

STUDIES OF MATING SYSTEMS IN SEED STANDS SUGGEST POSSIBLE CAUSES OF VARIABLE OUTCROSSING RATES IN NATURAL POPULATIONS OF *ACACIA MANGIUM*¹

Penny Butcher¹, Chris Harwood¹ & Tran Ho Quang²

¹) CSIRO Forestry and Forest Products, Canberra, ACT, Australia

²) Research Centre for Forest Tree Improvement Forest Science Institute of Vietnam

ABSTRACT

Acacia mangium, a tropical tree species planted widely for pulpwood production, occurs naturally on the margins of rainforest in north-eastern Australia and in the tropical lowlands of New Guinea. It is a pioneer species that establishes from seed after fire or other disturbance. The species has low levels of genetic diversity but high genetic differentiation among populations in different geographic regions. Outcrossing rates in natural populations, estimated using microsatellite markers, range from complete selfing in outlying populations with low genetic diversity to complete outcrossing in the more variable New Guinea populations. This is in contrast to other diploid acacias that are predominantly outcrossing. Studies in *A. mangium* seed orchards have shown that outcrossing rates are determined not only by the evolutionary history of the natural population used to establish the orchard but also the flowering patterns in the orchard. While the species is preferentially outcrossing, reductions in the number of flowering trees will result in a marked increase in the level of selfing and a decline in genetic diversity in the next generation. This clearly demonstrates the impact of a genetic bottleneck and explains the correlation between outcrossing rate and genetic diversity in natural populations. The adaptability of the breeding system in *A. mangium* may explain its success as a pioneer species.

Keywords: *Acacia mangium*, outcrossing, mating system

INTRODUCTION

Acacia mangium is an insect-pollinated (SEDGLEY *et al.* 1992a), mass flowering forest tree. It occurs on the margins of rainforest in north-east Queensland, Australia, in the tropical lowlands of Western Province, Papua New Guinea (PNG) and in the adjacent lowlands of south-eastern Irian Jaya. There are outlying populations in western Irian Jaya and on several of the Moluccu islands of eastern Indonesia. About one million hectares of plantations of *A. mangium* have been established in south-east Asia for pulpwood production (TURNBULL *et al.* 1998). Surveys of natural populations using isozymes (MORAN *et al.* 1989) and restriction fragment length polymorphisms (RFLPs) (BUTCHER *et al.* 1998) showed the species is unusual amongst forest trees in having relatively low levels of genetic diversity and marked differences in the level of diversity among natural populations.

A comparison of outcrossing rates using microsatellites showed that populations from New Guinea with high levels of diversity were completely

outcrossing, while populations with low diversity produce a high proportion of selfed seed (BUTCHER *et al.* 1999). Populations with high levels of selfing occur at the limits of the species geographic distribution. The southern-most populations in Queensland, Australia, are relatively small in size and fragmented (BUTCHER *et al.* 1998). They have low outcrossing rates (~30%). This is consistent with the evolution of high levels of selfing in response to genetic bottlenecks. A population in Sidei, Irian Jaya, is almost completely selfing (BUTCHER unpubl. data) and has extremely low levels of genetic diversity, consistent with population establishment following a founder event (BUTCHER *et al.* 1998).

Studies of breeding systems in seed orchard populations showed that (i) a seed orchard planted at Subanjeriji in Sumatra, Indonesia, based on Queensland natural populations with high levels of selfing, displayed similarly high levels of selfing (BUTCHER *et al.* 1999) while (ii) a seed orchard planted near Cardwell, Queensland, based on highly outcrossing Papua New Guinea populations, produced outcrossed seed (BUTCHER unpub. data). This

¹ This paper has been presented at the IUFRO Symposium on Population and Evolutionary Genetics of Forest Trees held in Stará Lesná, Slovakia, on August 25-29, 2002.

seed orchard was located in a region where natural populations produced a high proportion of selfed seed. These data suggested outcrossing rates in *A. mangium* were determined by the genetic characteristics of the population of origin, rather than the environmental characteristics of sites where it was planted.

Selfing provides reproductive assurance and is a common attribute among colonizing species (PRICE & JAIN 1981). Preliminary evidence that selfing rates of natural populations were maintained in planted populations in different environments suggested that some individuals would continue to self whether or not cross-pollen was available. However, recent data collected in seed orchards in Vietnam indicate that *A. mangium* maintains a highly flexible mating system. The level of selfing in seed orchards appears to be primarily influenced by the proportion of flowering trees in the population and its impact on pollinator behaviour.

In this study, the level of inbreeding in six seed orchards is reported and possible causes of variation in outcrossing rates among natural and planted populations explored.

METHODS

Genetic material

Leaves and seed pods were collected from 12 open-pollinated trees in each of five seed orchards and 10 trees in a sixth seed orchard in Vietnam. Mother trees were sampled as well as progeny so that alleles could be assigned to ovules and pollen and the number of paternal genotypes contributing to seed crops estimated. Seed orchards were selected to cover the range of provenances introduced to *A. mangium* breeding programs in Vietnam, and to represent different climatic regions in the country.

Location, seed source, age of seed orchards and the percentage of trees in the orchards carrying seed crops at the time of sampling are listed in Table 1. The number of seeds set per pod was recorded for each of 20 pods per tree and four seeds per pod germinated. Seed set and germination rates were recorded for the 70 families. Differences in seed set among seed orchards were tested by one-way ANOVA and paired *t*-tests. The relationship between seed set, germination and outcrossing rate among the 70 families was examined by linear regression.

DNA procedures

DNA was extracted from one seedling from each of 12 pods per tree using the FastDNA® kit (BIO 101). Six of the most polymorphic primer pairs had been selected from a set of 30 microsatellite primers (BUTCHER *et al.* 2000) based on screening a subset of 32 progeny; one progeny from each of eight trees from four natural populations. Each of the six microsatellites were selected from different linkage groups (BUTCHER & MORAN 2000) to ensure independence of loci. Mendelian inheritance of these loci had previously been demonstrated using full-sib progeny arrays from controlled crosses of *A. mangium* (BUTCHER *et al.* 2000). Procedures for microsatellite amplification and analysis followed BUTCHER *et al.* (2000).

Data analysis

Genetic diversity

Allelic richness of each seed orchard was compared based on the number of alleles detected in mothers and progeny at each of six microsatellite loci. Genetic diversity in the 10–12 sampled trees in each seed orchard was also compared with that in 10 trees in each of four natural stands (Bimadebun in New Guinea, Daintree and Claudie River in Australia,

Table 1. Latitude, longitude, seed source, area of seed stand, stand density, age and the estimated proportion of trees with seed crops in sampled seed orchards in Vietnam.

Seed orchard	Latitude (°N)	Longitude (°E)	Seed source	Area (ha)	Stand density (trees ha ⁻¹)	Age (years)	Percentage of trees with seed crops
Bau Bang	11°15'	106°38'	Daintree (Qld)	0.5	200	12	>60 but sampled at end of seed crop
Ba Vi Fortip	21°08'	105°28'	PNG, Claudie River (Qld)	2	400	4	18
Ba Vi Pongaki	21°10'	105°20'	Pongaki (PNG)	2	250	8	not available
Dong Ha	16°47'	107°03'	PNG, Claudie River (Qld)	3	400	5	60
Ham Yen ^a	22°02'	106°02'	Queensland ^a	2	200	14	>80
Phong Chau	21°30'	105°13'	Cardwell (Qld)	2	200	>10	>80

^a The seed sources for this seed orchard were from Queensland but the provenances are not known.

Aru in the Moluccu islands) (BUTCHER unpubl. data) based on the following measures; mean number of alleles per locus (A); observed heterozygosity (H_o); and expected panmictic heterozygosity (H_e). These were calculated using the formulae in WEIR (1996) and the GDA program (LEWIS & ZAYKIN 1999).

Mating system

For the mating system analysis, single-locus (t_s) and multilocus (t_m) estimates of the proportion of progeny arising from outcrossing in each seed orchard, and each family, were made using maximum likelihood procedures and the MLTR program (RITLAND 2002). Multilocus estimates were compared with single-locus ($t_m - t_s$) to determine the lower bound for selfing due to biparental inbreeding (BROWN 1990). The probability that two outcrossed progeny drawn from a progeny array were full-sibs (r_p) and the correlated selfing rate (r_s), which reflects variation in selfing rates among families, were also estimated using MLTR.

Where there was evidence of significant selfing in seed orchards, the minimum number of paternal genotypes was estimated for each progeny array using the program GERUD 1.0 (JONES 2001). This was to determine whether selfing was associated with restricted pollen flow.

RESULTS

Genetic Diversity

There were marked differences in allelic richness among the six seed orchards, ranging from 6 alleles per locus in Ba Vi Fortip to 23 alleles per locus in Dong Ha (Table 2). The level of diversity in the seed orchards reflected that in the source populations (Table 3) with the exception of Ba Vi Fortip. In general, seed orchards established from the southern-most Australia populations (Bau Bang, Phong Chau and Ham Yen) had half the allelic richness and 30 % less heterozygosity than seed

Table 2. Number of alleles detected at six microsatellite loci in parents and progeny from six seed orchards in Vietnam.

Population	n^a	Microsatellite locus						Mean
		Am164	Am173	Am018	Am387	Am465	Am041	
Bau Bang	141	15	4	6	8	11	13	9.5
Ba Vi Fortip	130	7	3	5	6	7	6	5.7
Ba Vi Pongaki	155	43	7	12	13	22	22	19.8
Dong Ha	154	54	10	18	12	22	23	23.2
Ham Yen	153	34	5	7	9	9	15	13.2
Phong Chau	154	22	3	5	8	8	14	10.0

^a n = number of individuals sampled in each population.

Table 3. Number of sampled seed trees (n), measures of genetic diversity (A = number of alleles per locus; H_e = expected heterozygosity and H_o = observed heterozygosity) for natural populations and seed orchards (SO) of *Acacia mangium* in Vietnam, estimated from six microsatellite loci.

	N	A	H_e	H_o
Daintree (Qld)	10	5.2	0.638	0.433
Claudie River (Qld)	10	5.0	0.646	0.633
Bimadebun (PNG)	10	4.3	0.590	0.700
Aru (Moluccu)	10	10.3	0.898	0.900
Sidei (Irian Jaya)	10	6.0	0.688	0.533
Bau Bang SO	12	5.2	0.682	0.375
Ba Vi Fortip SO	10	4.2	0.520	0.333
Ba Vi Pongaki SO	12	9.7	0.871	0.861
Dong Ha SO	12	11.7	0.884	0.806
Ham Yen SO	12	5.7	0.620	0.556
Phong Chau SO	12	5.2	0.664	0.694

Table 4. Multilocus outcrossing rates (t_m), difference between t_m and mean single-locus outcrossing rates (t_s), correlated selfing (r_s), correlated paternity (r_p) and the range of family outcrossing rates in six seed orchards of *Acacia mangium* in Vietnam.

Population	t_m	$t_m - t_s$	r_s	r_p	Family t_m
Bau Bang	0.488 (.129)	0.155 (.044)	0.517 (.117)	0.499 (.067)	0.00–0.93
Ba Vi Fortip	0.130 (.035)	0.047 (.016)	0.295 (.201)	0.816 (.329)	0.00–0.33
Ba Vi Pongaki	1.007 (.324)	0.156 (.288)	0.312 (.428)	0.398 (.130)	0.42–1.00
Dong Ha	1.000 (.010)	0.017 (.021)	0.001 (.003)	0.345 (.055)	all 1.00
Ham Yen	0.945 (.028)	0.051 (.021)	0.086 (.341)	0.430 (.063)	0.67–1.00
Phong Chau	0.926 (.023)	0.035 (.023)	0.100 (.291)	0.335 (.035)	0.67–1.00

orchards established from PNG seed sources (Dong Ha and Ba Vi). The exception was the Ba Vi Fortip orchard which had half the allelic richness of trees in the Dong Ha seed orchard, despite being established from the same seed sources in PNG and Claudie River, Australia (Table 1).

Mating system

Mating system parameters for the six seed orchards in Vietnam are presented in Table 4. Trees in four of the seed orchards were highly outcrossing. The Ba Vi Fortip seed orchard had the highest selfing rate (87%) while no selfing was detected in the Dong Ha orchard.

Single locus outcrossing rates were significantly less than multilocus estimates in Ba Vi Fortip and Bau Bang, indicating biparental inbreeding. The Ba Vi Fortip seed orchard had the highest level of correlated paternity indicating repeated matings with the same pollen donor. This is supported by estimates of only 1–3 paternal genotypes for each progeny array in Ba Vi Fortip and 1–4 paternal genotypes in Bau Bang calculated using the GERUD program. The Bau Bang seed orchard had the greatest variation in outcrossing rates (Table 4), ranging from 0 to 93% and this is reflected in significant estimates of correlated selfing.

Seed set ranged from a mean of 6.1 seeds per pod in Bau Bang to 9.2 seeds per pod in Ba Vi Pongaki. There were significant differences ($P < 0.01$) among seed orchards but paired t -tests showed this was attributable to lower seed set in the Bau Bang seed orchard. Mean seed set in the other five seed orchards ranged from 8.7 to 9.2 seeds per pod. Bau Bang is located in the south of Vietnam where seed matures earlier than in central and northern Vietnam. The lower seed set in Bau Bang most likely reflects the fact that collections were made from the last of the seed crop. Interestingly, the highly selfing Ba Vi Fortip trees produced 9.0 seeds per pod, indicating self-fertilization does not result

in high levels of seed abortion. Weak positive associations ($r^2 = 0.08$; $P = 0.02$) between outcrossing rate and seed set/pod and between outcrossing rate and germination ($r^2 = 0.06$; $P = 0.04$) suggest slight selective pressure in favour of outcrossed progeny.

DISCUSSION

Outcrossing rates in hermaphroditic plants are influenced by self-incompatibility mechanisms, the degree of protogyny or protandry, behaviour of pollinators within and among plants and selective abortion of selfs (MURAWSKI & HAMRICK 1991).

Acacia flowers do not have nectaries and the main attractant to pollinators is pollen (SORNSATHAPORNKUL & OWENS 1998) produced in abundant inflorescences (up to 130,000 spikes per tree for *A. mangium*: SEDGLEY *et al.* 1992a). In *A. mangium* flowers have a short female phase and this reduces the effectiveness of the protogynous outcrossing mechanism reported in other acacias (SEDGLEY *et al.* 1992b). Andromonoecy is common, with reports of 3–88% of staminate flowers per spike (SEDGLEY *et al.* 1992b). Flowers open sequentially along the spike so that an entire spike is never in a distinctly female or male phase (SEDGLEY 1992b). Geitonogamous pollination is likely as insects move between flowers on the same spike and/or tree. SEDGLEY *et al.* (1992b) reported little difference in penetration of pollen tubes into ovules following self- and cross-pollination.

The high level of selfing and low percentage of trees with seed crops in the Ba Vi Fortip seed orchard suggest that the density of flowering trees is a major determinant of outcrossing rates in *A. mangium*. Studies in several other tropical tree species have failed to find an association between flowering tree density and outcrossing (for example *Carapa guianensis*, HALL *et al.* 1994; *Shorea trapezifolia*, MURAWSKI *et al.* 1994; *Pithecellobium elegans*, HALL *et al.* 1996). However, these species were self-

incompatible; flowering density will therefore influence seed set but not outcrossing rates. A positive correlation between flowering tree density and outcrossing rate was reported in populations of the self-compatible rainforest tree *Cavanillesia platanifolia* (MURAWSKI *et al.* 1990). Isolated trees were highly or completely selfed indicating low levels of interplant movement by pollinators. Similarly, in the Ba Vi Fortip seed orchard, a low proportion of trees with seed crops was indicative of poor flowering resulting in high levels of selfing. The seed orchard was only four years old when sampled and less than 20 % of trees produced seed crops; crops were light compared to the other five orchards. If the proportion of flowering trees in a seed orchard is low and flowering is light, pollinators are not attracted to the stand and pollen transfer between trees becomes limiting. Trees that are self-compatible could still produce seed crops from pollen transfer between flowers within the tree, with, or perhaps without the assistance of pollinators. Only one of the sampled trees in Ba Vi Fortip received pollen from more than two pollen donors indicating pollen flow was indeed limited.

The sampled adult trees in the Ba Vi Fortip seed orchard had low genetic diversity indicating that seed production was limited to a narrow range of self-compatible genotypes that flowered at a relatively young age. This seed orchard was established from a broad genetic base of 84 open-pollinated families using the same seed sources (PNG and Claudie River in Queensland, Australia) as were planted at Dong Ha. Of the ten trees sample at Ba Vi Fortip, eight were from different families and yet the number of alleles detected in the parent trees was less than half that in Dong Ha (Table 3). Presumably, limiting seed production to self-compatible genotypes reduces the diversity of the sampled trees. Significant estimates of correlated selfing in Bau Bang seed orchard provide evidence that self-compatibility varies between genotypes. Correlated selfing could also result from differences in outcrossing rates among trees associated with population substructure. In Bau Bang, family outcrossing rates ranged from 0 to 93% (Table 4) and differences in single- and multilocus outcrossing rates suggest biparental inbreeding. However, neighbourhood structuring is unlikely in seed orchards, and biparental inbreeding is more likely to be due to differences in flowering phenology among families. Seed was collected in the Bau Bang orchard from the last of the trees holding seed, and would therefore include late-flowering phenotypes.

The results from the Ba Vi Fortip seed orchard demonstrate the immediate impact of a genetic

bottleneck. If the number of flowering trees in a population is reduced through fragmentation or rapid decline in population size, genetic diversity in the next generation will be markedly reduced. The impact of the bottleneck would be compounded by variation in the level of self-compatibility among mother trees. If only self-compatible trees contribute alleles to the next generation, the loss in allelic diversity will be greater than if it is simply a case of reduced numbers of outcrossing parents. Allelic richness in the parents and progeny of Ba Vi Fortip was one quarter (6 alleles per locus) that of the Dong Ha seed orchard (23 alleles per locus), yet both were established using open-pollinated seed from PNG and Claudie River regions. If the fecundity advantage to selfing, due to pollinator limitation of seed production by outcrossing, was greater than the negative effects of inbreeding depression this would lead to selection for self-compatibility within fragmented populations (LANDE & SCHEMSKE 1985).

The relatively low levels of variation and high levels of inbreeding in the natural populations in the Daintree region of Queensland (BUTCHER *et al.* 1998; 1999) also suggest past genetic bottlenecks. Of interest is what maintains the high level of selfing in these populations – a factor that is apparently not operating in the Ham Yen and Phong Chau seed orchards. These seed orchards had relatively low levels of diversity compared with PNG sourced seed orchards yet were predominantly outcrossing. This suggests maternal resources are preferentially allocated to outcrossed seeds. For selfed seed to be produced requires limited pollen flow, through fragmentation of populations, low density of flowering trees or inadequate pollen transfer by pollinators.

The fact that selfing was common in the Ba Vi Fortip orchard (primarily PNG origin) yet has not been detected in natural populations in PNG or in the Dong Ha orchard (primarily PNG origin) also suggests that maternal resources may be preferentially allocated to outcrossed embryos. However, seed set within pods does not appear to be affected by selfing, as the number of seeds per pod in Ba Vi Fortip did not differ from that in Dong Ha. In acacias, pollen is dispersed in polyads containing 16 pollen grains; the number of pollen grains in a polyad exceeds the number of ovules in the flower so full pod set can result from a single pollination (MUONA *et al.* 1991). This strategy reduces intra-ovary competition between sibling seeds that are all genetically similar (KRESS 1981). Competition for maternal resources may therefore operate between pods, with selfed pods failing to develop, rather than

between individual embryos within pods.

Implications for conservation and breeding programs

Acacia mangium has a highly flexible mating system, typical of colonizing species. Self-compatibility allows founder populations to expand, whether or not cross-pollen is available. Bottlenecks that reduce the number of reproductive individuals, for example in fragmented populations in the Daintree region (BUTCHER *et al.* 1998), will result in an increase in the level of inbreeding and a reduction in allelic richness. Evidence from the Ba Vi Fortip seed orchard indicates that this will occur regardless of the geographic origin and history of source populations. It might be expected that if seed orchards were established from natural populations with high levels of inbreeding, for example Daintree with 70% selfing, the probability of producing inbred seed would increase as there has been past selection for self-compatible genotypes. However, the high outcrossing rates in the Phong Chau seed orchard in Vietnam, established using seed sourced from Cardwell, a small natural population in the south of the Daintree region, indicate that preferential allocation of maternal resources to pods containing outcrossed seeds may limit the evolution of selfing.

High outcrossing rates in seed orchards are important to maximise gains from recurrent selection in open-pollinated breeding programs. Our study shows that in Vietnam, high outcrossing rates can be maintained in planted populations which have less than half the allelic richness of the more genetically diverse PNG populations, as long as there is a high proportion of heavily flowering trees and sufficient pollinator activity. The high level of selfing previously reported in the Subanjeriji orchard (70%) (BUTCHER *et al.* 1999) may reflect asynchronous, patchy flowering of *A. mangium* in this equatorial location. Volume growth from this seed source was one third of that of PNG provenances (TUOMELA *et al.* 1996). While inbreeding does not appear to affect seed set in *A. mangium*, it may impact on germination, seedling vigor and growth rates. Trials have been established in Vietnam to directly compare the performance of selfed and outcrossed progeny and quantify the impact of inbreeding on plantation performance. These trials should also clarify to what extent the poor performance of material sourced from the Daintree region is due to inbreeding per se as opposed to loss of adaptive potential associated with low genetic diversity.

ACKNOWLEDGMENTS

The study of Vietnamese seed orchards was funded in part by the Australian Agency for International Development. Additional financial and logistic support was provided by CSIRO Forestry and Forest Products and the Research Centre for Forest Tree Improvement, Forest Science Institute of Vietnam and the Agricultural Genetics Institute of Vietnam.

REFERENCES

- BUTCHER, P.A., MORAN, G.F. & PERKINS, H.D. 1998: RFLP diversity in the nuclear genome of *Acacia mangium*. *Heredity* **81**: 205–213.
- BUTCHER, P.A., GLAUBITZ, J.C. & MORAN, G.F. 1999: Applications for microsatellite markers in the domestication and conservation of forest trees. *For. Genet. Res. Infor.* **27**:34–42.
- BUTCHER, P.A. & MORAN G.F. 2000: Genetic linkage mapping in *Acacia mangium*. 2. Development of an integrated map from two outbred pedigrees using RFLP and microsatellite loci. *Theor. Appl. Genet.* **101**: 594–605.
- BUTCHER, P.A., DECROOQ, S., GRAY, Y. & MORAN G.F. 2000: Development, inheritance and cross-species amplification of microsatellite markers from *Acacia mangium*. *Theor. Appl. Genet.* **101**: 1282–1290.
- BROWN, A.H.D. 1990: Genetic characterisation of plant mating systems. Chapter 9. *In*: Plant Population Genetics, Breeding and Genetic Resources. Brown, A. H. D., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds) Sinauer Assoc. Inc., Massachusetts, pp. 145–162.
- HALL, P., ORRELL, L.C. & BAWA, K.S. 1994: Genetic diversity and mating system in a tropical tree, *Carapa guianensis* (Meliaceae). *Amer. J. Bot.* **81**: 104–111.
- HALL, P., WALKER, S. & BAWA, K.S. 1996: Effects of forest fragmentation on diversity and mating systems in a tropical tree *Pithecellobium elegans*. *Conserv. Biol.* **10**: 757–768.
- JONES, A.G. 2001: GERUD1.0 A computer program for the reconstruction of parental genotypes from progeny arrays using multi-locus DNA data. *Mol. Ecol. Notes* **1**: 33–39.
- KRESS, W.J. 1981: Sibling competition and evolution of pollen unit, ovule number and pollen vector in Angiosperms. *Syst. Bot.* **6**: 101–112.
- LANDE, R. & SCHEMSKE, D. W. 1985: The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**: 24–40.
- LEWIS, P.O. & ZAYKIN, D. 1999: Genetic Data Analysis: Computer programme for the analysis of allelic data. Version 1.0 (d13). Free programme distributed from the GDA Home Page at <http://alleyn.eeb.uconn.edu/gda/>
- MORAN, G.F, MUONA, O. & BELL, J.C. 1989: *Acacia mangium*: a tropical forest tree of the coastal lowlands with low genetic diversity. *Evolution* **43**: 231–235.
- MUONA, O., MORAN, G.F. AND BELL, J.C. 1991: Hierarchical patterns of correlated mating in *Acacia*

- melanoxylon*. *Genetics* **127**: 619-626.
- MURAWSKI, D.A., HAMRICK, J.L., HUBBELL, S.B. & FOSTER, R.B. 1990: Mating systems of two Bombacaceous trees of a neotropical moist forest. *Oecologia* **82**: 501-506.
- MURAWSKI, D.A. & HAMRICK, J.L. 1991: The effect of the density of flowering individuals on the mating systems of nine tropical tree species. *Heredity* **67**: 167-174.
- MURAWSKI, D.A., DAYANANDAN, B. & BAWA, K.S. 1994: Outcrossing rates of two endemic *Shorea* species from Sri Lankan rain forests. *Biotropica* **26**: 23-29.
- PRICE, S.C. & JAIN, S.K. 1981: Are inbreeders better colonizers? *Oecologia* **49**: 283-286.
- RITLAND, K. 2002: Extensions of models for the estimation of mating systems using n independent loci. *Heredity* **88**: 221-228.
- SEDGLEY, M., YONG, W.C., NEWMAN, V., HARBAR, J., SMITH, R.M., GHAN, K.K. & TAFUDDIN, A. 1992a: Phenology of *Acacia mangium* and *A. auriculiformis* in Australia and Malaysia. In: Carron, L.T. and Aken, K.M. (eds) Breeding Technologies for Tropical Acacias. ACIAR Proceedings **37**, Canberra, pp. 36-44.
- SEDGLEY, M., HARBAR, J., SMITH, R.-M.M., WICKNESWARI, R. & GRIFFIN A.R. 1992b: Reproductive biology and interspecific hybridisation of *Acacia mangium* and *Acacia auriculiformis* A. Cunn. ex Benth. (Leguminosae: Mimosoideae). *Austr. J. Bot.* **40**: 37-48.
- SORNSATHAPORNKUL, P. & OWENS, J.N. 1998: Pollination biology in a tropical *Acacia* hybrid (*A. mangium* Willd. × *A. auriculiformis* A.Cunn ex Benth.). *Ann. Bot.* **81**: 631-645.
- TURNBULL, J.W., MIDGLEY, S.J. & COSSALTER, C. 1998: Tropical acacias planted in Asia: An overview. In: Turnbull, J.W., Crompton, H.R. and Pinyopusarerk, K. (eds) Recent Developments in Acacia Planting. ACIAR Proceedings 82, Canberra, pp. 14-28.
- TUOMELA, K., A. OTSAMO, J. KUUSIPALO, R. VUOKKO & G. NIKLES 1996: Effect of provenance variation and singling and pruning on early growth of *Acacia mangium* Willd. plantation on *Imperata cylindrica* (L.) Beauv. dominated grassland. *For. Ecol. Manage.* **84**: 241-249.
- WEIR, B.S. 1996: Genetic Data Analysis II. Methods for Discrete Population Genetic Data. Sinauer Associates, Sunderland, MA.