ESTIMATION OF OUTCROSSING RATES IN *KOOMPASSIA MALACCENSIS* FROM AN OPEN-POLLINATED POPULATION IN PENINSULAR MALAYSIA USING MICROSATELLITE MARKERS

CT Lee[1, *], SL Lee[1], KKS Ng[1], QZ Faridah[2], SS Siraj[3], M Norwati[1]

[1]*Forest Research Institute Malaysia, 52109 Kepong, Selangor Darul Ehsan, Malaysia*
[2]*Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*
[3]*Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*

Received September 2010

LEE CT, LEE SL, NG KKS, FARIDAH QZ, SIRAJ SS & NORWATI M. 2011. Estimation of outcrossing rates in *Koompassia malaccensis* from an open-pollinated population in Peninsular Malaysia using microsatellite markers. *Koompassia malaccensis* (Leguminosae), locally known as kempas, is an important tropical timber species in South-East Asia. Although studies have shown that most tropical tree species are predominantly outcrossing, there is no empirical support for this species prior to this study, with regard to its mating system. Information on its reproductive biology is also scanty. We report the estimation of the outcrossing rates of *K. malaccensis* using microsatellite markers, based on a fruiting season at the Semangkok Forest Reserve, Selangor. Microsatellite analysis was performed for an average of 46 seeds each from nine adult *K. malaccensis* trees, using four polymorphic microsatellite loci (*Kma050*, *Kma067*, *Kma147* and *Kma180*). Single and multilocus population outcrossing estimates (t_s and t_m respectively) were determined using the software MLTR version 3.0. Results showed that this timber species was predominantly outcrossing ($t_m = 0.890$). Biparental mating ($t_m - t_s$) was very low, only 0.026, suggesting low tendency of mating between relatives. Outcrossing estimates obtained for individual mother trees were in the range of 0.637 to 0.994. The relatively lower outcrossing rates exhibited by a few progeny arrays indicated that *K. malaccensis* was not completely self-incompatible.

Keywords: Kempas, tropical rain forest, Leguminosae, bee-pollinated

LEE CT, LEE SL, NG KKS, FARIDAH QZ, SIRAJ SS & NORWATI M. 2011. Penganggaran kadar kacukan luar *Koompassia malaccensis* dalam satu populasi dari Semenanjung Malaysia yang melalui pendebungaan secara terbuka menggunakan penanda mikrosatelit. *Koompassia malaccensis* (Leguminosae), atau dikenali dengan nama tempatannya kempas, merupakan satu spesies balak tropika yang penting di Asia Tenggara. Sungguhpun terdapat kajian yang menunjukkan bahawa kebanyakan spesies pokok tropika mengamalkan kacukan luar secara pradominan, namun sebelum kajian ini, tiada sokongan empirikal yang dilaporkan berkenaan sistem kacukan spesies ini. Maklumat tentang biologi pembiakannya juga amat kurang. Kami melaporkan penganggaran kadar kacukan luar untuk *K. malaccensis* menggunakan penanda mikrosatelit berdasarkan satu musim buah di Hutan Simpan Semangkok, Selangor. Analisis mikrosatelit telah dijalankan untuk progeni daripada sembilan pokok *K. malaccensis* dewasa, dengan purata 46 biji setiap pokok, menggunakan empat lokus mikrosatelit yang polimorfik (*Kma050*, *Kma067*, *Kma147* dan *Kma180*). Anggaran kacukan luar populasi secara lokus tunggal dan multilokus (masing-masing t_s dan t_m) telah ditentukan dengan menggunakan program MLTR versi 3.0. Keputusan menunjukkan bahawa spesies balak ini mengamalkan kacukan luar dengan $t_m = 0.890$. Kacukan dwiinduk ($t_m - t_s$) didapati sangat rendah, hanya 0.026; ini mencadangkan bahawa kecenderungan kacukan antara individu yang berpertalian rapat adalah rendah. Anggaran kacukan luar yang diperoleh untuk pokok induk individu adalah dalam lingkungan 0.637 hingga 0.994. Kadar kacukan luar yang lebih rendah secara relatif yang ditunjukkan oleh beberapa kumpulan progeni menunjukkan bahawa *K. malaccensis* bukan takswaserasi sepenuhnya.

INTRODUCTION

Mating system is the pattern by which gametes are united to transmit genes from one generation to the next to produce the offspring of a population (Stern & Roche 1974). It is one of the factors that shape the genetic variation of a plant species. Plants have a wide spectrum of sexual reproduction systems. Some plants display self-incompatibility while some are

**E-mail: leechait@frim.gov.my*

predominantly selfing. In between are species that, despite being self-compatible, have other mechanisms to avoid inbreeding (Geburek 2005). Mating systems are grouped into five categories, namely, predominantly selfing, predominantly outcrossing, mixed selfing and outcrossing, apomictic, and haploid selfing (Brown 1990).

Traditionally, plant mating systems were inferred from the floral biology and intraspecific crossing compatibility of the species (Bawa 1974). However, only limited species could be tested due to the constraint of logistical difficulties involved in controlled pollinations of very tall trees. Such limitations have been overcome with the application of genetic markers, which can provide information on mating pattern and level of inbreeding through the analysis of progeny arrays (O'Malley & Bawa 1987). In particular, codominant markers such as isozymes and microsatellites are useful in the study of mating systems for plants (Fritsch & Riesberg 1992, Pace & Qualset 1995, Obayashi et al. 2002, Karasawa et al. 2007).

Microsatellites, also called simple sequence repeats (SSRs), are informative DNA markers because they are codominant and highly polymorphic. These markers consist of tandemly repeated DNA motifs of 6 bp or less, widely dispersed throughout all prokaryotic and eukarotic genomes (Tautz 1989), such as $(CT)_n$ or $(GAG)_n$. Once specific primers have been established, SSRs can be amplified from small amount of genomic DNA by polymerase chain reaction (PCR). These attributes make microsatellite markers powerful molecular tools for various genetic analyses.

Koompassia malaccensis, or locally known as kempas, is one of the major commercial timber species in South-East Asia (Soerinegara & Lemmens 1994). It belongs to the family Leguminosae (Fabaceae), subfamily Caesalpinioideae. In Peninsular Malaysia, kempas was one of the top five sawntimber species exported from January till November 2010, with a volume of 31 631 m^3 valued at RM24.19 mil (Malaysian Timber Industry Board 2011). However, little is known about its mating system and reproductive biology. Mating system studies in forest genetics are very important to define optimal conservation strategies (Brown et al. 1985). Mating system is one of the major factors that shape the genetic variation of a plant species as well as an important determinant of the genetic structure of plant populations (Loveless & Hamrick 1984, Hamrick & Godt 1989). Thus, this study was carried out to better understand the mating system of this tropical emergent tree. The specific objective of this study was to estimate the outcrossing rates of *K. malaccensis* based on a single fruiting season in a natural population.

MATERIALS AND METHODS

During a fruiting season in September 2005, open-pollinated seed samples were collected from nine kempas trees located at the Semangkok Forest Reserve, Kuala Kubu Bharu, Selangor, on the wayside of the Kuala Kubu Bharu Road to Fraser's Hill. As these trees were distant from one another, seed samples were collected separately from beneath the mother trees. In the laboratory, the seeds were soaked in water after removing the papery wings. Then the inner coats were removed and the embryos excised for DNA extraction.

DNA was extracted from embryo tissue using the modified CTAB method (Murray & Thompson 1980). Four polymorphic loci, namely, *Kma050*, *Kma067*, *Kma147* and *Kma180*, (Lee et al. 2006) were used for the estimation of the outcrossing rates (Table 1). The forward primers were fluorescently labelled with 6-FAM (Bio Basic Inc). Microsatellite analysis was carried out for a total of 432 seeds, 48 putative progenies for each half-sib family. The PCR amplifications were carried out using a GeneAmp PCR System 9700 (Applied Biosystems) in a 10 µl reaction volume, with approximately 10 ng of template DNA, 50 mM of KCl, 20 mM of Tris-HCl (pH 8.0), 1.5 mM of $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP and 0.5 unit of *Taq* DNA polymerase (Promega). After an initial denaturing step of 3 min at 94 °C, PCR amplifications were performed for 35 cycles at 94 °C (30 s), annealing temperatures (30 s) and 72 °C (30 s), followed by a final extension step of 7 min at 72 °C. The annealing temperature for all the loci was 45 °C except for locus *Kma050*, which was 55 °C. For genotyping, the PCR products were subjected to fragment analysis using an ABI PRISM 377 DNA sequencer (Applied Biosystems). Allele sizes were assigned against GeneScan ROX 400 (Applied Biosystems) internal size standard using GENESCAN version 3.7.1 and genotyped using GENOTYPER version 3.7 software (Applied Biosystems). False progenies detected through

microsatellite analysis were later excluded from the data analysis.

Calculations of the allele frequencies by locus for all the half-sib families were performed using the MICROSATELLITE TOOLKIT (Park 2001). The mating system parameters were estimated using the software MLTR version 3.0 (Ritland 2002), which implements the mixed mating model. The underlying assumptions included: (1) each mating event was a random outcross or self-fertilisation event, (2) the probability of an outcross was independent of maternal genotype, (3) outcross pollen allele frequencies were homogeneous among maternal trees, (4) no occurrence of selection between fertilisation and the analysis of progeny arrays, and (5) alleles at different loci segregated independently (for multilocus estimates) (Ritland & Jain 1981). This model finds maximum likelihood solutions for the multilocus outcrossing rates (t_m), single locus outcrossing rates (t_s) and biparental inbreeding ($t_m - t_s$). Outcrossing rates were also obtained for indvidual mother trees. The variance of the estimates was generated by bootstrapping 1000 times using families as the resampling unit.

RESULTS

Maternal genotypes by locus for the nine adult trees are given in Table 2. Mother trees no. 2, 3, 4 and 6 exhibited heterozygous genotypes for all the four loci investigated. From the microsatellite analysis, 16 illegitimate progenies were detected based on their genotypic data which lacked maternal alleles. They were subsequently discarded from further analysis. Thus the progenies analysed were half-sibs of the respective mother trees. The number of alleles and allele frequencies for all the half-sib families by locus are given in Table 3. All the loci employed were highly polymorphic, with an average of about 12 observed alleles per locus. The total number of alleles for each family per locus ranged from 4 to 11.

Based on the microsatellite analysis, it was demonstrated that *K. malaccensis* was

Table 1 Primer sequences of the microsatellite loci applied in this study

Locus	GenBank accession no.	Repeat motif	Primer sequence (5'–3')
Kma050	DQ356308	$(CT)_{15}$	Forward: CAGTAAAGATGATAGTGCAGACAA Reverse: GTATCCGTTCCAATCAGTAAT
Kma067	DQ356311	$(CT)_{9}$	Forward: TCGGTCATTGGGAAACTCT Reverse: AGGAAGATTTGGGAGTCA
Kma147	DQ356323	$(AGG)_{6}$	Forward: ATGGGGTAATTTTCCGTCA Reverse: GTTTGTTTTCACGGTAATGG
Kma180	DQ356329	$(GA)_{13}$	Forward: AGCCTAAAACCCCAATGA Reverse: CAGGCTGCAGTGAGTTAAAC

Table 2 Genotypes of the *Koompassia malaccensis* mother trees investigated

Mother tree	Locus			
	Kma050	*Kma067*	*Kma147*	*Kma180*
1	102/108	171/175	344/344	246/254
2	104/116	157/171	337/344	248/252
3	108/116	163/171	337/342	244/248
4	110/116	171/195	342/345	246/260
5	114/116	173/191	344/344	250/262
6	98/102	171/173	337/342	250/252
7	116/120	171/171	337/346	250/255
8	116/118	171/175	344/344	246/246
9	114/116	175/177	346/349	250/250

Table 3 Allele frequencies by locus for all the *Koompassia malaccensis* half-sib families assayed

Locus	Allele	Allele frequency (%) for half-sib families								
		Family 1	Family 2	Family 3	Family 4	Family 5	Family 6	Family 7	Family 8	Family 9
Kma050	98	4.44	4.55	2.08	2.38	7.50	29.07	6.25	1.06	1.14
	102	28.89	2.27	3.13	1.19	0.00	37.21	3.13	1.06	1.14
	104	4.44	29.55	9.38	1.19	2.50	4.65	2.08	14.89	3.41
	106	1.11	0.00	0.00	0.00	2.50	0.00	0.00	0.00	0.00
	108	34.44	5.68	33.33	8.33	5.00	4.65	4.17	2.13	4.55
	110	1.11	0.00	3.13	35.71	1.25	0.00	0.00	0.00	0.00
	112	5.56	1.14	2.08	3.57	3.75	2.33	5.21	7.45	7.95
	114	3.33	1.14	2.08	1.19	27.50	2.33	9.38	3.19	31.82
	116	8.89	54.55	33.33	41.67	45.00	12.79	38.54	32.98	38.64
	118	7.78	0.00	6.25	4.76	1.25	3.49	1.04	35.11	9.09
	120	0.00	0.00	5.21	0.00	3.75	1.16	29.17	2.13	0.00
	122	0.00	1.14	0.00	0.00	0.00	2.33	1.04	0.00	2.27
Total no. of alleles		10	8	10	9	10	10	10	9	9
Kma067	157	0.00	36.05	3.13	1.14	2.38	0.00	0.00	1.06	1.14
	163	1.11	2.33	23.96	0.00	0.00	0.00	0.00	0.00	0.00
	169	2.22	1.16	0.00	1.14	0.00	0.00	0.00	0.00	0.00
	171	33.33	52.33	56.25	57.95	32.14	63.95	85.42	58.51	30.68
	173	44.44	4.65	8.33	3.41	29.76	31.40	10.42	12.77	5.68
	175	13.33	0.00	2.08	1.14	1.19	1.16	1.04	26.60	25.00
	177	2.22	1.16	4.17	1.14	1.19	3.49	3.13	0.00	31.82
	183	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.14
	191	2.22	2.33	2.08	2.27	33.33	0.00	0.00	1.06	3.41
	193	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.14
	195	1.11	0.00	0.00	31.82	0.00	0.00	0.00	0.00	0.00
Total no. of alleles		8	7	7	8	6	4	4	5	8
Kma147	337	2.22	43.02	32.98	0.00	2.44	24.36	30.85	4.35	1.11
	341	2.22	0.00	0.00	0.00	0.00	1.28	0.00	0.00	1.11
	342	12.22	6.98	35.11	43.02	8.54	41.03	9.57	14.13	11.11
	343	3.33	0.00	2.13	2.33	0.00	1.28	1.06	0.00	1.11
	344	70.00	39.53	12.77	9.30	70.73	11.54	19.15	76.09	15.56
	345	3.33	2.33	9.57	40.70	3.66	7.69	3.19	0.00	12.22
	346	4.44	4.65	3.19	1.16	9.76	12.82	35.11	4.35	31.11
	347	0.00	0.00	4.26	0.00	0.00	0.00	0.00	1.09	0.00
	348	2.22	1.16	0.00	0.00	0.00	0.00	0.00	0.00	2.22
	349	0.00	0.00	0.00	2.33	1.22	0.00	0.00	0.00	24.44
	350	0.00	2.33	0.00	0.00	3.66	0.00	0.00	0.00	0.00
	356	0.00	0.00	0.00	1.16	0.00	0.00	1.06	0.00	0.00
Total no. of alleles		8	7	7	7	7	7	7	5	9
Kma180	244	2.22	4.55	27.08	2.27	7.14	7.50	14.89	0.00	2.63
	246	36.67	11.36	9.38	50.00	7.14	15.00	6.38	71.05	13.16
	248	10.00	38.64	35.42	4.55	10.71	5.00	7.45	10.53	10.53
	250	2.22	1.14	2.08	3.41	29.76	18.75	23.40	13.16	57.89
	252	1.11	37.50	7.29	1.14	4.76	40.00	5.32	0.00	0.00
	254	28.89	2.27	4.17	2.27	0.00	1.25	4.26	1.32	3.95
	255	5.56	0.00	10.42	4.55	2.38	6.25	32.98	2.63	1.32
	256	2.22	2.27	0.00	1.14	3.57	2.50	2.13	0.00	0.00
	258	0.00	0.00	0.00	1.14	0.00	0.00	0.00	1.32	0.00
	260	1.11	2.27	3.13	29.55	4.76	1.25	1.06	0.00	3.95
	262	6.67	0.00	1.04	0.00	29.76	2.50	2.13	0.00	3.95
	266	3.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.32
	271	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.32
Total no. of alleles		11	8	9	10	9	10	10	6	10

predominantly outcrossing. Table 4 shows the estimated multilocus outcrossing rates of each half-sib family, ranging from 0.637 to 0.994. The multilocus outcrossing rate (t_m) by population was 0.890 (SD 0.041), whereas the single locus outcrossing rate (t_s) was 0.864 (SD 0.042), inferring a minimal biparental mating ($t_m - t_s$) of 0.026 (SD 0.013).

DISCUSSION

To date, no study has been reported on the mating system of *K. malaccensis*. Information on the reproductive biology is also scarce. Bees (*Apis* spp.) are the main flower visitors (Appanah & Weinland 1993). From the microsatellite analysis of the progeny arrays, it was found that *K. malaccensis* from the Semangkok Forest Reserve exhibited highly outcrossing mating system, i.e. 89%. This is in general agreement with the other studies showing that most tropical tree species are predominantly outcrossing (Doligez & Joly 1997, Lee et al. 2000, Ward et al. 2005). It also corresponds with the life history traits of *K. malaccensis*, namely, widespread, having efficient gene flow and low population genetic structure, as supported by the genetic diversity study using microsatellites (Lee 2009).

From the microsatellite data, only minimal biparental mating (2.7%) was detected. This suggests a very low tendency of mating between relatives. This could be attributed to the bees which are the main visitors of its hermaphroditic flowers. Weak spatial genetic structure has been reported to be associated with long-distance seed dispersal and pollen flow (Epperson & Chung 2001, Parker et al. 2001, Ng et al. 2006). Although there are no reported empirical data on the spatial genetic structure, *K. malaccensis* most likely has weak spatial genetic structure, since it has seeds with single pod which are flat, oblong and surrounded by papery wing, characteristics fit for wind dispersal, apart from having energetic pollinators.

Among the outcrossing rates of individual mother trees, some values were relatively lower (mother tree 2: $t_m = 0.789$, 4: $t_m = 0.637$, 8: $t_m = 0.713$; Table 4) than the rest, inferring that *K. malaccensis* was not completely self-incompatible. Such phenomenon has been observed in various tropical tree species by controlled crossing experiments (Bawa 1974) as well as through molecular marker analysis (Murawski et al. 1994, Lemes et al. 2003). It is reported that one main advantage of self-compatibility is the increased probability of successful pollination which can be of considerable advantage in the face of pollination unpredictability or lack of pollinators (Bawa 1974). This idea was confirmed by recent theoretical papers showing that quantitative variation in self-incompatibility might reflect an evolutionarily stable mating strategy (Busch & Schoen 2008).

One possible reason for the low outcrossing rate observed in mother tree 4 could be that it was relatively more isolated or distant from the rest of the flowering adult trees. Since the seeds were collected from mother trees located by the roadside, and not from an ecological plot, data of the tree positions were not available to support this postulation. Future gene flow study of *K. malaccensis* based on a mapped population will

Table 4 Outcrossing rates of *Koompassia malaccensis* from Semangkok Forest Reserve based on microsatellite analyses

Mother tree	Number of progenies analysed	Multilocus outcrossing rate (t_m)
1	48	0.991 (0.021)
2	47	0.789 (0.077)
3	48	0.989 (0.008)
4	44	0.637 (0.209)
5	44	0.918 (0.074)
6	44	0.938 (0.035)
7	48	0.981 (0.018)
8	48	0.713 (0.163)
9	45	0.994 (0.011)

Standard deviations are given in parentheses.

be able to provide a more conclusive inference in this aspect.

CONCLUSIONS

Results from the microsatellite analysis of the progeny arrays collected from the Semangkok Forest Reserve showed that *K. malaccensis* had a mixed mating system which was predominantly outcrossing (89.0%) with a low level of consanguineous mating (2.7%). Low observed biparental mating is explainable by its energetic pollinators (bees) and mode of seed dispersal (wind). Variable multilocus population outcrossing rates obtained (t_m ranged from 0.637 to 0.994) inferred that *K. malaccensis* was not completely self-incompatible. The reasons for the observed spatial heterogeneity in the outcrossing rates can be further ascertained with gene flow study in the future.

ACKNOWLEDGEMENTS

This research was funded by the Forest Research Institute Malaysia (Project No. 40-30-04-04-019). We thank P Ramli, J Ghazali and M Yahya for their field assistance and D Mariam and T Sharifah for their technical assistance. The cooperation from the Forest Department of Peninsular Malaysia is much appreciated.

REFERENCES

Appanah S & Weinland G. 1993. *Planting Quality Timber Trees in Peninsular Malaysia: A Review.* Malayan Forest Records No. 38. Forest Research Institute Malaysia, Kepong.

Bawa KS. 1974. Breeding systems of tree species of a lowland tropical community. *Evolution* 28: 85–92.

Brown AHD. 1990. Genetic characterization of plant mating systems. Pp 145–162 in Brown AHD et al. (Eds) *Plant Population Genetics, Breeding, and Genetic Resources.* Sinauer Associates, Sunderland.

Brown AHD, Barrett SCH & Moran GF. 1985. Mating system estimation in forest trees, models, methods and meanings. Pp 32–49 in Gregarius HR (Ed) *Population Genetics in Forestry.* Springer-Verlag, Berlin.

Busch JW & Schoen D. 2008. The evolution of self-incompatibility when mates are limiting. *Trends in Plant Science* 13: 128–136.

Doligez A & Joly HI. 1997. Mating system of *Carapa procera* (Meliaceae) in the French Guiana tropical forest. *American Journal of Botany* 84: 461–470.

Epperson BK & Chung MG. 2001. Spatial genetic structure of allozyme polymorphisms within populations of *Pinus strobes* (Pinaceae). *American Journal of Botany* 88: 1006–1010.

Fritsch P & Reisberg LH. 1992. High outcrossing rates maintain male and hermaphrodite individuals in populations of the flowering plant *Datisca glomereta*. *Nature* 359: 633–636.

Geburek T. 2005. Sexual reproduction of forest trees. Pp171–198 in Geburek T & Turok J (Eds) *Conservation and Management of Forest Genetic Resources in Europe.* Arbora Publishers, Zvolen.

Hamrick JL & Godt MJW. 1989. Allozyme diversity in plant species. Pp 43–63 in Brown AHD et al. (Eds) *Plant Population Genetics, Breeding and Genetic Resources*, Sinauer Associates, Sunderland.

Karasawa MMG, Vencovsky R, Silva CM, Zucchi MI, Oliveira GCX & Veasy EA. 2007. Mating system of Brazilian *Oryza glumaepatula* populations studied with microsatellite markers. *Annals of Botany* 99: 245–253.

Lee CT. 2009. Development of microsatellite markers and genetic diversity assessment of kempas (*Koompassia malaccensis*) in Peninsular Malaysia. PhD thesis, Universiti Putra Malaysia, Serdang.

Lee CT, Lee SL, Faridah QZ, Siraj SS, Ng KKS, Norlia B & Mat-Isa MN. 2006. Isolation and characterization of microsatellite markers in *Koompassia malaccensis* (Leguminosae), an important tropical timber species. *Molecular Ecology Notes* 6: 1198–1201.

Lee SL, Wickneswari R, Mahani MC & Zakri AH. 2000. Mating system parameters in a tropical tree species, *Shorea leprosula* Miq. (Dipterocarpaceae), from Malaysian lowland dipterocarp forest. *Biotropica* 32: 693–702.

Lemes MR, Gribel R, Proctor J & Grattapaglia D. 2003. Population genetic structure of mahogany (*Swietenia macrophylla* King, Meliaceae) across the Brazilian Amazon, based on variation at microsatellite loci: implications for conservation. *Molecular Ecology* 12: 2875–2883.

Loveless MD & Hamrick JL. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.

Malaysian Timber Industry Board. 2011. Statistics of Peninsular Malaysia: export of sawntimber (by major species). http://www.mtib.gov.my. Accessed on 2 August 2011.

Murawski DA, Gunatilleke IAUN & Bawa KS. 1994. The effects of selective logging on inbreeding in *Shorea megistophylla* (Dipterocarpaceae) from Sri Lanka. *Conservation Biology* 8: 997–1002.

Murray M & Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8: 4321–4325.

Ng KKS, Lee SL, Saw LG, Plotkin JB & Koh CL. 2006. Spatial structure and genetic diversity of three tropical tree species with different habitat preferences within a natural forest. *Tree Genetics and Genomes* 2: 121–131.

O'Malley M & Bawa KS. 1987. Mating system of a tropical rain forest tree species. *American Journal of Botany* 74: 1143–1149.

Obayashi K, Tsumura Y, Ihara-Ujino T, Niiyama K, Tanouchi H, Suyama Y, Washitani I, Lee CT, Lee SL & Norwati M. 2002. Genetic diversity and outcrossing rate between undisturbed and selectively logged forests of *Shorea curtisii* (Dipterocarpaceae) using microsatellite

DNA analysis. *International Journal of Plant Science* 163: 151–158.

Pace CD & Qualset CO. 1995. Mating system and genetic differentiation in *Dasypyrum villosum* (Poaceae) in Italy. *Plant Systematics and Evolution* 197: 123–147.

Park SDE. 2001. Trypano tolerance in West African cattle and the population genetic effects of selection. PhD thesis, University of Dublin, Dublin. Software available at http://www.animalgenomics.ucd.ie/sdepark/ms-toolkit/

Parker KG, Hamrick JL, Parker AL & Nason JD. 2001. Fine-scale genetic structure in *Pinus clausa* (Pinaceae) populations: effects of disturbance history. *Heredity* 87: 99–113.

Ritland K. 2002. Extensions of models for the estimation of mating systems using n independent loci. *Heredity* 88: 221–228.

Ritland K & Jain S. 1981. A model for the estimation of outcrossing rate and gene frequencies using n independent loci. *Heredity* 47: 35–52.

Soerinegara I & Lemmens RHMJ (Eds). 1994. *Plant Resources of South-East Asia 5 (1) Timber Trees: Major Commercial Timbers. Bogor, Indonesia.* Pudoc Scientific Publishers, Wageningen.

Stern K & Roche L. 1974. *Genetics of Forest Ecosystem.* Springer-Verlag, New York.

Tautz D. 1989. Hypervariability of simple sequences as a general source of polymorphic DNA markers. *Nucleic Acids Research* 17: 6463–6471.

Ward MC, Dick W, Gribel R & Lowe AJ. 2005. To self, or not to self… a review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* 95: 246–254.

