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Spatial structure and genetic diversity of two tropical tree species with contrasting breeding systems and different ploidy levels

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Abstract

Analyses of the spatial distribution pattern, spatial genetic structure and of genetic diversity were carried out in two tropical tree species with contrasting breeding systems and different ploidy levels using a 50-ha demographic plot in a lowland dipterocarp forest in Peninsular Malaysia. *Shorea leprosula* is a diploid and predominantly outcrossed species, whereas *S. ovalis* ssp. *sericea* is an autotetraploid species with apomictic mode of reproduction. Genetic diversity parameters estimated for *S. leprosula* using microsatellite were consistently higher than using allozyme. In comparisons with *S. leprosula* and other tropical tree species, *S. ovalis* ssp. *sericea* also displayed relatively high levels of genetic diversity. This might be explained by the lower pressure of genetic drift due to tetrasomic inheritance, and for autotetraploids each locus can accommodate up to four different alleles and this allows maintenance of more alleles at individual loci. The observed high levels of genetic diversity in *S. ovalis* ssp. *sericea* can also be due to a random retention of more heterogeneous individuals in the past, and the apomictic mode of reproduction might be an evolutionary strategy, which allows the species to maintain high levels of genetic diversity. The spatial distribution pattern analyses of both species showed significant levels of aggregation at small and medium but random distribution at the big diameter-class. The decrease in magnitude of spatial aggregation from small- to large-diameter classes might be due to compensatory mortality during recruitment and survival under competitive thinning process. Spatial genetic structure analyses for both species revealed significant spatial genetic structure for short distances in all the three diameter-classes. The magnitude of spatial genetic structure in both species was observed to be decreasing from smaller- to larger-diameter classes. The high spatial genetic structuring observed in *S. ovalis* ssp. *sericea* at the small-diameter class is due primarily to limited seed dispersal and apomictic mode of reproduction. The similar observation in *S. leprosula*, however, can be explained by limited seed and pollen dispersal, which supports further the fact that the species is pollinated by weak fliers, mainly of *Thrips* and *Megalurothrips* in the lowland dipterocarp forest.

Keywords: allozyme, genetic diversity, microsatellite, *Shorea*, spatial distribution pattern, spatial genetic structure, tropical tree species

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Introduction

The spatial distribution pattern, within plant populations, is influenced by various ecological and evolutionary processes such as seed dispersal, intra- and interspecific competition and environmental heterogeneity which take place during the life history of a plant. Seed shadow basically determines the spatial distribution pattern of cohorts. After seed dispersal, the compensatory mortality due to intra- and interspecific competitions, environmental heterogeneity, herbivores and plant diseases would

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affect the spatial distribution pattern. The degree to which individuals are aggregated affects plant mating systems and also how plant species use resources or can be used as resources (Condit *et al*. 2000).

The spatial genetic structure within plant populations, in addition to the ecological and evolutionary processes that affect spatial distribution patterns, can also be influenced by limited pollen dispersal, local genetic drift, inbreeding and selection favouring the same or different genotypes (Wright 1943; Levin & Kerster 1974; Turner *et al*. 1982; Heywood 1991; Epperson 1992, 1995). Studies of spatial genetic structure in plant populations can reveal the operation of key evolutionary processes. When spatial genetic structure develops, it may influence the patterns of local breeding and evolution. Hence, understanding of spatial genetic structure at the population level is crucial for the management and conservation of genetic resources.

Information on spatial genetic structure within natural stands have been reported extensively for temperate forest trees species but somewhat limited for tropical forest trees. For temperate forest tree species, the majority of the studies showed weak spatial genetic structure, consistent with long-distance seed and pollen dispersal (e.g. Epperson & Allard 1989; Berg & Hamrick 1995; Schnabel & Hamrick 1995; Leonardi *et al*. 1996; Chung *et al*. 2000; Epperson & Chung 2001; Parker *et al*. 2001). However, some studies showed significant spatial genetic structure and these were explained by limited seed dispersal (e.g. Schnabel *et al*. 1991; Chung & Epperson 2000).

For tropical forest tree species, Hamrick *et al*. (1993) showed that spatial genetic structure was present in small- and intermediate-diameter classes for *Platypodium elegans*, *Alseis blackiana* and *Swartzia simplex* in Panama. Boshier *et al*. (1995), in their study of *Cordia alliodora*, showed that near neighbours were related genetically more highly than more distant trees. Doligez & Joly (1997) revealed no clearcut spatial pattern of *Carapa procera* in French Guiana. Degen *et al*. (2001a), when applying a multilocus approach using a random amplified polymorphic DNA (RAPD) marker on eight tropical tree species revealed significant spatial genetic structure at distances up to 300 m for some of these species. Spatial structure varies with life history stages (Epperson & Alvarez-Buylla 1997). However, there are few studies of the spatial distribution of genetic variation in different age or size classes of plants. Analysis of spatial genetic structure without consideration of life stage or age may fail to observe spatial structure and might lead to misinterpretation of the underlying ecological and evolutionary processes (Aldrich *et al*. 1998; Schnabel *et al*. 1998; Caron *et al*. 2000; Kalisz *et al*. 2001).

Shorea leprosula and *S. ovalis* ssp. *sericea* are both common dipterocarps and distributed widely in Peninsular Malaysia (Symington 1943; Ashton 1982). Previous studies on *S. leprosula* showed that it is a diploid ($2n = 14$; Jong & Lethbridge 1967), reproduced mainly through outcrossing (Chan 1981; Lee *et al*. 2000a), and with low energy flower thrips (Thysanoptera), mainly *Thrips* and *Megalurothrips* as the primary pollinators (Chan & Appanah 1980; Appanah & Chan 1981). *S. ovalis* ssp. *sericea* is known to be a tetraploid ($4n = 28$; Jong & Lethbridge 1967; Jong & Kaur 1979) and reproduce largely apomictically through adventive polyembryony, in which outgrowth of the nucellar or integument cells produce an embryo (Kaur *et al*. 1978, 1986). Although both species produce winged seeds, seed dispersal is due mainly to gravity and seldom exceeds 50 m radius from the mother trees (Webber 1934; Chan 1980).

Outcrossing plants in general exhibit higher levels of genetic diversity than selfing plants (Hamrick & Godt 1996). Allozyme study of *S. leprosula* throughout Malaysia showed that the species exhibited high levels of genetic diversity and most of the diversity was partitioned within populations (Lee *et al*. 2000b). Literature on the genetic diversity of apomictic plant species is relatively sparse. Some studies using allozymes indicated that apomicts harboured substantial levels of genetic diversity (e.g. Ellstrand & Roose 1987; Widen *et al*. 1994). It was suggested that apomixis may be a way of retaining heterozygosity in species exposed to high rates of autogamy, by combining vegetative reproduction and seed dispersal and by preserving locally adapted genotypes from recombination (Durand *et al*. 2000). The strong association between apomixis and polyploidy has been noted for many years (Asker & Jerling 1992; Mogie 1992) even though, at present, strong evidence is still lacking to show how polyploidy initiates apomixis directly. In a comprehensive review on the association between polyploidy and apomixis, Gustafsson (1946, 1947) concluded that although apomixis can be induced in diploids by favourable mutation, the action of many apomixis-inducing genes is stronger at the polyploidy level.

Brown & Young (2000), in their study of an endangered autotetraploid daisy (*Rutidosis leptorrhynchoides*) population, observed increase in allelic richness compared to their diploid populations. Similarly, other studies also showed that autotetraploid species exhibited high levels of genetic diversity in comparison with their closely related diploid species (e.g. Soltis & Rieseberg 1986; Mahy *et al*. 2000; Hardy & Vekemans 2001). To our knowledge, to date, genetic diversity studies on apomictic and tetraploid species have never been reported for tropical tree species.

The aims of this study were to investigate the spatial distribution pattern, spatial genetic structure and genetic diversity of two tropical tree species, with contrasting breeding systems and different ploidy levels using allozyme and microsatellite markers. *S. leprosula* is outcrossed with limited pollen and seed dispersal. We would therefore postulate the species to exhibit high levels of genetic diversity and display significant levels of spatial aggregation and spatial genetic structure. Similarly, autotetraploidy

and apomictic mode of reproduction allows us to postulate significant levels of spatial aggregation and spatial genetic structure, and high levels of genetic diversity in *S. ovalis* ssp. *sericea*.

Materials and methods

Study site and sample collections

This study was conducted at a 50-ha demographic plot in Pasoh Forest Reserve (Negeri Sembilan, 2°59′ N, 102°19′ E), Peninsular Malaysia (Fig. 1). In September 2001 a total of 348 individuals of *S. leprosula* and *S. ovalis* ssp. *sericea* were identified and sampled. All samples collected were classified according to diameter at breast height (dbh) into three diameter classes: large (BIG, dbh > 40 cm), medium (MED, dbh 8–15 cm) and small (SMA, dbh 1–4 cm). Of the 178 individuals collected for *S. leprosula*, 64, 60 and 54 individuals were classified into large-, medium- and small-diameter classes, respectively (Fig. 2a). For *S. ovalis* ssp. *sericea*, of the 170 individuals collected 58 were classified as large, 55 as medium and 57 as small (Fig. 2b).

Genetic analysis

Allozyme analysis was carried out according to Lee *et al.* (2000c) for four enzyme systems: glucose phosphate isomerase (GPI), diphosphogluconate pyrophosphatase (UGP), phosphoglucomutase (PGM) and malate dehydrogenase (MDH). Assignment of genotypes was conducted in accordance with the known enzyme substructure (Weeden & Wendel 1989). The allozyme analysis was carried out only for *S. leprosula* and not for *S. ovalis* ssp. *sericea*, because *S. ovalis* ssp. *sericea* exhibited tetrasomic banding patterns, which were too complicated to be interpreted into genotypic data.

For microsatellite analysis, genomic DNA was extracted from leaves or inner bark tissues using the procedure of Murray & Thompson (1980) with modifications. The extracted DNAs were purified further using a high pure polymerase chain reaction (PCR) template preparation kit (Boehringer Mannheim). The samples were genotyped for seven microsatellite loci, developed for *S. curtisii* (Ujino *et al.* 1998), i.e. *Shc*01, *Shc*02, *Shc*03, *Shc*04, *Shc*07, *Shc*09 and *Shc*17. Microsatellites amplification were performed in a 25 μL reaction volume, containing 10 ng DNA, 50 mM KCl, 20 mM Tris-HCl (pH 8.0) and 1.5 mM $MgCl_2$, 0.2 μM of each primer, 0.2 mM of each dNTP and 1 unit of platinum *Taq* DNA polymerase (Gibco-BRL). The polymerase chain reaction (PCR) was carried out on a GeneAmp 9700 thermal cycler (Applied Biosystems) for an initial denaturing step at 94 °C for 4 min, followed by 35 cycles each at 94 °C for 1 min, 52–54 °C for 30 s and 72 °C for 45 s. A final extension step at 72 °C for 30 min was performed after the 35 cycles. Genotyping was performed on 5% denaturing (6 M urea) polyacrylamide gels. Electrophoresis was carried out with 1× Tris-borate-EDTA (TBE) buffer on an ABI Prism 377 automated DNA sequencer (Applied Biosystems). Allele sizes were scored against the internal size standard and the individuals were genotyped using GENESCAN 3.1 and GENOTYPER 2.1 software (Applied Biosystems).

Statistical analysis

The levels of genetic diversity of *S. leprosula* were estimated for mean number of alleles per locus (A_a), effective number of alleles per locus (A_e; Crow & Kimura 1970), allelic

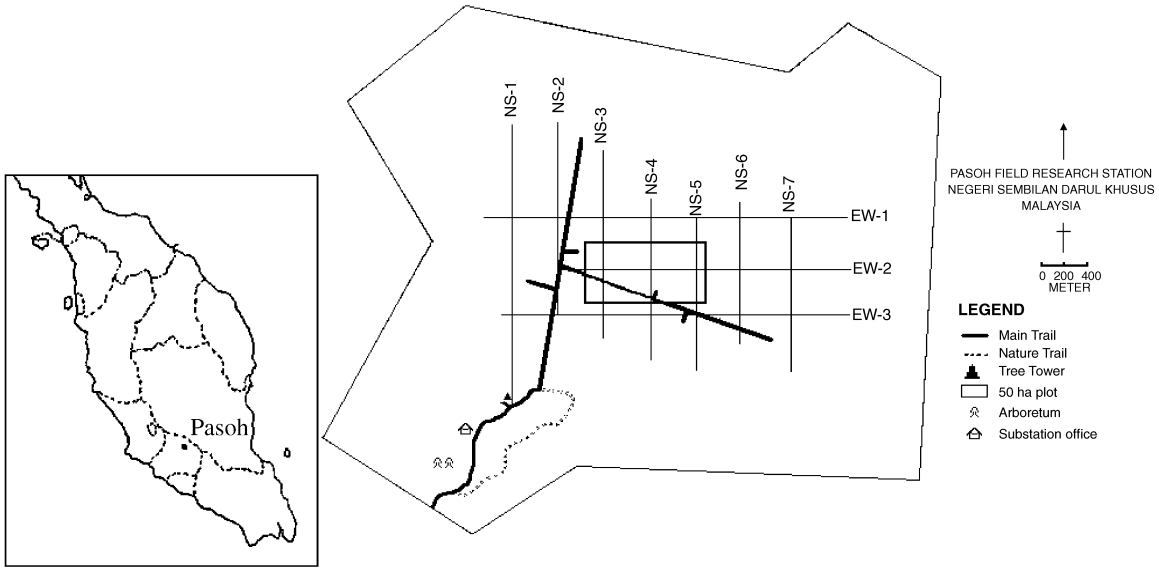

Fig. 1 Location of the Pasoh Forest Reserve in Negeri Sembilan and the map description of the Pasoh Field Research Station.

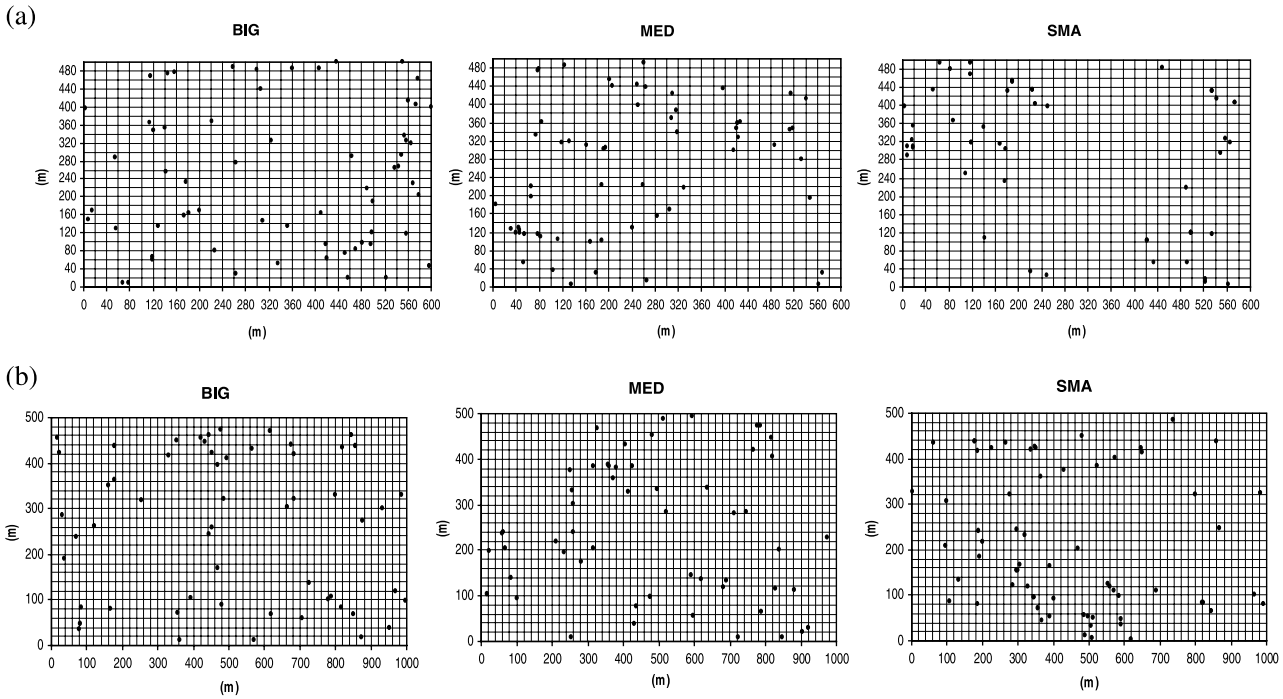

Fig. 2 (a) Distribution of *Shorea leprosula* individuals within the 30-ha plot in the Pasoh Forest Reserve. BIG: 64 individuals (dbh > 40 cm); MED: 60 individuals (8–15 cm); and SMA: 54 individuals (1–4 cm). (b) Distribution of *S. ovalis* ssp. *sericea* individuals within the 50-ha plot in the Pasoh Forest Reserve. BIG: 58 individuals (dbh > 40 cm); MED: 55 individuals (8–15 cm); and SMA: 57 individuals (1–4 cm).

richness (R_s; Petit *et al*. 1998), observed heterozygosity (H_O) and expected heterozygosity (H_E; Nei 1987) with the assistance of programs BIOSYS-1 (Swofford & Selander 1981), POPGENE version 1.31 (Yeh *et al*. 1999) and FSTAT version 2.9.3.2 (Goudet 2002). Wright's (1965) fixation index (F_{IS}), averaged over all loci, was calculated to assess the overall departure from Hardy–Weinberg equilibrium for each diameter class. For *S. ovalis* ssp. *sericea*, the following parameters were calculated using AUTOTET program (Thrall & Young 2000): mean number of alleles per locus (A_a), observed heterozygosity (H_O), expected heterozygosity under random mating and assuming random chromosomal segregation (e.g. no double reduction) (H_E; Geiringer 1949; Wricke & Weber 1986; Bever & Felber 1992) and fixation index (F_{IS}). To test whether mean F_{IS} values were significantly different from zero, an approximation of a 95% confidence level was determined by multiplying the standard error by 1.96 (Loiselle *et al*. 1995).

The spatial distribution of the three diameter classes for both species were tested for clumping using univariate second-order spatial pattern analysis, based on Ripley's (1976) *K*-function. This method considers all individuals of trees within a given radius t of the focal individual. The estimator of the function $K(t)$ used is:

$$K(t) = n^{-2}A\sum\sum_{i\neq j} w_{ij}^{-1} I_t(u_{ij})$$

where n is the number of plants in the plot, A is the area of the plot in square metres (m²), I_t is a counter variable, u_{ij} is the distance between trees i and j and w_{ij} is a weighting factor to correct for edge effects (Haase 1995). The $K(t)$ was calculated separately for each distance t (0–245 m in 35-m increments). Results were displayed as a plot of $\sqrt{[K(t)/\pi]} - t$, and then plot $K(t)$ vs. t to examine the spatial dispersion at all distance classes t. To test the significant deviation from a random distribution, Monte Carlo computer-generated data were used. To construct a 99% confidence envelope, 99 simulations were run and the sample statistic was compared to this envelope. These calculations were performed using the program SPATIAL POINT PATTERN ANALYSIS (Haase 1995).

Spatial genetic structure for *S. leprosula* was analysed for Moran's *I* coefficient (Moran 1950). The correlograms are computed as an indication of spatial scale of genetic substructuring (Sokal & Oden 1978; Sokal & Wartenberg 1983). Allele frequencies greater than 5% were calculated. Average Moran's *I* coefficients were calculated for all alleles as a summary statistic. A permutation procedure using Monte Carlo simulations was applied to test significant deviation from random spatial distribution of each calculated measure (Manly 1997). Each permutation consisted of a random redistribution of multilocus genotypes over the spatial coordinate of the sampled trees. For each of the spatial distance classes, observed values were compared with the distribution obtained after 1000 permutations.

A 99% confidence interval for the parameters was constructed as the interval (Streiff *et al*. 1998). These calculations were performed using the program SPATIAL GENETIC SOFTWARE-SGS (Degen *et al*. 2001b).

The Moran's *I* coefficient has been used widely, but recently many studies have also used the kinship coefficient for spatial genetic structure analysis (e.g. Loiselle *et al*. 1995; Hardy & Vekemans 2001; Kalisz *et al*. 2001; Parker *et al*. 2001; Chung *et al*. 2002, 2003; Dutech *et al*. 2002; Erickson & Hamrick 2003). The kinship coefficient, a measure of coancestry (F_{ij}) was used in the analysis of spatial genetic structure of *S. ovalis* ssp. *sericea*. This coefficient can estimate between pairs of mapped individuals *i* and *j* or the probability that genes in different individuals within subpopulations are identical by descent (Cockerham 1969). This statistic was computed between all pairs of individuals belonging to the same ploidal using multilocus estimates obtained following Loiselle *et al*. (1995), where the contribution of each allele is weighted by its respective polymorphism $p_{lu}(1 - p_{lu})$, with p_{lu} being the *u*th allele frequency at locus *l*. The average F_{ij} over pairs of individuals was computed for distance intervals of 35 m. The standard error over loci was estimated using the jackknife method. The absence of spatial genetic structure was tested within each class using a permutation method (1000 permutations); spatial distances were permuted randomly among pairs of individuals, and the estimated value of the average kinship coefficient was compared to the distribution after permutations. These calculations were performed using the program AUTOCORG 2.1 (Hardy & Vekemans 2001; available at ohardy@ulb.ac.be).

Results

Genetic diversity

For *S. leprosula*, a total of four allozyme and seven microsatellite loci were resolved consistently. Comparatively, genetic diversity parameters estimated using allozyme loci (Table 1) were consistently lower than microsatellite loci (Table 2). The number of alleles observed for allozyme loci ranged from two (*Pgm-1* and *Mdh-1*) to six (*Gpi-1*), and for microsatellite loci from three (*Shc*17) to 20 (*Shc*07). The mean number of alleles per locus for allozyme loci ranged from 2.5 (SMA) to 3.5 (BIG and MED) and for microsatellite loci from 11.0 (SMA) to 11.4 (BIG). The mean expected heterozygosity was relatively similar across the three diameter classes for allozyme loci (BIG = 0.49, MED = 0.51 and SMA = 0.48) and microsatellite loci (BIG = 0.70, MED = 0.71 and SMA = 0.69). However, the mean effective number of alleles per locus and allelic richness for allozyme were highest in MED (A_e = 2.11 and R_s = 3.48), followed by BIG (A_e = 1.98 and R_s = 3.08) and SMA (A_e = 1.91 and R_s = 2.47). The mean effective number of alleles per locus for microsatellite loci also shows a similar trend to allozyme loci, with the highest value in MED (6.21) followed by BIG (5.50) and SMA (4.93). However, mean allelic richness values for microsatellite loci showed the highest value in

Diameter class/ locus	*N*	A_a	A_e	R_s	H_O	H_E
BIG						
Gpi-1	62	6	2.25	4.87	0.53	0.56
Ugp-1	62	4	2.13	3.43	0.45	0.54
Pgm-1	55	2	1.96	2.00	0.24	0.49
Mdh-1	58	2	1.60	2.00	0.22	0.38
Mean	59.3	3.5	1.98	3.08	0.36	0.49
SE	1.7	1.0	0.14	0.69	0.03	0.04
MED						
Gpi-1	36	5	2.68	4.92	0.73	0.64
Ugp-1	36	5	2.33	4.98	0.42	0.58
Pgm-1	35	2	1.94	2.00	0.37	0.49
Mdh-1	33	2	1.50	2.00	0.18	0.34
Mean	35.0	3.5	2.11	3.48	0.42	0.51
SE	0.7	0.9	0.26	0.85	0.06	0.07
SMA						
Gpi-1	38	3	2.11	2.98	0.55	0.53
Ugp-1	37	3	2.05	2.89	0.43	0.52
Pgm-1	34	2	1.84	2.00	0.35	0.46
Mdh-1	38	2	1.63	2.00	0.16	0.39
Mean	36.8	2.5	1.91	2.47	0.37	0.48
SE	0.9	0.3	0.11	0.27	0.02	0.03

Table 1 Levels of genetic diversity of *Shorea leprosula* in three diameter classes (BIG, MED and SMA) based on four allozyme loci. Number of individuals (*N*); mean number of alleles per locus (A_a); effective number of alleles per locus (A_e); allelic richness (R_s); observed heterozygosity (H_O) and expected heterozygosity (H_E)

Table 2 Levels of genetic diversity of *Shorea leprosula* and *Shorea ovalis* ssp. *sericea* in three diameter classes (BIG, MED and SMA) based on seven microsatellite loci

	Shorea leprosula						*Shorea ovalis* ssp. *sericea*			
Diameter class/locus	N	A_a	A_e	R_s	H_O	H_E	N	A_a	H_O	H_E
BIG										
*Shc*01	63	18	10.43	17.22	0.60	0.91	50	12	0.25	0.83
*Shc*02	63	7	2.27	6.89	0.70	0.57	45	6	0.87	0.77
*Shc*03	62	5	2.27	4.95	0.58	0.56	56	3	0.06	0.14
*Shc*04	58	17	9.63	16.58	0.83	0.90	52	13	0.78	0.87
*Shc*07	62	19	8.90	18.42	0.73	0.90	41	18	0.48	0.85
*Shc*09	63	10	3.47	9.23	0.57	0.72	52	9	0.74	0.77
*Shc*17	63	4	1.55	3.76	0.37	0.35	52	4	0.31	0.48
Mean	62.0	11.4	5.50	11.01	0.63	0.70	49.7	9.3	0.50	0.67
SE	0.7	2.4	1.49	2.36	0.06	0.08	1.9	2.0	0.04	0.10
MED										
*Shc*01	56	16	11.06	15.90	0.59	0.92	53	20	0.45	0.91
*Shc*02	60	7	2.87	6.80	0.78	0.66	47	6	0.79	0.70
*Shc*03	60	5	2.27	4.99	0.58	0.56	54	2	0.01	0.02
*Shc*04	57	15	9.06	14.79	0.84	0.90	53	12	0.89	0.86
*Shc*07	57	20	12.97	19.58	0.98	0.93	40	18	0.33	0.91
*Shc*09	58	12	3.86	11.45	0.53	0.75	55	8	0.83	0.78
*Shc*17	60	3	1.35	3.00	0.28	0.26	48	2	0.28	0.33
Mean	58.3	11.1	6.21	10.93	0.66	0.71	50.0	9.7	0.51	0.64
SE	0.6	2.4	1.78	2.34	0.09	0.09	2.0	2.8	0.05	0.13
SMA										
*Shc*01	52	17	8.50	16.68	0.62	0.89	49	10	0.55	0.87
*Shc*02	54	7	2.51	6.78	0.79	0.61	57	6	0.80	0.69
*Shc*03	54	5	1.67	4.89	0.43	0.41	57	3	0.09	0.13
*Shc*04	48	16	9.40	16.00	0.73	0.90	57	11	0.85	0.83
*Shc*07	54	17	6.67	16.51	0.85	0.86	55	14	0.22	0.74
*Shc*09	54	11	4.14	10.86	0.59	0.77	56	8	0.75	0.80
*Shc*17	54	4	1.62	3.78	0.44	0.39	57	2	0.26	0.31
Mean	52.9	11.0	4.93	10.79	0.64	0.69	55.4	7.7	0.50	0.62
SE	0.9	2.2	1.23	2.15	0.06	0.08	1.1	1.7	0.04	0.11

BIG (11.01) followed by MED (10.93) and SMA (10.79). For *S. ovalis* ssp. *sericea*, seven microsatellite loci were resolved consistently. In general, *S. ovalis* ssp. *sericea* exhibited relatively high levels of genetic diversity for all three diameter classes (Table 2). The mean number of alleles per locus was found to be highest in MED (9.7), followed by BIG (9.3) and SMA (7.7). The expected heterozygosity was found to be relatively similar across all three diameter classes (BIG = 0.67, MED = 0.64 and SMA = 0.62).

Fixation index

For *S. leprosula*, the F_{IS} values were found to be significantly different from zero in BIG for both the allozyme (0.27) and microsatellite loci (0.08), but was not significantly different from zero in MED and SMA for both markers (Table 3).

Table 3 Fixation indices (F_{IS}) in three diameter classes (BIG, MED and SMA) based on four allozyme and seven microsatellite loci in *Shorea leprosula* and seven microsatellite loci in *S. ovalis* ssp. *sericea*

Species/diameter class	Microsatellite	Allozyme
Shorea leprosula		
BIG	0.08* (0.02)	0.27* (0.11)
MED	0.05 (0.03)	0.18 (0.13)
SMA	0.03 (0.03)	0.22 (0.14)
Shorea ovalis ssp. *sericea*		
BIG	0.30* (0.04)	NA
MED	0.20* (0.04)	NA
SMA	0.20* (0.04)	NA

*Significantly different from zero ($P < 0.05$).
NA: not available.

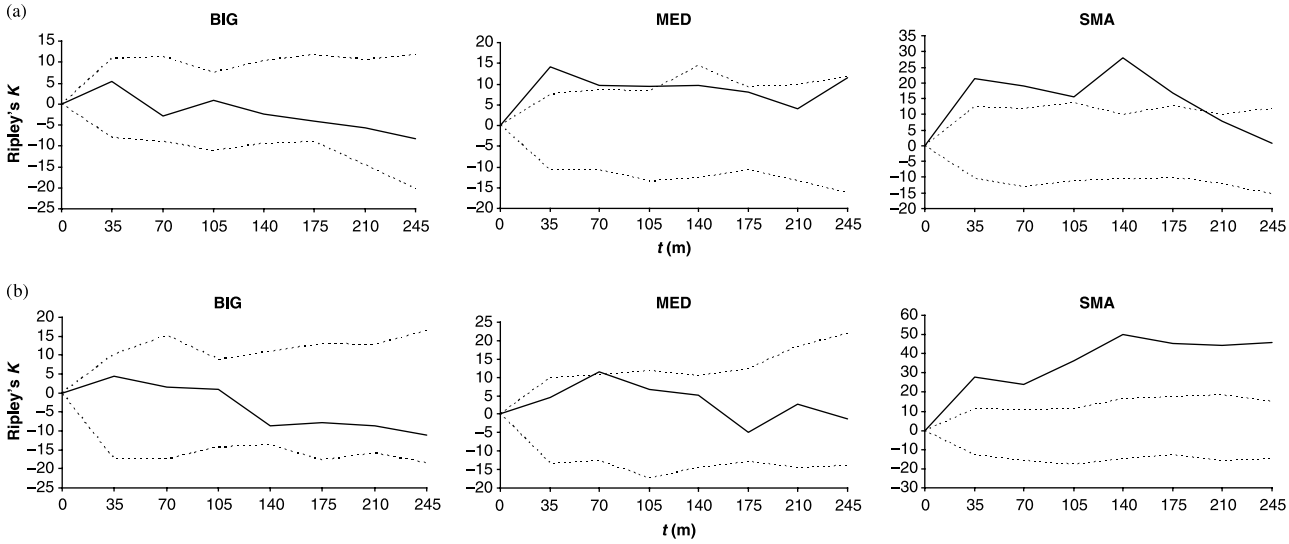

Fig. 3 The spatial distribution pattern analysis using Ripley's *K* for each diameter-class across the 30-ha plot for *Shorea leprosula* (a) and across the 50-ha plot for *S. ovalis* ssp. *sericea* (b). Continuous lines represent the sample statistic ($\sqrt{[K(t)/\pi]} - (t)$ and dashed lines represent 99% confidence envelope over t = 0–245 m.

The F_{IS} values calculated for *S. ovalis* ssp. *sericea* were found to be significantly different from zero in all diameter classes (BIG = 0.30, MED and SMA = 0.20; Table 3). The significant positive mean of F_{IS} values might suggest excess of homozygotes.

Spatial distribution

The results of the spatial distribution pattern analysis of *S. leprosula* and *S. ovalis* ssp. *sericea* are shown in Fig. 3a,b, respectively. For *S. leprosula*, significant spatial aggregation were observed for SMA (t = 0–175 m) and MED (t = 0–105 m) but random distribution in BIG (t = 0). Similarly, *S. ovalis* ssp. *sericea* also showed significant spatial aggregation in SMA (t = 0–245) and MED (t = ~70–81 m) but random distribution in BIG (t = 0). The magnitude of spatial aggregation was observed from high aggregation to random distribution as diameter class increases for both species (SMA > MED > BIG). Therefore, decrease in spatial aggregation with age was observed in both the outcrossed and apomictic species.

Spatial genetic structure

For *S. leprosula*, allozyme data revealed significant spatial genetic structure in MED (105 m) and SMA (35 m) but not in BIG (Fig. 4a). However, analyses based on microsatellite data showed significant spatial genetic structure mainly for short distances in all the diameter classes (Fig. 4b), with a decrease in magnitude of spatial genetic structure from smaller- to larger-diameter classes (SMA = 140 m > MED = 70 m > BIG = 35 m). Similarly, a significant decrease of spatial genetic structure with age was also observed for *S. ovalis* ssp. *sericea* (SMA = 140 m > MED = 70 m > BIG = 35 m; Fig. 4c).

Discussion

Genetic diversity

Two different biparentally inherited codominant genetic markers were used on *S. leprosula*, one originating from coding sequences (allozyme) and the other from noncoding sequences (as it is common for microsatellites; Tautz 1989). It is generally agreed that microsatellite sequences mutate at a high rate (due to DNA polymerase slippage), creating much higher variability (more alleles) in comparison to allozyme sequences (Schlotterer & Pemberton 1998). In this study, we found higher levels of genetic diversity for microsatellite than allozyme and this is agreeable with several studies on forest trees species (Chase *et al*. 1996; Echt *et al*. 1998; Streiff *et al*. 1998; Butcher *et al*. 1999; Degen *et al*. 1999).

A major impediment for microsatellite analysis in the past was the need to develop PCR primers for every species. However, many recent studies have shown that microsatellites and their flanking regions are conserved across closely related species (e.g. Konuma *et al*. 2000; Stacy *et al*. 2001). Some studies even showed that microsatellite region was conserved over phylogenetic distances of several million years (FitzSimmons *et al*. 1995; Rico *et al*. 1996). Molecular phylogeny analysis of Dipterocarpaceae revealed close affinities among *Shorea*, *Hopea*, *Dryobalanops* and *Neobalanocarpus* (Tsumura *et al*. 1996; Kajita *et al*. 1998; Dayanandan

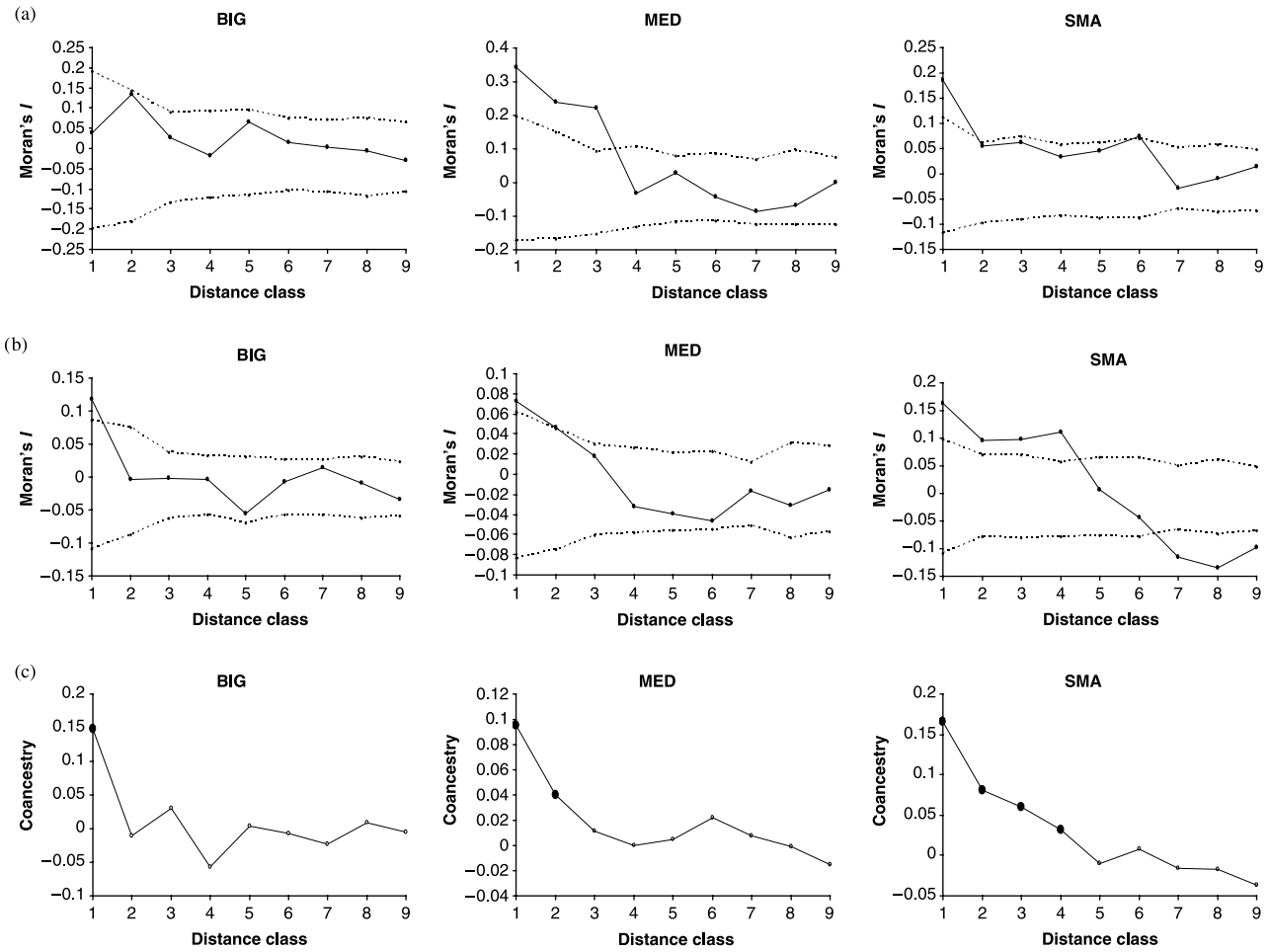

Fig. 4 (a, b) Correlograms of estimated Moran's *I* coefficient for *Shorea leprosula* using allozyme and microsatellite, respectively. Dashed lines represent upper and lower 99% confidence limits around zero relationship. (c) Correlograms of estimated coancestry (F_{ij}) using microsatellite for *S. ovalis* ssp. *sericea*. The filled circles in each of the diameter classes refer to values that deviate significantly from zero ($P < 0.01$). For (a), (b) and (c), distance class was defined at nine intervals; each interval is 35 m, from 0 to 35 m (distance class 1) up to 280–315 m (distance class 9).

et al. 1999). In this study, we have amplified successfully seven microsatellite primers developed for *S. curtisii* on *S. leprosula* and *S. ovalis* ssp. *sericea*, and this might support further that affinities among these species are relatively closed.

The observed mean number of alleles per locus (A_a) in all three diameter classes of *S. leprosula* (A_a = 11.0–11.4) was higher in comparisons with *S. curtisii* (A_a = 7.9 based on 40 samples over eight loci; Ujino *et al*. 1998), *Neobalanocarpus heimii* (A_a = 8.8 based on 30 samples over four loci; Konuma *et al*. 2000) and *Dryobalanops aromatica* (A_a = 5.1 based on seven loci over five populations, each about 18 samples; Lim *et al*. 2002). However, in terms of expected heterozygosities (H_E), the values of *S. leprosula* (0.69–0.71) were relatively similar to *S. curtisii* (0.64), *N. heimii* (0.78) and *D. aromatica* (0.71). The value of A_a is affected greatly by sample size (Leberg 2002). Hence, the higher values of A_a observed in *S. leprosula* could be due to the larger number of individuals being used in the present study. However, it could also be due to the characteristic of the species, where the species had been reported to exhibit remarkably high levels of genetic diversity (Lee *et al*. 2000b). The value of H_E is determined mainly by the evenness of allele frequencies (Marshall & Brown 1975). In comparison with *S. curtisii*, *N. heimii* and *D. aromatica*, *S. leprosula* exhibited the highest value of A_a. However, many of these alleles were present at low frequencies and this could be the reason why the H_E of *S. leprosula* is relatively similar to *S. curtisii*, *N. heimii* and *D. aromatica*.

Shorea ovalis ssp. *sericea* (Table 2) displayed comparable levels of genetic diversity with *S. leprosula* (present study), *S. curtisii* (Ujino *et al*. 1998), *N. heimii* (Konuma *et al*. 2000) and *D. aromatica* (Lim *et al*. 2002). Similarly, many studies also showed that autotetraploid species exhibited high

levels of genetic diversity (e.g. *Tolmiea menziesii*: Soltis & Rieseberg 1986; *Rutidosis leptorrhynchoides*: Brown & Young 2000; *Vaccinium oxycoccos*: Mahy *et al*. 2000; *Centaurea jacea*: Hardy & Vekemans 2001). This can be explained by the lower pressure of genetic drift due to the effects of tetrasomic inheritance on autotetraploid species (Stebbins 1980; Moody *et al*. 1993; Mahy *et al*. 2000). In addition, for autotetraploids, each locus can accommodate up to four different alleles and this allows maintenance of more alleles at individual loci. The observed high levels of genetic diversity in *S. ovalis* ssp. *sericea* could also be due to a random retention of more heterogeneous individuals in the past, and the apomictic mode of reproduction might be an evolutionary strategy which allows the species to maintain high levels of genetic diversity.

Fixation index

Shorea ovalis ssp. *sericea* is an apomictic species and due to the absence of segregation and recombination during reproduction, the value of F_{IS} may possibly indicate either an excess of heterozygotes or that of homozygotes. However, in this study an excess of homozygotes was observed. The significant positive value of F_{IS} for *S. leprosula* at large-diameter class might indicate an excess of homozygotes in a manner consistent with inbreeding due to selfing or biparental mating (Lee *et al*. 2000a).

The proportion of homozygotes has been reported frequently to decrease with age and this was explained by the selection in favour of particular heterozygote genotype individuals or the selection against homozygote individuals resulting from inbreeding depression (Schaal & Levin 1976; El Kassaby *et al*. 1987; Hamrick *et al*. 1993; Tonsor *et al*. 1993; Alvarez-Buylla *et al*. 1996). However, in this study the F_{IS} values calculated for both species (Table 3) showed a contrary result; the proportion of heterozygotes reduces as diameter classes increases. Similar observations have also been reported on *Pinus attenuate* (Strauss 1986) and *Pinus clausa* (Parker *et al*. 2001). This might indicate the lack of heterozygote advantage and selection might be in favour of particular homozygous genotypes. Nevertheless, it is still not clear how homozygosity and heterozygosity affect survival and their response to different selection forces. If the hypothesis of heterozygote advantage is preferable then the homozygous genotype individuals might show inferior qualities, but they can still manage to survive and reach maturity under favourable environmental conditions.

Spatial distribution pattern

Spatial distribution pattern analyses showed that the majority of tropical tree species aggregated at various diameter classes (Hubbell 1979; He *et al*. 1997; Itoh *et al*. 1997; Okuda *et al*. 1997; Condit *et al*. 2000; Plotkin *et al*. 2000). Similarly, in this study we found significant spatial aggregation in medium- and small-diameter classes for both the *S. leprosula* and *S. ovalis* ssp. *sericea* (small-diameter class trees are generally more clumped than medium-diameter class trees). The possible mechanisms of clumping have been discussed by other groups from the viewpoint of seed dispersal (Plotkin *et al*. 2000), gap recruitment (Itoh *et al*. 1997; Plotkin *et al*. 2000), distance-dependent mortality (Itoh *et al*. 1997), density-dependent recruitment (Okuda *et al*. 1997), topography (Plotkin *et al*. 2000), pest effect (Wills & Condit 1999; Harms *et al*. 2000), herbivores and plant diseases (Condit *et al*. 2000) and species density (Condit *et al*. 2000). For *S. leprosula* and *S. ovalis* ssp. *sericea*, seed shadow due to limited seed dispersal determines basically the clumping of small-diameter class trees. After seed dispersal, the compensatory mortality due to environmental heterogeneity (e.g. formation of gaps due to canopy opening) and intra- and interspecific competition on small-diameter class trees might lead to less clumping on medium-diameter class trees. Subsequently, the compensatory mortality due to microenvironmental selection, herbivores and plant diseases on medium-diameter class trees will aggravate the thinning process so that only few individuals will be able to survive and form the future adults, and this might cause the random distribution on large-diameter class trees.

Spatial genetic structure

The use of allozyme markers on *S. leprosula* could detect spatial genetic structure in only small- and medium-diameter class trees, while microsatellite markers were able to detect spatial genetic structure for all three diameter classes. Because we used the same set of individuals for both markers, the microsatellite marker can be considered to have higher sensitivity (because microsatellite loci carry more alleles) than allozyme markers to detect spatial genetic structure, and this merit has also been reported in other studies (e.g. Peakall *et al*. 1995; Powell *et al*. 1996; Smouse & Peakall 1999; Degen *et al*. 2001a).

The ecological and evolutionary processes (e.g. limited seed dispersal, competitive thinning, predation and environmental heterogeneity) that affect spatial distribution patterns can also be contributing factors to the observed spatial genetic structure. The decrease in magnitude of spatial genetic structure from smaller- to larger-diameter classes was observed in both species, which is in accordance with other studies (Hamrick *et al*. 1993; Berg & Hamrick 1995; Epperson & Alvarez-Buylla 1997). This can be explained by the compensatory mortality and competitive thinning process during recruitment and selection in favour of different genotypes. In addition, the presence of different levels of spatial genetic structure at different diameter classes indicated that analysis of spatial genetic structure

study based on pooled life stages may fail to observe genetic structure and might lead to misinterpretation of the underlying ecological and evolutionary processes.

Spatial genetic structure can also be affected by limited pollen dispersal, local genetic drift and inbreeding (Wright 1943; Levin & Kerster 1974; Turner *et al*. 1982; Heywood 1991; Epperson 1995). The major causes of spatial genetic structure within populations have been reported to be due to restricted pollen and seed dispersal (Wright 1943; Latta *et al*. 1998). The high spatial genetic structure observed in *S. ovalis* ssp. *sericea* at the smaller-diameter class is due probably to limited seed dispersal and apomictic modes of reproduction. Similar observations in *S. leprosula*, however, can be explained by limited seed and pollen dispersal, which support further the fact that the species is pollinated by weak fliers, mainly of *Thrips* and *Megalurothrips* in the lowland dipterocarp forest (Chan & Appanah 1980; Appanah & Chan 1981). Lee *et al*. (2000a) has reported a high outcrossing rate in *S. leprosula*. However, a high outcrossing rate might not necessarily indicate long-distance pollen flow, as mating can occur among nearby trees, depending upon the pollinators. Lee *et al*. (2000a) also reported a high rate of biparental mating in *S. leprosula* and this supported further the fact that mating among nearby trees is common and the species is pollinated by low energetic insects.

Knowledge of spatial genetic structure at different life stages of forest tree species can be used as part of the practical guidelines for forest harvesting systems. Logging activities have been reported to increase the proportion of seeds set by selfing (Murawski *et al*. 1994; Doligez & Joly 1997). However, if a species is, to some extent, structured genetically within a population, logging activities may reduce inbreeding caused by biparental mating (Lee 2000). This study showed that both the outcrossing and the autotetraploidy–apomitic species are spatially genetically structured (in which individuals within each clump are more similar genetically compared to individuals of other clumps). If the clumps consist of many individuals, to avoid biparental mating the logging activities can be designed in such a way that only a few individuals are left behind for each clump. This may reduce the chances of mating among relatives and at the same time maintain stand-scale genetic diversity.

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This study forms part of the PhD study of K. K. S. Ng and it was initiated by S. L. Lee, both of whom shared a similar interest in population genetics, reproductive biology and ecology of tropical tree species, in particular Dipterocarpaceae. C. L. Koh is a Professor of genetics with a broad interest in molecular genetics and population genetics of humans and plants.
