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Reproductive Biology and Interspecific Hybridisation of *Acacia mangium* and *Acacia auriculiformis* A. Cunn. ex Benth. (Leguminosae: Mimosoideae)

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Abstract

Acacia mangium and *A. auriculiformis* flowered between February and May, producing mature pods between October and April. The flowers of both species were similar in structure and showed weak protogyny and variable levels of andromonoecy. Male flowers either lacked pistils completely or had small sterile pistils. Controlled hand pollination resulted in pollen tubes in the pistil and penetration of the ovules following self and cross intraspecific and interspecific pollination. The cross *A. auriculiformis* × *A. mangium* was more successful than the reciprocal, but fertile seed was produced following interspecific pollination in both directions and all seedlings were shown to be hybrid by isozyme analysis of parents and seedlings. There were relatively few insect visitors to the flowering branches, but the same suite of insects was observed foraging for pollen on both species. Native bees belonging to the Halictidae carried most polyads on their hairy bodies and may act as pollinating agents.

There appeared to be no major fertility barriers to interspecific hybridisation between *Acacia mangium* and *A. auriculiformis*, and hybrids could occur spontaneously via synchronous flowering and common insect visitors.

Introduction

Acacia mangium Willd. and *A. auriculiformis* A. Cunn. ex Benth. (Leguminosae: Mimosoideae) are two tropical arborescent species native to areas of northern Australia and southern New Guinea (Pedley 1975; Maslin and Pedley 1982). There is interest in both species due to their potential for plantation forestry in a number of countries, and *A. auriculiformis* is widely planted in tropical environments as an ornamental (Turnbull 1987).

The fact that *Acacia* flowers are very small and are grouped into inflorescences presents problems in the investigation of reproductive biology (Sedgley 1987, 1989). Most research has been conducted on species native to temperate southern Australia, and has identified a complex breeding system involving andromonoecy, protogyny and self-incompatibility with pollen transfer via bees, other insects or birds (Ford and Forde 1976; Bernhardt and Walker 1984, 1985; Kenrick and Knox 1985; Kenrick *et al.* 1986). Not surprisingly many of the southern species show high variability (Philp and Sherry 1946; Moffett 1956; Coaldrake 1971), but recent work involving isozyme analysis of both *A. mangium* and *A. auriculiformis* has shown low genetic diversity (Moran *et al.* 1989*a*, 1989*b*).

Recent reports from Malaysia describe interspecific hybrids between *A. mangium* and *A. auriculiformis* (Turnbull 1987; Carron and Aken 1992). Hybridisation of *Acacia* has been reported previously (Cheel 1935; Philp and Sherry 1946; Leach and Whiffin 1978; Ali and Qaiser 1980) but is not common in the section *Juliflorae* to which *A. mangium* and *A. auriculiformis* belong (Pedley 1982). The aim of this study is to

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investigate the reproductive biology of the two species with regard to the occurrence of interspecific hybridisation. This is of interest from the ecological viewpoint with regard to potential competition between the hybrid and the parent species. It is also important commercially, as superior genotypes of many genera for both horticulture and forestry have been produced via interspecific hybridisation (Sedgley and Griffin 1989).

Materials and Methods

Plant Material

At least five mature flowering trees of both species were studied at each of Atherton (latitude 17° S, longitude 145·5° E) in north Queensland, Australia, and near Kuala Lumpur (latitude 3° N, longitude 102° E) and Tawau (latitude 4° N, longitude 118° E) in Malaysia. Most of the trees were planted specimens for either amenity or forestry use, and herbarium specimens were lodged with the Australian National Herbarium, Canberra.

Phenology

Ten trees of each species at Atherton were selected, and observed at fortnightly intervals for 3 years. At each observation an assessment was made of the proportion of the canopy with open flowers or mature fruits. Climate data for Atherton are shown in Fig. 1.

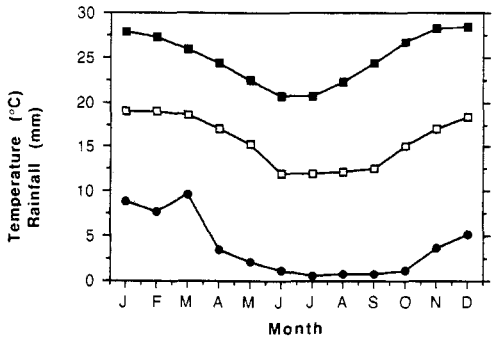


Fig. 1. Eighteen-year mean data for daily maximum (■) and minimum (□) temperature (°C) and rainfall (●) per month for Atherton, Australia.

Floral Biology

Twenty spikes with flowers just prior to anthesis on three trees of each species at Atherton were observed at hourly intervals for the opening sequence of individual flowers with regard to extension of the style and of the stamens. A further 20 spikes were collected from trees of both species in Australia and Malaysia. Each flower on every spike was observed using a binocular microscope, and scored for the presence or absence of a fully developed pistil. Results were analysed using ANOVA. The diameter of polyads and pistils from 50 flowers of each species was measured using a binocular microscope.

Flowers were sampled in Australia and Malaysia and fixed in FPA50 (formalin 5 : propionic acid 5 : 50% ethanol 90). For scanning electron microscopy (SEM), they were dehydrated through an ethanol series, critical-point dried and sputter-coated with gold. Pistils from 20 hermaphrodite and 20 male flowers were processed for SEM and the dimensions measured from photomicrographs. Ovaries from five hermaphrodite and five male flowers of *A. mangium* were fixed, embedded in glycol methacrylate and longitudinal sections stained with periodic acid–Schiff's reagent and toluidine blue O for observation using light microscopy (Feder and O'Brien 1968).

Controlled Pollination

At least 10 spikes were selected per treatment on three trees of each species and subjected to either self or cross intraspecific pollination, or to interspecific pollination. Spike-bearing branches were labelled and bagged one day prior to anthesis as the flowers changed from green to yellow. The bags were removed at anthesis when the first open flowers were in the female stage. The number of flowers

to be used as the female parent was reduced to approximately 20 per spike, and any unopened buds were removed. The remaining flowers were emasculated by removing the stamens with undehisced anthers from around the style with fine forceps. Spikes to be used as the pollen source were air dried for up to 4 h until the anthers had dehisced. The spikes were then sieved through a 53-μm stainless steel sieve and the pollen collected on a piece of smooth black plastic. Pollen was collected with a fine brush with black hairs against which the pollen could be seen, and transferred to the stigma. The spikes were then rebagged and the bags removed after 3 days. The flowers were removed for fixation in Carnoy's fluid (ethanol 6 : chloroform 3 : acetic acid 1) or the spikes were left to set seed.

The pistils were dissected from the fixed flowers and the stigma/style was severed at the junction with the ovary. The tissue was hydrated through an alcohol series, softened in 0·8 N sodium hydroxide and stained with decolorised aniline blue (Martin 1959). The stigma/styles were squashed directly onto microscope slides, whereas the ovaries were scored along the suture, and oriented on the slide such that the ovules were displayed following squashing. The slides were observed using fluorescence microscopy, and pollen tubes were counted in the stigma/style and in the ovary. Pods resulting from controlled pollination were collected at maturity, and the number of seeds per pod counted. Results were analysed using ANOVA and the Mann-Whitney test. The percentage pod set results were analysed by fitting a binomial model followed by the χ^2 test. The seeds were germinated, and the number of surviving seedlings and their height were recorded after 6 months.

Isozyme Analysis

Leaf tissues from all seedlings and parent trees were subjected to starch gel electrophoresis (Moran and Bell 1983). The gels were stained for the enzyme systems aspartate amino transferase (AAT) and glutamate dehydrogenase (GDH) which have been shown to discriminate the two species (Moran *et al.* 1989*a*, 1989*b*; Wickneswari 1989; Wickneswari and Norwati 1991). For each enzyme system the fastest migrating zone of activity was designated locus 1 and the next, locus 2, and the fastest migrating band within a locus was allele 1 and the next, allele 2.

Insect Visitors

Insect visitors to flowering branches were observed hourly over a 5-day period on five trees of each species at Atherton. Observations were made on whether insect visitors foraged for pollen or for nectar, and specimens were collected and stored individually in gelatin capsules. Following identification, specimens were prepared for observation by SEM by placing the insect on its back on a stub and coating with gold. Each insect was examined for pollen on its ventral surface, and the number of *Acacia* polyads and foreign pollen grains was recorded.

Results

Phenology

The peak flowering period for both species at Atherton was between February and August, with mature pods present between September and April (Fig. 2). Flowering was not continuous, but flowering and non-flowering periods occurred in cycles. Flowering lasted from 4 to 8 days with non-flowering breaks of between 3 and 18 days. The *Acacia mangium* trees produced pods each year, but *A. auriculiformis* showed poor pod production over the period of observation.

Floral Biology

Floral biology was similar between species, between trees and between locations. Following the opening of the petals, the stigma/style was the first floral organ to extend (Fig. 3). Stylar extension was followed by extension of the numerous stamens (Fig. 4), followed by anther dehiscence to release the single polyad per spore sac. The period between extension of the style and extension of the filaments was only a few hours for each individual flower, and the flowers opened sequentially along the spike so that an entire spike was never in a distinctly female or male phase. The polyads of both species were composite structures consisting of 16 pollen grains. They were of similar diameter (36·5±0·4 μm in *A. mangium* and 38·9±0·5 μm in *A. auriculiformis*) and

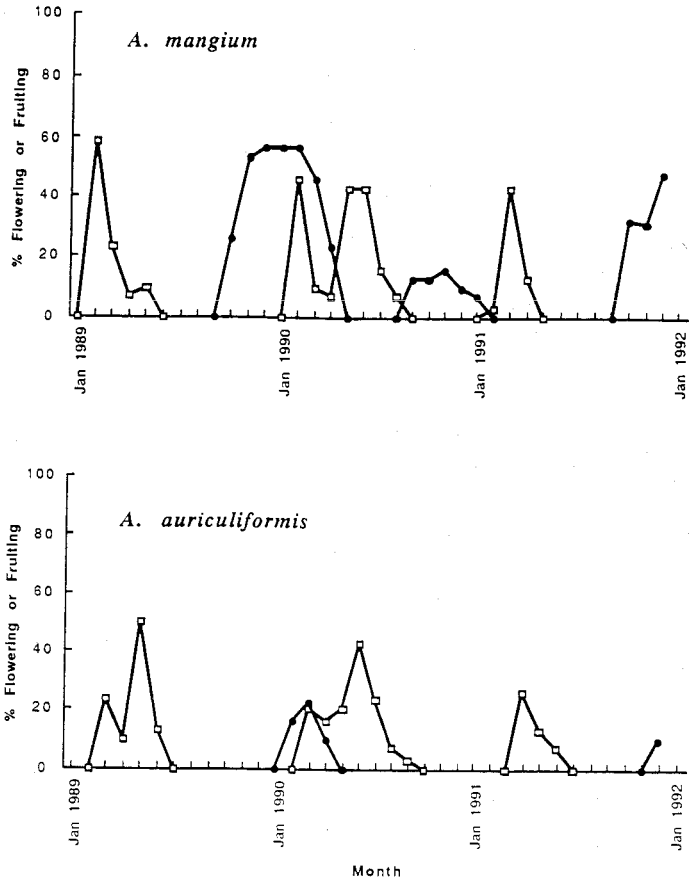


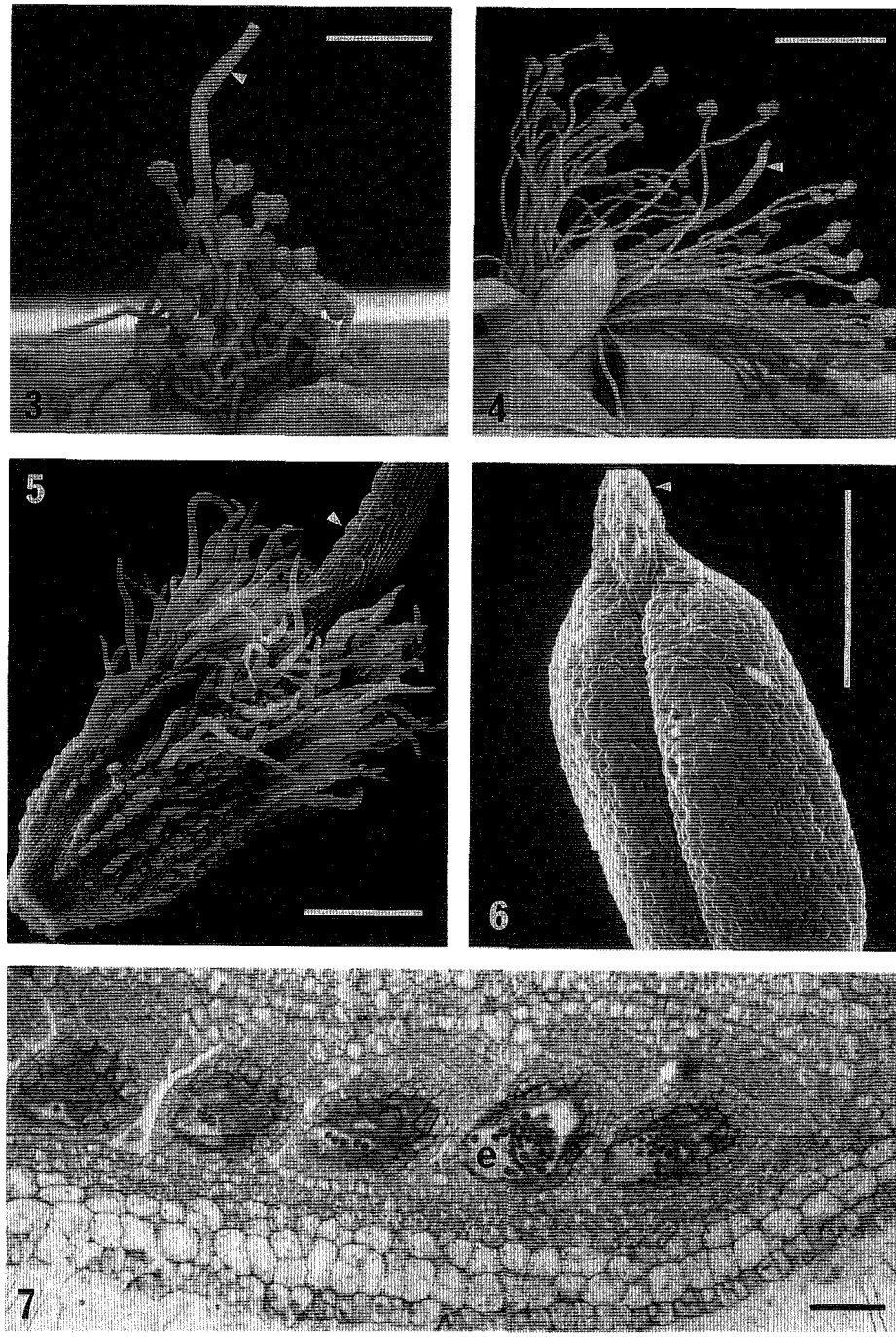
Fig. 2. Flowering (□) and fruiting (●) of *Acacia mangium* and *A. auriculiformis* at Atherton, Australia, over 3 years.

had similar exine patterning. The single pistil had a long style, with a blunt concave stigma of 80·9±1·5 μm diameter in *A. mangium* and 68·7±1·2 μm diameter in *A. auriculiformis*.

The number of flowers per spike was highly variable and male flowers, with absent or small pistils, represented a varying proportion of the flowers on spikes of both species (Table 1). Trees of *A. mangium* had between 102 and 225 flowers per spike with 3·5–

Table 1. Mean number of flowers (± s.e.) per spike of trees of *Acacia mangium* and *A. auriculiformis*

	Total no. of flowers	Percentage of male flowers
A. mangium		
Australia 1989	202·1 (15·1)	31·8 (19·2)
Australia 1990	212·9 (4·8)	23·4 (8·6)
Malaysia 1991	158·9 (15·3)	57·4 (12·3)
A. auriculiformis		
Australia 1989	56·0 (3·8)	5·1 (3·8)
Australia 1990	76·8 (5·9)	2·9 (1·5)
Malaysia 1990	92·2 (5·3)	3·4 (0·8)



Figs 3–6. Scanning electron micrographs. **Fig. 3.** Flower of *Acacia mangium* from Malaysia in the female stage showing extension of the style (*arrow*) but not of the stamens. Scale: 500 μm. **Fig. 4.** Flower of *Acacia auriculiformis* from Australia in the bisexual stage showing both the style (*arrow*) and stamens extended. Scale: 1mm. **Fig. 5.** Pistil of hermaphrodite flower of *Acacia auriculiformis* from Malaysia showing the hairy ovary and the base of the style (*arrow*). Scale: 100 μm. **Fig. 6.** Pistil of male flower of *Acacia mangium* from Malaysia showing the small glabrous ovary and short style (*arrow*). Scale: 100 μm. **Fig. 7.** Light micrograph. Longitudinal section of ovary of hermaphrodite flower of *A. mangium* from Australia showing ovules with embryo sacs (e). Scale: 50 μm.

Table 2. Characteristics of normal and small pistils of *Acacia mangium* and *A. auriculiformis* (mean ± s.e.)

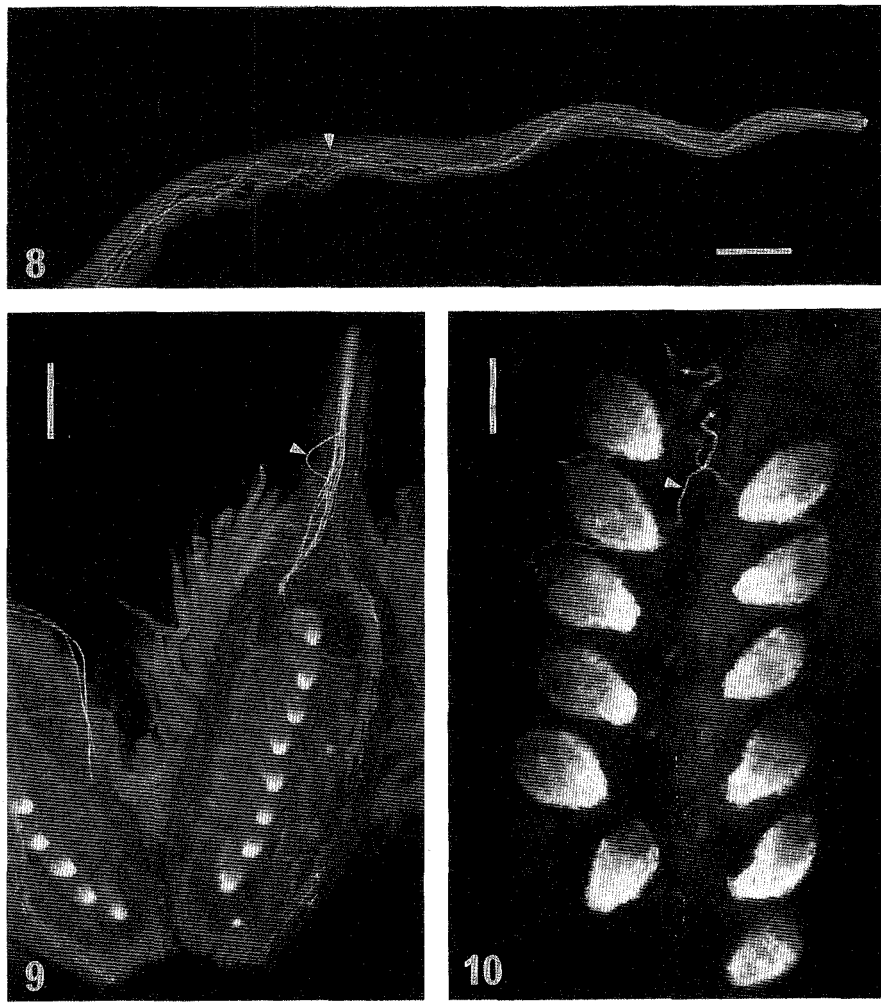
	A. mangium		*A. auriculiformis*	
	Normal	Small	Normal	Small
Length of style (μm)	3360±270	39±2	2860±110	28±3
Length of ovary (μm)	660±10	230±10	680±30	280±10
Diameter of ovary (μm)	440±20	130±3	350±10	140±10
Number of ovules per ovary	13·3±0·2	6·2±2·2	16·0±0·2	13·2±0·5
Length of ovule (μm)	80·5±2·7	36·2±5·6	99·2±1·6	98·0±3·7
Diameter of ovule (μm)	48·5±1·1	16·7±1·7	59·2±1·6	48·0±2·8

88·3% males, whereas those of *A. auriculiformis* had between 43 and 118 flowers per spike with 0·1–19·9% males. There were significant differences ($P<0·001$) in both total flower number and the percentage of male flowers between trees of both species within locations, but not between locations or between years. *A. mangium* had significantly more flowers per spike than *A. auriculiformis* ($P<0·001$), but there was no significant difference in the percentage of male flowers. There tended to be more male flowers toward the base of the spike. There was no relationship between the proportion of male flowers and the total number of flowers per tree.

Most male flowers had no pistil, but some had small pistils (Table 2). Occasional flowers had up to four small pistils, or a pistil of intermediate dimensions between the normal and the small pistils (ovary length 430±30 μm and 390±20 μm in *A. mangium* and *A. auriculiformis* respectively), but these were observed only rarely. The normal pistils had a long style and a hairy ovary (Fig. 5), whereas the small pistils had a very short style with no concave stigmatic cup, and lacked the ovary hairs (Fig. 6). There were fewer ovules in the small compared with the normal ovaries of both species. Normal ovules of *A. mangium* had fully developed embryo sacs (Fig. 7) whereas small ovules lacked embryo sacs.

Controlled Pollination

Pollen tube growth results were similar between trees and between locations, and results were pooled. Following controlled hand pollination, between 26 and 81% of the flowers had a polyad adhering to the stigma (Table 3). Some polyad–pistil combinations resulted in germination of a majority of the grains (Figs 8, 9) and penetration of a number of ovules (Figs 9, 10), but others produced little or no pollen tube growth. It was not determined whether lack of pollen tube growth in some pistils was due to a deficiency in the pistil or in the pollen. There was little difference between self and cross intraspecific pollination, and interspecific pollination in both directions resulted in ovule penetration. Penetration of ovules was lowest in the cross *A. mangium* by *A. auriculiformis*, as pollen tubes were regularly observed in the ovary but relatively few showed directional growth toward an ovule. Interspecific pollination resulted in between 26 and 35% of spikes with pods containing fertile seed, which represented between 0 and 20% set of the total flowers pollinated per cross (Table 4). More seed was produced per pod by the trees in Australia than by those in Malaysia ($P<0·01$), and there was variability among the Malaysian trees in the percentage pod set ($P<0·001$). Germination was variable, with 48–90% of the seeds germinating per cross, and 41–62% survival after 6 months (Table 5). The seedlings showed intermediate morphology between the parent species, and all Australian crosses showed variability in seedling height. The Malaysian crosses germinated poorly, and the whole seedling was used for isozyme analysis.



Figs. 8–10. Fluorescence micrographs. **Fig. 8.** Stigma/style of self-pollinated *Acacia mangium* pistil from Australia showing pollen tubes (*arrow*). Scale: 1mm. **Fig. 9.** Ovary of *Acacia auriculiformis* from Australia showing pollen tubes (*arrow*). Scale: 350 μm. **Fig. 10.** Ovary of *Acacia mangium* from Malaysia showing pollen tubes (*arrow*) penetrating the ovules. Scale: 150 μm.

Table 3. Mean numbers of polyads and pollen tubes (± s.e.) per pistil of *Acacia mangium* (Am) and *A. auriculiformis* (Aa) following intraspecific and interspecific pollination

Pollination	No. of polyads on stigma	No. of pollen tubes in style	No. of penetrated ovules
Am × Am			
Self	0·81 (0·09)	4·31 (0·26)	1·59 (0·14)
Cross	0·71 (0·05)	1·55 (0·44)	0·93 (0·27)
Am × Aa	0·61 (0·05)	1·58 (0·18)	0·27 (0·04)
Aa × Aa			
Self	0·26 (0·05)	1·30 (0·23)	0·98 (0·17)
Cross	0·34 (0·05)	1·77 (0·36)	0·96 (0·20)
Aa × Am	0·43 (0·08)	1·44 (0·11)	0·97 (0·07)

Table 4. Hybrid seeds produced following controlled hand pollination of *Acacia mangium* (Am) and *A. auriculiformis* (Aa)

Cross	Number of: spikes pollinated	flowers pollinated	mature pods	seeds	Percentage of pods	Seeds per pod	Percentage of hybrid seedlings
			Atherton				
Aa2×Am7	17	116	0	0	0	0	—
Aa8×Am3–6	3	20	4	29	20	7·2	100
Aa8×Am7	16	185	7	73	3·8	10·4	100
Aa9×Am6	3	31	0	0	0	0	—
Aa9×Am3–6	4	32	0	0	0	0	—
Am5×Aa8'9	29	416	14	75	3·4	5·4	100
Am6×Aa11'12	15	286	0	0	0	0	—
			Malaysia				
Aa1×Am1–20	60	1254	65	118	5·2	1·8	100
Aa2×Am1–20	53	1051	27	43	2·6	1·6	100
Aa3×Am1–20	37	539	0	0	0	0	—
Aa4×Am1–20	61	1128	8	13	0·7	1·6	100
Aa5×Am1–20	40	638	23	89	3·6	3·9	100
Aa6×Am1–20	20	186	3	4	1·6	1·3	100
Aa7×Am1–20	21	330	20	52	6·1	2·6	100
Aa8×Am1–20	22	249	10	15	4·0	1·5	100

Isozyme Analysis

Both AAT and GDH gave different banding patterns between the two species (Table 6). The alleles of the AAT-1 locus differed between the Australian and Malaysian populations, but were consistent for all of the parental trees used in the crossing program. All seedlings from controlled hand pollination were shown to be hybrid using the two enzyme systems.

Insect Visitors

Insects were not abundant on the flowering branches, with up to 10 individuals of each type observed per flowering branch per day. There was no single dominant group of insect visitors to the flowers, but the native halictid bees carried most polyads on their hairy bodies (Table 7; Figs 11, 12). All insect types foraged for pollen only, and the native bees collected from many individual flowers on up to 20 spikes at each visit. The same suite of insects was observed on both *A. mangium* and *A. auriculiformis* in similar numbers, and none of the insects carried pollen of any other genus than *Acacia*.

Table 5. Survival and height of Australian hybrid seedlings between *Acacia mangium* (Am) and *A. auriculiformis* (Aa) at 6 months

Cross	Percentage of germinated seeds	Percentage survival	Height of surviving seedlings Mean (±s.e.)	Range
Aa8×Am3–6	89·7	58·6	154·3 (24·0)	51–285
Aa8×Am7	79·4	61·6	178·4 (20·4)	20–535
Am5×Aa8'9	48·0	41·3	181·3 (34·9)	40–414

Table 6. Isozyme phenotypes of parents and seedlings from controlled hand pollination of *Acacia mangium* and *A. auriculiformis*

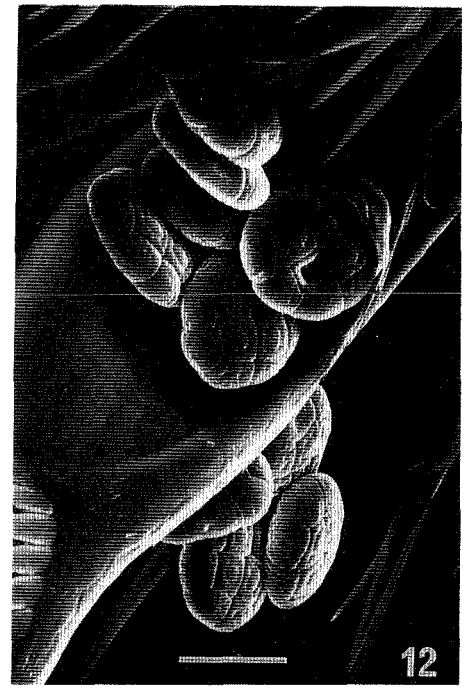
Locus	Isozyme phenotypes		
	A. auriculiformis parents	Seedlings	*A. mangium* parents
Australia			
AAT-1	2,2	2,3	3,3
GDH-1	1,1	1,2	2,2
Malaysia			
AAT-1	1,1	1,2	2,2
GDH-1	1,1	1,2	2,2

Discussion

This research has demonstrated interspecific hybridisation between *A. auriculiformis* and *A. mangium*. Hybridisation could occur naturally as the two species are both native to Cape York Peninsula, flower synchronously and are visited by the same suite of insects. Moreover, there is little pistil–pollen incongruity between the two species, and controlled interspecific pollination results in hybrid progeny as demonstrated by isozyme analysis. The major limitation to hybridisation in the wild is probably geographical, as *A. mangium* is a coastal species while *A. auriculiformis* grows inland. Hybridisation is most likely to occur in disturbed habitats, and where the two species are grown together under cultivation, as has happened in Malaysia. Following hand pollination, the hybrid families produced variable seedling populations. There was a high rate of seedling death, and plants ranged from extremely stunted to very vigorous. This is not unusual for interspecific crosses, but from the commercial point of view it highlights the need for rigorous selection of planting material at the seedling stage. Despite the difference in style length between the species, similar numbers of interspecific pollen tubes reached the ovary following crossing in both directions, but there appeared to be weak attraction by *A. mangium* ovules for *A. auriculiformis* pollen tubes, which showed disoriented growth and few penetrations. As a result, *A. auriculiformis* was more fertile than *A. mangium* as female parent, and *A. auriculiformis* × *A. mangium* seedlings also showed higher germination and survival. This raises implications for proposed hybridisation programs, and tree to tree variation in andromonoecy and interspecific fertility are also important considerations. Selection criteria for breeding trees must include fertility as well as silvicultural parameters.

Table 7. Pollen on bodies of insects collected from trees of *Acacia mangium* and *A. auriculiformis* at Atherton (mean ± s.e.)

Insect type	No. of *Acacia* polyads per insect	No. of non-*Acacia* pollen grains per insect	Collecting pollen (P) or nectar (N)
Hymenoptera			
Halictidae	298·4±16·7	0	P
Apidae	44·0±5·4	0	P
Diptera			
Syphidae	77·0±7·1	0	P
Bibionidae	0	0	P
Coleoptera	1·7±1·6	0	P
Hemiptera	0	0	P



Figs. 11–12. Scanning electron micrographs. **Fig. 11.** Halictid bee collected from a spike of *Acacia mangium* in Australia showing hairy body. Scale: 500 μm. **Fig. 12.** Polyads of *Acacia auriculiformis* on the leg of a halictid bee from Australia. Scale: 25 μm.

Flowering of the trees coincided with the warmest and wettest time of year with no flowers produced during the relatively cool and dry winter months. Our phenology results for *A. mangium* are similar to those of Hopkins and Graham (1989) recorded in north-eastern Australia over a period of 4 years. They observed flowering between January and May with developing pods present on the trees between May and October. The floral structure of the two species was very similar and also similar to that of other *Acacia* species which have been studied. In contrast to some other species, however, the female phase of the flower was very short thus reducing the effectiveness of the protogynous outcrossing mechanism. The flowers shared the condition of andromonoecy with some other *Acacia* species, and there was variation in the proportion of male flowers. As with other species of *Acacia*, bees appear to be the most effective pollen vectors (Bernhardt and Walker 1984, 1985). The halictid bees were attracted to the flowers by the pollen which adhered to all parts of the body. They were faithful to the *Acacia* blossom indicating that they could be efficient pollen vectors during the periods of flowering of the two species. Honeybees also visit the flowers of both species, and hives could be introduced to seed orchards for pollination at flowering time. *Acacia* flowers lack nectaries but have extrafloral secretory glands located on the phyllodes (Boughton 1981; Marginson *et al.* 1985). These did not appear to be a source of attraction to the insects visiting these two species.

Neither species showed pistil–pollen self-incompatibility, although seed set experiments have shown a high level of self-incompatibility in *A. auriculiformis* and variable levels in *A. mangium* (Ibrahim 1991). Self-incompatibility has been recorded in many other species of *Acacia* (Kenrick and Knox 1989), with inhibition of pollen tube growth in the nucellus of *A. retinodes* (Kenrick *et al.* 1986). There is a close physical match

between the diameter of the *Acacia* polyad and the diameter of the stigma, and the composite pollen grain is considered to be a mechanism for ensuring full pod set following a single pollination event (Kenrick and Knox 1982). This is in keeping with the observation that in both species, as in many other acacias, the maximum number of ovules was the same as the number of pollen grains in the polyad. The polyad is the sole product of a single spore sac, and so the pollen grains of a polyad, and the resulting seeds of a pod, reflect the genetic composition of only one paternal tree. Thus the adaptation to dependence on a single pollination event involving one polyad per pistil is an evolutionary compromise resulting in reduced opportunity for variability between progeny from a single pod. Despite this, *Acacia* is one of the most widespread genera in Australia, indicating that the benefits of mass flowering and efficient pollination mechanism outweigh the potential for reduced variability.

Acknowledgments

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