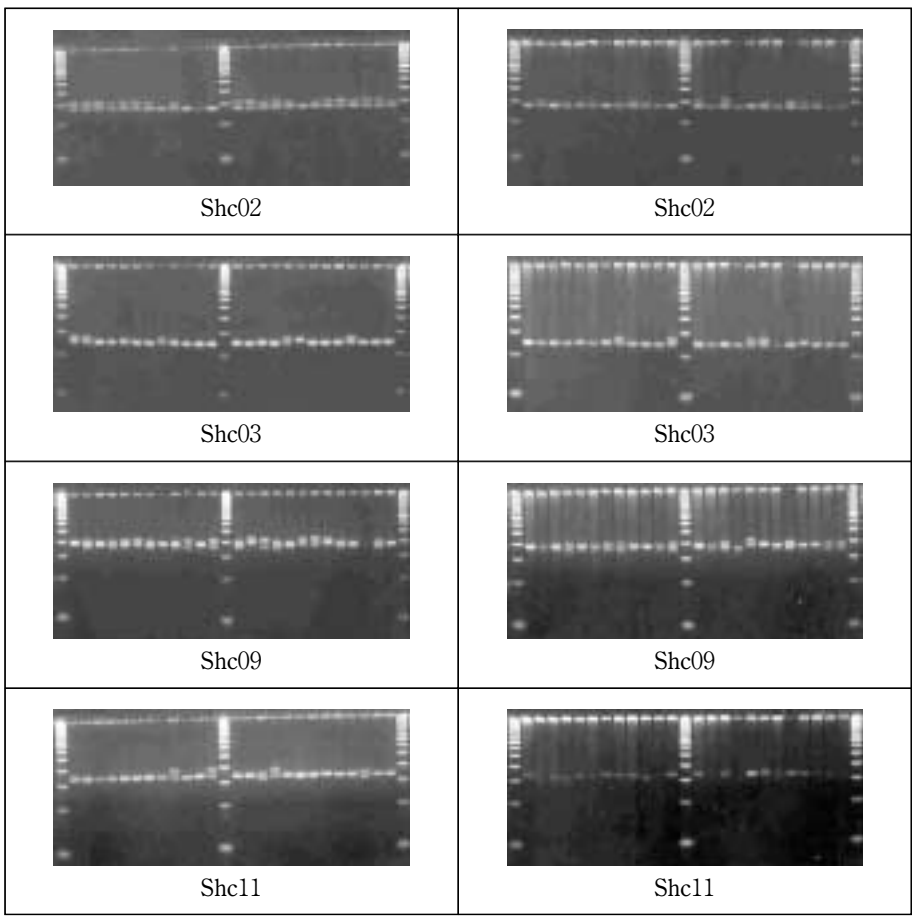

Fig. 2A. SSR polymorphisms in some *S. leprosula* samples from adults (a) and saplings (b) generated by four SSR loci. A 50 bp DNA ladder was used as a DNA size marker.

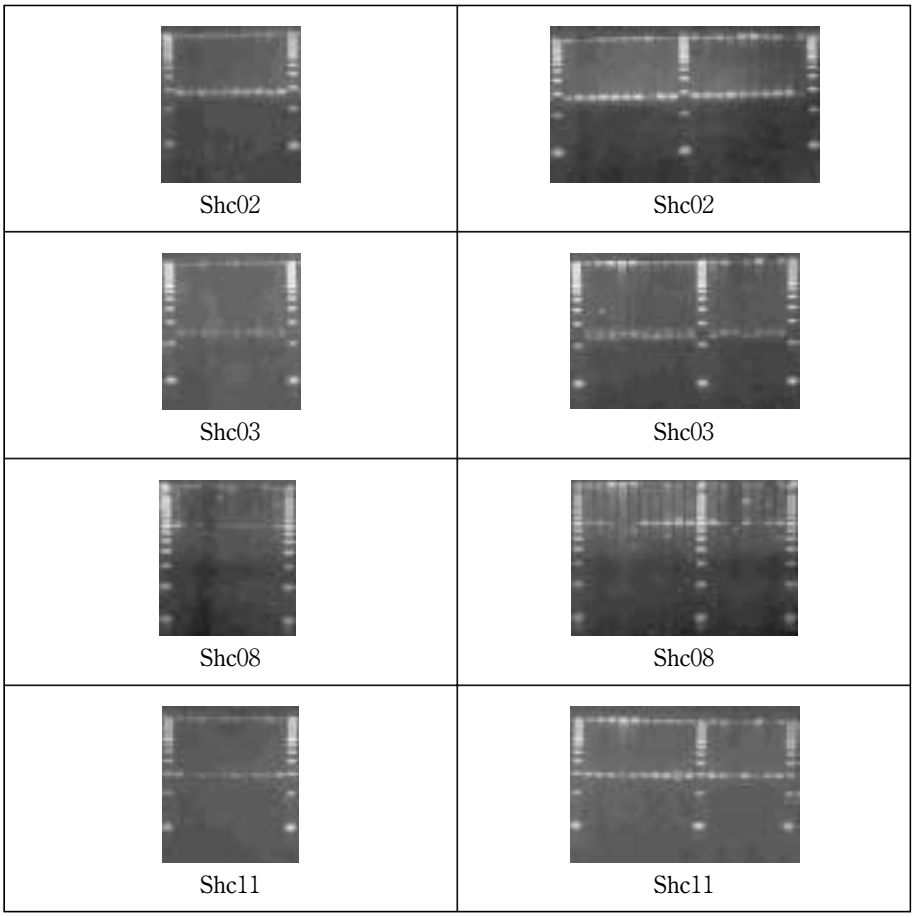

Fig. 2B. SSR polymorphisms in some *D. cornutus* samples from adults (a) and saplings (b) generated by four SSR loci. A 50 bp DNA ladder was used as a DNA size marker.

Reactions were stopped with 5 μl loading dye (15% Ficoll, 0.25% Bromophenol blue, and 0.25% Xylene cyanol FF). 10 μl of the PCR product was separated using 3.5% Metaphor™ (FMC Bioproducts, Rockland, Maine) agarose gel in 1x TBE buffer. It should be noted that the 3% Metaphor™ agarose gels run in 1x TBE buffer have a degree of resolution equal to that of a 6% polyacrylamide gel for the targeted PCR product size range (FMC Bioproducts manual). The gels were run at approximately 80 V for 3 to 4 hours. After staining with Gelstar® nucleic acid gel stain (FMC Bioproducts, Rockland, Maine) for one hour, the gel was visualised under ultraviolet light. Polaroid 667 film was used to document the gels (Figure 2A and 2B).

Statistical analysis

The genetic relatedness among all the 24 *S. leprosula* mother trees and, 10 *D. cornutus* mother trees was calculated using the Biosys-1 software (Swofford & Selander, 1981). The probability, *P* that two different mother trees exhibit identical genotype profiles was also estimated as the mean genetic identity raised to the power of the mean number of alleles per mother tree. In general, relatedness is the probability of sharing an identical-by-descent gene between two individuals (Konuma *et al*., 2000). Cluster analysis using the genetic identity values via the unweighted pairwise groups with arithmetic averaging (UPGMA, Sneath & Sokol, 1973) was conducted using an NTSYS-pc computer program (Rohlf, 1990). The spatial distance was also calculated between all mother trees for both species. The correlation between genetic relatedness and spatial distances among these trees was performed using the Mantel test (Mantel, 1967) in an NTSYS-pc computer program (Rohlf, 1990) with 5000 times randomization.

Paternity assignment was conducted by simple exclusion based on multilocus genotypes for the 24 and 10 mother trees

of *S. leprosula* and *D. cornutus*, respectively, using CERVUS 2.0 software (Marshall *et al*., 1998). Parentage analysis was also conducted by comparing alleles detected between saplings and adults using genotype data at four SSR loci. Alleles at every loci for each sapling were compared with those of adult trees, and adults which did not share any alleles at each locus were excluded as candidate parents.

RESULTS AND DISCUSSION

Genetic diversity

All the four SSR loci surveyed were polymorphic in saplings and adults of *S. leprosula* assayed (Table 2). The number of alleles per locus ranged from three to seven (Table 1) and the average was 4.25 for saplings and 5.0 for adults. The mean expected heterozygosity (H_e) detected in saplings and adults for *S. leprosula* was 0.532 and 0.640, respectively. These values are much higher than previously reported for the same species using allozyme markers; the mean expected heterozygosity was 0.369 (Lee *et al*., 2001).

Table 2. Estimates of genetic diversity parameters for *S. leprosula* and *D. cornutus* in Compartment 17, Labis Forest Reserve. Figures in parentheses are the standard errors.

	N	n_a	P (%)	H_o	H_e	r_b	Q
S. leprosula							
Adults	24.0	5.0 (0.82)	100	0.490 (0.152)	0.640 (0.074)	0.091	0.891
Saplings	23.8	4.25 (0.95)	100	0.273 (0.059)	0.532 (0.107)	0.169	--
D. cornutus							
Adults	10.0	2.8 (0.5)	100	0.125 (0.075)	0.464 (0.102)	0.232	0.637
Saplings	32.0	3.25 (0.63)	100	0.313 (0.171)	0.499 (0.133)	0.124	--

Note:
N = mean sample size per locus; n_a = mean number of alleles per locus;
P = percentage of polymorphic loci; H_o = mean observed heterozygosity per locus;
H_e = mean expected heterozygosity per locus (Nei's 1978);
r_b = frequency of null alleles, $(H_e - H_o / 1 + H_e$, Brookfield 1996);
Q = paternity exclusion probability

In *D. cornutus*, the number of alleles per locus ranged from two to five and the average was 3.25 for saplings and 2.75 for adults based on four polymorphic SSR loci (Table 2). Meanwhile, the mean expected heterozygosity (H_e) detected in saplings and adults of *D. cornutus* was 0.532 and 0.640, respectively.

Genetic relatedness

Genetic identities between the *S. leprosula* mother trees varied between 0.077 (SM13 and SM24) and 1.000 (SM4 and SM7, and SM6 and SM14) (Table 3). The mean genetic identity of the *S. leprosula* mother trees was 0.471 (N = 276 pairwise comparisons). Thus, the probability of two mother trees being identical by chance is $P = 5.8 \times 10^{-2}$.

The dendrogram for *S. leprosula* revealed four main clusters, i.e. Cluster I: SM1; Cluster II: SM2, SM3, SM4, SM5, SM6, SM7, SM8, SM11, SM13, SM14, SM20 and SM23; Cluster III: SM10, SM12, SM17, SM21, SM22, SM24, SM25, SM27 and SM1S, and Cluster IV: SM9 and SM15 (Figure 3). Individual SM21 was distinctly different from the other individuals within cluster III. Based on Figure 3, four mother trees, SM1, SM9, SM15 and SM21, are not closely related and could be used as seed sources.

In *D. cornutus*, genetic identities among pairs of mother trees varied between 0.000 (DM5 and DM9) and 1.000 (DM2 and DM3) (Table 4). The mean genetic identity between mother trees within the *D. cornutus* SPA was 0.557 (N = 45 pairwise comparisons), whereas the probability of two mother trees being identical by chance was $P = 1.7 \times 10^{-1}$. However,

larger *P* values have been reported when the plants involved were closely related, e.g. *P* was about 10^{-2} for *Acer negundo* (Nybom & Rogstad, 1990) and 10^{-3} among closely related blackberry cultivars (Nybom *et al*., 1989). Nevertheless, in some plant species, the chance of fingerprint identity can be relatively low, for instance distance rice cultivars give an identity probability of 10^{-11} (Dallas, 1988).

The UPGMA based on Nei's (1978) genetic identity among 10 mother trees of *D. cornutus* are presented in Figure 4. Three distinct clusters were formed; Cluster I: SM1, SM6, SM8 and SM10; Cluster II: SM4, SM5 and SM7, and Cluster III: SM2, SM3 and SM9. Within cluster I individual SM8 was different from the other individuals, whereas in cluster II individual M5 was distinctly different from individuals M4 and M7. The cluster analysis showed that cluster I was more closely related to cluster II than to cluster III. Based on Figure 4, the individuals SM2 or SM3, SM5, SM8 and SM9 are not closely related and can be used for seed production.

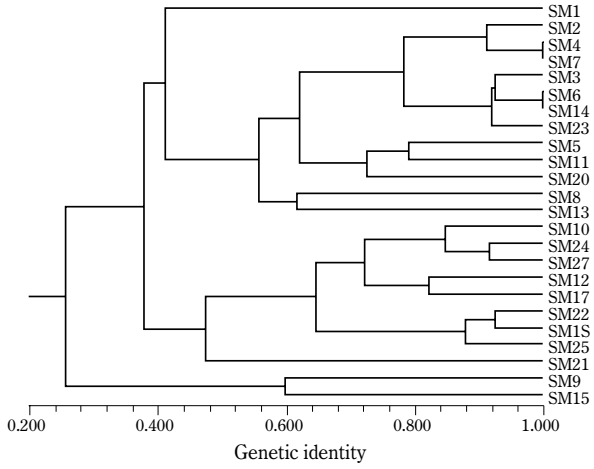

Fig. 3. UPGMA cluster analysis based on Nei's (1978) genetic identity among 24 mother trees of *S. leprosula* in a 1 hectare SPA of *S. leprosula* in Compartment 17, Labis Forest Reserve, Segamat, Johor.

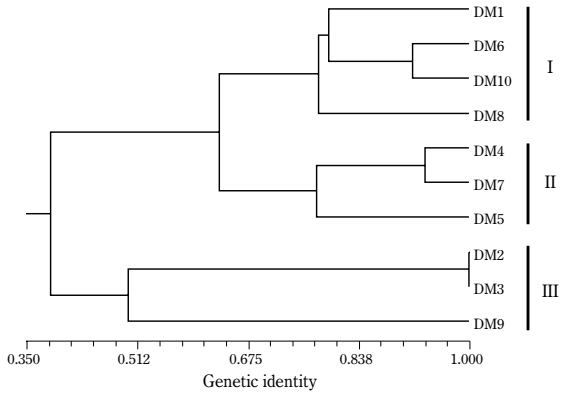

Fig. 4. UPGMA cluster analysis based on Nei's (1978) genetic identity among 10 mother trees of *D. cornutus* in a 0.9 hectare SPA of *D. cornutus* in Compartment 17, Labis Forest Reserve, Segamat, Johor.

Table 3. Genetic identity between pairs of mother trees of *S. leprosula*

	SM1	SM2	SM3	SM4	SM5	SM6	SM7	SM8	SM9	SM10	SM11	SM12	SM13	SM14	SM15	SM17	SM20	SM21	SM22	SM23	SM24	SM25	SM27	SM1S
SM1	1.000																							
SM2	0.400	1.000																						
SM3	0.676	0.676	1.000																					
SM4	0.365	0.913	0.772	1.000																				
SM5	0.300	0.700	0.676	0.730	1.000																			
SM6	0.548	0.730	0.926	0.833	0.730	1.000																		
SMT7	0.365	0.913	0.772	1.000	0.730	0.833	1.000																	
SM8	0.507	0.507	0.714	0.463	0.592	0.617	0.463	1.000																
SM9	0.100	0.200	0.085	0.183	0.400	0.091	0.183	0.338	1.000															
SM10	0.338	0.423	0.429	0.540	0.338	0.540	0.540	0.286	0.338	1.000														
SM11	0.316	0.632	0.668	0.722	0.791	0.722	0.722	0.535	0.316	0.535	1.000													
SM12	0.365	0.365	0.463	0.500	0.456	0.500	0.500	0.309	0.365	0.772	0.577	1.000												
SM13	0.183	0.639	0.463	0.583	0.730	0.500	0.583	0.617	0.548	0.154	0.433	0.250	1.000											
SM14	0.548	0.730	0.926	0.833	0.730	1.000	0.833	0.617	0.091	0.540	0.722	0.500	0.500	1.000										
SM15	0.300	0.100	0.085	0.091	0.400	0.091	0.091	0.338	0.600	0.169	0.158	0.365	0.548	0.091	1.000									
SM17	0.400	0.500	0.507	0.639	0.400	0.639	0.639	0.169	0.400	0.676	0.474	0.822	0.365	0.548	0.200	1.000								
SM20	0.183	0.548	0.386	0.417	0.730	0.417	0.417	0.540	0.548	0.154	0.722	0.167	0.583	0.417	0.365	0.091	1.000							
SM21	0.338	0.169	0.286	0.154	0.254	0.231	0.154	0.429	0.338	0.571	0.267	0.540	0.154	0.231	0.254	0.338	0.154	1.000						
SM22	0.274	0.274	0.386	0.417	0.365	0.500	0.417	0.154	0.456	0.849	0.577	0.667	0.083	0.500	0.183	0.639	0.333	0.463	1.000					
SM23	0.548	0.730	0.926	0.833	0.822	0.917	0.833	0.617	0.091	0.463	0.722	0.583	0.500	0.917	0.183	0.548	0.417	0.309	0.417	1.000				
SM24	0.548	0.456	0.617	0.583	0.365	0.583	0.583	0.309	0.274	0.849	0.433	0.667	0.167	0.583	0.091	0.730	0.083	0.540	0.750	0.583	1.000			
SM25	0.274	0.274	0.231	0.250	0.365	0.250	0.250	0.154	0.548	0.617	0.577	0.500	0.083	0.250	0.183	0.456	0.583	0.463	0.833	0.250	0.583	1.000		
SM27	0.365	0.548	0.463	0.667	0.365	0.500	0.667	0.154	0.365	0.849	0.433	0.667	0.250	0.500	0.091	0.822	0.083	0.463	0.750	0.500	0.917	0.583	1.000	
SM1S	0.254	0.254	0.357	0.386	0.423	0.386	0.386	0.143	0.507	0.714	0.668	0.617	0.077	0.386	0.169	0.592	0.463	0.429	0.926	0.386	0.694	0.926	0.694	1.000
	SM1	SM2	SM3	SM4	SM5	SM6	SM7	SM8	SM9	SM10	SM11	SM12	SM13	SM14	SM15	SM17	SM20	SM21	SM22	SM23	SM24	SM25	SM27	SM1S

Note: The mean pairwise genetic identity among 24 mother trees of *S. leprosula* is 0.471.

Table 4. Genetic identity between pairs of mother trees of *D. cornutus*

	DM1	DM2	DM3	DM4	DM5	DM6	DM7	DM8	DM9	DM10
DM1	1.000									
DM2	0.750	1.000								
DM3	0.750	1.000	1.000							
DM4	0.535	0.267	0.267	1.000						
DM5	0.750	0.500	0.500	0.802	1.000					
DM6	0.866	0.577	0.577	0.617	0.722	1.000				
DM7	0.500	0.250	0.250	0.935	0.750	0.577	1.000			
DM8	0.750	0.500	0.500	0.668	0.500	0.722	0.750	1.000		
DM9	0.250	0.500	0.500	0.134	0.000	0.144	0.250	0.500	1.000	
DM10	0.722	0.433	0.433	0.694	0.577	0.917	0.722	0.866	0.289	1.000
	DM1	DM2	DM3	DM4	DM5	DM6	DM7	DM8	DM9	DM10

Note: The mean pairwise genetic identity among 10 mother trees of *D. cornutus* is 0.557.

Lefort *et al*. (1998) used nine SSR loci to determine the genetic relatedness among elite oaks (*Quercus robus* L.) trees. They found that five selected trees were not closely related and would therefore form suitable seed sources for an advanced breeding program.

Genetic structure

Figure 5 shows the plot of all pairwise genetic identity between *S. leprosula* and *D. cornutus* mother trees against the corresponding geographical distance. A negative correlation was detected in the *S. leprosula* population (r = -0.112, P = 0.07, Mantel test, Figure 5A), meanwhile the *D. cornutus* population showed a negative and significant correlation (r = -0.467, P = 0.003, Mantel test, Figure 5B).

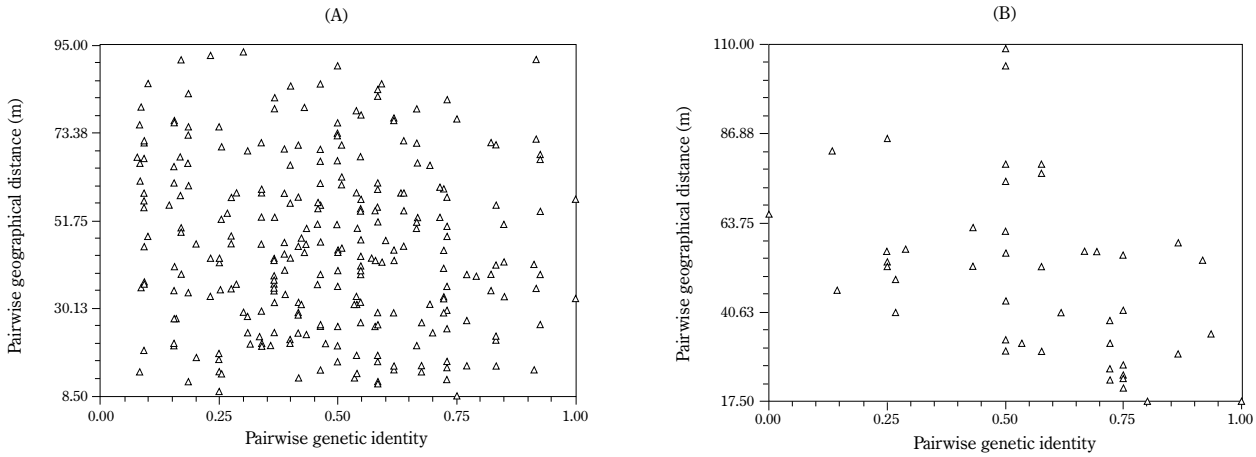

Fig. 5. Relationship between pairwise geographical distance and pairwise genetic identity for the *S. leprosula* population (A) and the *D. cornutus* population (B).

If there was a genetic structure within these mother trees, there would be a clear negative correlation and a high r-value (Mantel test) would be expected between these parameters. Genetic structure, the genetic differentiation within a population, is induced when gene flow by pollen and seed dispersal is limited (Konuma *et al*., 2000). Therefore, these results indicate that the spatial genetic structure among the 24 and 10 mother trees of *S. leprosula* and *D. cornutus*, respectively, in the SPA was low. This further implies extensive gene flow in previous generations in the populations. A similar result was also detected in 30 mother trees of *Neobalanocarpus heimii* in a 42-ha study plot, Pasoh Forest Reserve (Konuma *et al*., 2000). They suggested that long-distance gene flow and seed migration are responsible for the poorly developed genetic structure in this species.

According to Isagi *et al*. (2000), if a population is genetically structured and inbreeding or outbreeding depression occurs, as indicated by the genetic relatedness of adult trees, some kind of selection on pollen grains could have occurred. Dow and Ashley (1996) presumed the existence of a mechanism allowing female flowers of *Quercus macrocarpa* to

preferentially select pollen from distant sources rather than pollen produced by neighbouring trees.

Chase *et al*. (1996) revealed that most mating events in *Pithecellobium elegans* were not between the closest neighbours, because of variations in phenology or flowering behaviour among adult trees. Many tree species show large fluctuations in flowering among years with or without synchronisation between trees in a population. In the case of episodic flowering without synchronisation in a population, only some of the trees in a population can contribute to reproduction in a given year and this may have resulted from pollination between distant trees. Isagi *et al*. (2000) reported that more than 70% of the pollination events in *Magnolia obovata* occurred between non-nearest neighbours.

Parentage analysis

The total exclusion probability, the exclusion probability in the case of both parents being unknown (Marshall *et al*., 1998), over four SSR loci for *S. leprosula* and *D. cornutus* was 0.891 and 0.637, respectively (Table 2). The paternity exclusion probability detected in *S. curtisii* (K. Obayashi, personal communication) and *N. heimii* (Konuma *et al*., 2000) using the same primer systems (Ujino *et al*., 1998) was 0.97.

Pemberton *et al*., (1998) reported that the null alleles are responsible for mismatches between parent-offspring pairs, i.e. the offspring do not amplify an allele that is present in the parents. In addition, the presence of null alleles will bias the estimation of genotype and allele frequencies (Dowling *et al*., 1997). In this study, the frequency of null alleles detected in adult trees of *S. leprosula* and *D. cornutus* were 9.1% and 23.2%, respectively (Table 2). Callen *et al*. (1993) found that in a survey of $(AC)_n$ SSR markers in human, 7 or 30% of the 23 markers surveyed demonstrated the presence of null alleles. In addition, Roa *et al*. (2000) in their study on cross-species amplification of *Manihot* species using 10 SSR loci found the presence of null alleles in Manihot species was on average about 11%. Null allele detected in locus Shc02 for *N. heimii* was probably caused by mismatches between parent and offspring (Konuma *et al*., 2000).

Based on the parentage analysis, about 13.3% of the alleles detected in *D. cornutus* saplings (Table 5A and 5B) probably originated from adult trees outside the SPA. This implies gene flow occurred in this species within the SPA. Isagi *et al*. (2000) found that about 57% of the possible parents of *Magnolia obovata* saplings were from outside the study plot in Ogawa Forest Reserve revealed by eight SSR loci.

Table 5A. Parentage analysis for *D. cornutus* in SPA, Compartment 17, Labis Forest Reserve.

Locus	Observed alleles	
	Adults	Saplings*
Shc02	A, B	A, B, *C*
Shc03	A, B, C	A, B, C
Shc08	A, C, D, E	A, *B*, C, D, E,
Shc11	A, B	A, B

Note:
Underlined alleles probably originated from adult trees within the seed production area (SPA).
Italicised alleles probably originated from adult trees outside the seed production area (SPA).

Table 5B. Parentage allele contribution in *D. cornutus* in SPA, Compartment 17, Labis Forest Reserve.

Locus	Parentage allele contribution (%)	
	Saplings	
	Within SPA	Outside SPA
Shc02	66.7	33.3
Shc03	100.0	0.0
Shc08	80.0	20.0
Shc11	100.0	0.0
Mean	86.7	13.3

Note:
Within SPA means alleles probably originated from adult trees within the SPA.
Outside SPA means alleles probably originated from adult trees outside the SPA.

In contrast, all the saplings of *S. leprosula* shared the same alleles with mother trees inside the *S. leprosula* SPA at each locus (Table 6A & 6B). This result suggests that these mother trees are probably their parents. Nevertheless, there are possibilities that these alleles detected in saplings may also come from *S. leprosula* mother trees outside the *S. leprosula* SPA

in Compartment 17. According to Isagi *et al*. (2000), more than 70% of the pollination events in *M. obovata* occurred between non-nearest neighbours. In fact, the average mating distance, 524 m, has been reported in *N. heimii* based on five reproductive trees in Pasoh Forest Reserve, a lowland tropical rainforest in Malaysia (Konuma *et al*. 2000).

Table 6A. Parentage analysis for *S. leprosula* in SPA, Compartment 17, Labis Forest Reserve.

Locus	Observed alleles	
	Adult	Sapling*
Shc02	A, B, C	A, B, C
Shc03	A, B, D	A, B, D
Shc09	A, B, C, D, E, F, G	A, B, C, D, E, F, G
Shc11	A, B, C, D, E	A, B, C, D

Note:
Underlined alleles probably originated from adult trees within the seed production area (SPA).

Table 6B. Parentage allele contribution in *S. leprosula* in SPA, Compartment 17, Labis Forest Reserve.

Locus	Parentage allele contribution (%)	
	Sapling	
	Within SPA	Outside SPA
Shc02	100.0	0.0
Shc03	100.0	0.0
Shc09	100.0	0.0
Shc11	100.0	0.0
Mean	100.0	0.0

Note:
Within SPA means alleles probably originated from adult trees within the seed production area (SPA).
Outside SPA means alleles probably originated from adult trees outside the seed production area (SPA).

CONCLUSION

The application of SSRs in the present study successfully distinguished four mother trees of *S. leprosula* (SM1, SM9, SM15 and SM21) and *D. cornutus* (DM2 or DM3, DM5 and DM8), from two SPAs in Compartment 17, Labis Forest Reserve, Segamat, Johor, respectively. These mother trees are not closely related and therefore form potential seed sources for an advanced breeding program. Low spatial genetic structure within the populations of mother trees was detected in *S. leprosula* and *D. cornutus* seed stands. This implies that gene flow occurred in these species within the SPA. This is validated in the present study for *D. cornutus* where about 13.3% of the sapling alleles detected seem to have originated from adult trees outside the SPA.

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