HIGH GENETIC DIVERGENCE BETWEEN GEOGRAPHIC REGIONS IN THE HIGHLY OUTCROSSING SPECIES *ACACIA AULACOCARPA* (CUNN. EX BENTH.)

M. McGranahan[1], J. C. Bell[2], G. F. Moran[2*] & M. Slee[1]

[1] Department of Forestry, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia
[2] CSIRO Forestry and Forest Products, PO Box E4008, Kingston, ACT 2604, Australia
*To whom correspondence should be addressed

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ABSTRACT

A study was undertaken to determine the patterns of genetic variation and mating system in *Acacia aulacocarpa*. The study of genetic variation analysed seedlings from 22 populations of *A. aulacocarpa* at 30 isozyme loci representing 19 enzyme systems. Cluster analysis revealed a subdivision of populations into five genetically distinct groups. Genetic distances between the five groups were high. The overall diversity ($H_T = 0.298$) was high compared to other Australian tree species with similar distributions. An exceptionally high proportion of this diversity (62.6%) was distributed between populations. However 86% of this interpopulation genetic diversity could be apportioned between the population groups which were largely geographically based. The study of the mating system in four populations of *A. aulacocarpa* at 10 isozyme loci revealed a very high level of outcrossing (mean multilocus outcrossing rate = 0.94) with little variation between populations.

Key words: *Acacia aulacocarpa*, genetic diversity, allozyme variation, outcrossing rates, mating system

INTRODUCTION

Allozyme variation has been widely studied in Australian tree species to provide information for domestication and breeding programs and for conservation of genetic resources (MORAN 1992). Three of these studies have involved acacia species which co-occur in northern Australia and New Guinea, namely *A. auriculiformis* (WICKNESWARI & NORWATI 1993), *A. crassicarpa* (MORAN *et al.* 1989a) and *A. mangium* (MORAN *et al.* 1989b). Levels of genetic diversity in these three tropical *Acacia* species were generally lower than in the few temperate acacias studied (MORAN 1992) or other tree groups (HAMRICK *et al.* 1992). In particular, *A. mangium* is genetically depauperate at least for allozyme variation. Despite the low diversity, the degree of genetic differentiation was generally higher in these acacias than other tree species. Of particular note in *A.auriculiformis* is the division of populations into three genetic groups which corresponded to broad geographic regions within the distribution, namely Queensland and Northern Territory (Australia) and Papua New Guinea.

Acacia aulacocarpa Cunn. ex Benth. has a widespread but disjunct distribution in tropical and subtropical northern and north-eastern Australia, southern Papua New Guinea and south-eastern Indonesia, ranging between 6°–31°S (BOLAND *et al.* 1984). This species also co-occurs with the above three species across parts of its natural range. These four acacias belong to what is considered the most primitive subgroup of the primitive section, *Juliflorae* of the Australian acacias (BOLAND *et al.* 1990). The four species have disjunct distributions; all occurring in southern New Guinea and north-eastern Queensland, with *A. aulacocarpa* and *A. auriculiformis* also occurring in the Northern Territory. It seems likely that these species, or their progenitors, occupied a wide range across the Australian geological plate (AUDLEY-CHARLES 1987) since the Tertiary period. Major marine transgressions and cycles of aridity in the Quaternary have probably contributed substantially to the current disjunct distributions of these species. *A. aulacocarpa* occurs in dry woodlands, open forest, and rainforests. Given the commonality of the evolutionary, historical and environmental factors for these species it might be expected that *A. aulacocarpa* would have a similar population genetic structure to the other species.

Inbreeding in plants is one of the major determinants of the extent of genetic differentiation within and between populations. Australian acacias are primarily insect pollinated and have hermaphroditic flowers (SEDGELY 1986). The few available estimates of outcrossing rates in natural populations of acacias (MORAN *et al.* 1989a, MUONA *et al.* 1991) are high and range between 0.89 and 0.96 indicating a predomi-

nantly outcrossing system. The presence of only low amounts of inbreeding in a number of acacia species has been attributed to a strong self-incompatibility system (BERNHARDT *et al.* 1984). However, KENRICK and KNOX (1989) have reported a range in levels of self-incompatability across a number of acacia species with some species of temperate distribution such as *A. paradoxa* and *A. ulicifolia* found to be self-compatible.

This study investigates the level and distribution of genetic diversity in, and the mating system of *Acacia aulacocarpa* using allozyme markers and compares its population genetic structure to that of three acacia species that co-occur with it across its intercontinental distribution.

MATERIALS AND METHODS

Seed collections

Acacia aulacocarpa mostly occurs as a shrub or small tree, 5–15 m tall, in open forest associations. However it also grows as a large tree, up to 35 m with a diameter of one metre, in rainforests in Queensland and also in Papua New Guinea and Indonesia (THOMSON 1994). Twenty-two populations of *Acacia aulacocarpa* were selected to represent the natural range of the species. Where possible, populations consisting of ten mother trees were selected. Populations 10, 11, 13, 16, and 20 are bulk seed collections, with each being made from five or more mother trees in the population. Details of the selected populations are given in Table 1 and their location shown in Figure 1.

Allozyme analysis

Seeds were germinated on moist filter paper and vermiculite in Petri dishes at 30°C for five to seven days. Fifty seedlings per population were assayed for enzyme systems using starch gel electrophoresis, following standard procedures described by MORAN and BELL (1983). An equal number of seedlings from each tree were assayed so that each population was represented by 50 seedlings. For populations 10,11, 13, 16 and 20 fifty seedlings were selected at random from the bulked seedlots. Each seedling was crushed in a 0.05 M borate extraction buffer (pH 9.0) containing 1 $mg{\cdot}mL^{-1}$ dithiothreitol and $20mg{\cdot}mL^{-1}$ polyvinylpyrrolidone (MW = 40,000). Nineteen enzyme systems were assayed. These were aconitate hydratase (AC), alanine aminotransferase (ALT), alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), catalase (CAT), esterase (EST), glucosephosphate isomerase (GPI), glutamate dehydrogenase (GDH), glycerate dehydrogenase (GLY), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), peptidase (PEP), peroxidase (PER), phosphoglucose

Table 1 Details of sampled populations of *Acacia aulacocarpa*

Population No. & location		Latitude (S)	Longitude (E)	Altitude (m)	No. of parents	CSIRO seedlot
1	Erambu-Bupul, Ind.	8°02'	141°00'	40	1	17850
2	Makapa, PNG *	7°56'	142°35'	15	10	16947
3	Wasua Pedeya, PNG	8°17'	142°52'	10	10	16950
4	Dimisisi, PNG	8°31'	142°13'	50	10	17560
5	Pongaki, PNG	8°40'	141°51'	30	4	16988
6	Bensbach-Balamuk, PNG	8°53'	141°17'	25	10	17551
7	Oriomo Sawmill, PNG	8°49'	143°06'	10	10	16998
8	14km S of Maningrida, NT	12°11'	134°18'	40	5	16180
9	Blackmore River, NT *	12°41'	130°56'	40	9	18576
10	Doongan, WA	15°23'	126°17'	200	5B	18877
11	Kununurra, WA	15°38'	128°40'	37	6B	18228
12	West Salt Creek, NT	15°01'	133°11'	100	6	18227
13	N of Borroloola, NT	15°38'	136°25'	30	7B	15715
14	Old Lockhart Airstrip, Qld	12°50'	143°18'	15	5	18358
15	26km NNW of Kuranda, Qld	16°40'	143°51'	460	12B	18285
16	Buckley L.A., Qld	17°09'	145°37'	720	5	13865
17	10km NW of Mt Molloy, Qld	16°40'	145°15'	420	5	17905
18	Garioch, Qld *	16°40'	145°18'	400	6	13866
19	183km S of Mt Larcom, Qld *	23°50'	151°00'	70	10	17739
20	70km NW of Biloela, Qld	24°00'	150°00'	200	5B	16919
21	Samford, Qld	27°17'	152°51'	50	5	17891
22	Pacific Hwy, MacLean, NSW	29°27'	153°13'	–	10	18984

* denotes population which were analysed for their mating system; B denotes a bulk collection

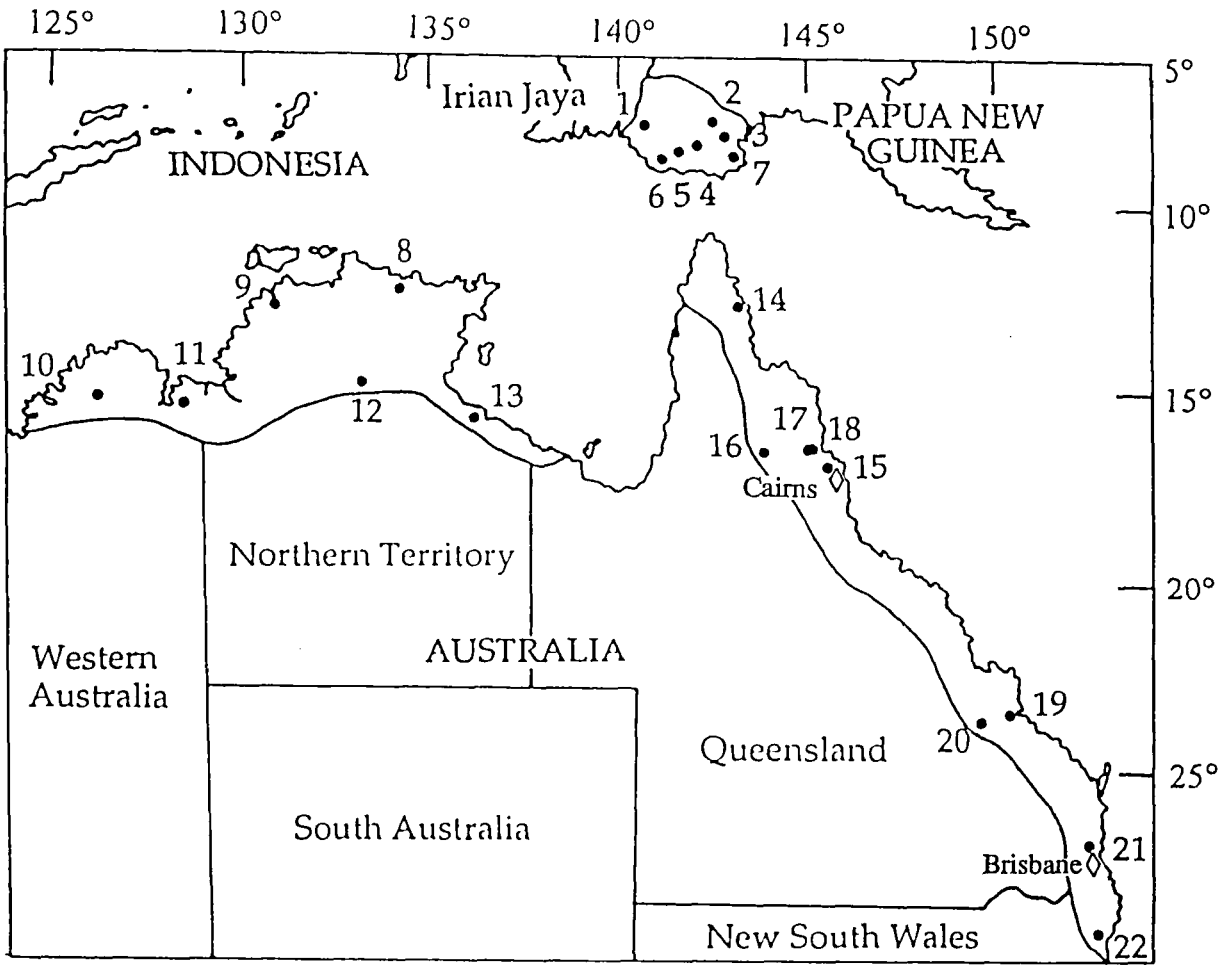

Figure 1 The distribution of *Acacia aulacocarpa* and location of 22 sampled populations. Details of populations are given in Table 1.

mutase (PGM), phosphogluconate dehydrogenase (PGD), shikimate dehydrogenase (SDH), superoxide dismutase (SOD) and uridine diphosphogluconic pyrophosphatase (UGP). Details of each enzyme system, including the buffer systems on which they were run are outlined in BREWER and SING (1970), PLAYFORD *et al.* (1993) and WICKNESWARI and NORWATI (1993). Recipes for enzyme systems are given in WENDEL and WEEDEN (1989) and STRAUSS and CONKLE (1986) and buffer systems in MORAN *et al.* (1989c)

Electrophoretic variants were genetically interpreted based on segregation patterns of progeny arrays of open-pollinated families. The loci within each enzyme system and the alleles within each locus were numbered according to their migration rates. The fastest migrating locus/allele was designated one, the next fastest two, and so on. Any bands which did not conform to Mendelian segregation patterns were omitted from the analysis.

The four populations selected for analysis of their mating systems were Makapa, Blackmore River, Garioch and Mt Larcom. For each population two hundred seedlings were assayed for isozyme genotypes at 10 loci. These seedlings came from ten mother trees for the Makapa and Larcom populations and from nine and six mother trees for the Blackmore and Garioch populations respectively. The seedlings were assayed for the ten most variable loci as determined from the rangewide population survey. The loci were *Ac, Aat–2, Aat–3, Est–1, Gpi–2, Mdh–3, Me, Per–1, Pgd–3,* and *Ugp–1.* However the number of loci that were variable in any one population ranged from three to five.

DATA ANALYSIS

Genotype arrays for each population were analysed using the BIOSYS–1 package (SWOFFORD & SELANDER 1981). Allelic frequencies were calculated and used to compute (1) the mean number of alleles per locus (A), (2) the proportion of polymorphic loci (P, 0.99 criterion), (3) observed heterozygosity (H_o), (4) expected panmictic heterozygosity (H_e), and (5) Wright's Fixation Index (F). Nei's (1978) genetic

distances were calculated and used to perform an hierarchical cluster analysis (unweighted pair group method with arithmetic averaging, UPGMA) of populations. Standard errors for genetic distances were calculated according to the method of RITLAND (1989).

The hierarchical gene statistics of NEI (1973) were used to examine the partitioning of gene diversity within and between populations and zones (groups). H_T, the total gene diversity of sampled populations can be partitioned into H_P, the mean diversity within populations, and D_{PT}, the mean gene diversity between populations. The proportion of genetic diversity between populations (G_{PT}) can be estimated from D_{PT} / H_T. On the basis of the cluster analysis five biological groups were evident. The relative degree of differentiation between these groups (or zones) was calculated by $H_T = H_P + D_{PZ} + D_{ZT}$, where D_{PZ} is the mean gene diversity between populations within groups, and D_{ZT} is the mean gene diversity between groups.

The mean frequency of alleles found in only one population (private alleles ($p(1)$)) was used to calculate the mean number of migrants exchanged between populations per generation (Nm) (SLATKIN & BARTON 1989). This method assumes genetic and demographic equilibrium, neutral migration and that electromorphs are allelic. These results were compared with estimates of gene flow using the method by SLATKIN (1987) which uses Wright's F_{ST} and is defined as (Nm)est = $(1/F_{ST} - 1)/4$. F_{ST} was equated with G_{PT} in the calculations. This method assumes an island model of migration and that the actual value of F_{ST} is the same for every allele.

Single locus estimates of outcrossing rates were made using the maximum likelihood procedure of BROWN *et al.* (1975). The heterogeneity of single locus outcrossing rates was tested with χ^2 tests (RAO 1973). Multilocus estimates of outcrossing rates (t) and their variances were calculated for the four populations using the method of RITLAND and JAIN (1981).

RESULTS

Genetic Diversity

Allelic frequencies were estimated from 30 loci for each of 22 populations. The allelic frequencies for 15 of the more variable and distinctive loci are listed in Table 2 (in Appendix). All loci were polymorphic except *Gdh* and *Gly–1*. Most of the loci had at least three alleles, with a maximum of eight alleles scored in *Sdh*. For half of the loci, there were marked differences in allelic frequencies between some populations and many corresponded to geographic clustering of populations. Thus the populations from New Guinea (NG) had different common alleles from Australian populations at the loci *Ac, Gpi–2, Mdh–2, Sod* and *Ugp–4*. The Northern Territory (NT) and Western Australian (WA) populations had very different allele frequencies at the loci *Aat–3, Aat–4* and *Per–1* compared to the other Australian populations. At the *Aat–1* locus the most common allele in populations from Queensland and New South Wales (NSW) was allele 3 whereas in the NT and NG populations it was allele 2.

Two north Queensland populations, Mt Molloy and Garioch, had very distinct allelic profiles. The common allele at the *Ac, Pep–2, Per–1, Ugp–1* and *Ugp–3* loci for these two populations differed not only from the other northern Queensland populations, but from all of the populations studied. The other three north Queensland populations were distinctly different in allelic frequencies from the four southern populations at *Idh–2,Ugp–1* and *Per–1* loci. On the basis of these differences in allelic frequencies five groups of populations were evident. These groups were termed "biological groups" since their separation was not completely geographical.

The genetic diversity measures for each population of *A. aulacocarpa* are listed in Table 3. A plot of expected heterozygosity and the number of parents sampled per population showed no apparent relationship between the number of parents and the level of expected heterozygosity. However population one had only seedlings from one tree and the data from this population should be interpreted with considerable caution. This population was include for completeness since it is the only seed collection made from Irian Jaya. Considerable variation between populations was apparent in each of the genetic diversity measures. The range for the mean number of alleles in each population (A) was between 1.37 and 2.23 (mean 1.85) and for the percentage of polymorphic loci (P) was between 20 to 73.3 (mean 53.1). Among populations there was a three-fold range in observed heterozygosities (0.055 –0.179) and expected heterozygosities (0.056–0.185). Fixation indices, except in two populations, were positive but not significantly departing from Hardy-Weinberg equilibrium.

The mean genetic diversity estimates in Table 3 were averaged for each biological group and pairwise comparisons made between groups. The estimates of mean number of alleles per locus (A), polymorphic loci (P), observed and expected heterozygosities (H_O and H_E) for the NG group were all lower than the Australian groups. Differences in genetic diversity between biological groups within Australia were quite small.

Genetic distances between populations were high. The mean genetic distance between populations was 0.260 (SD = 0.086). The UPGMA cluster analysis

Table 3 Genetic diversity estimates and fixation indices for *Acacia aulacocarpa* populations

Group	Population	*A*	*P*[a]	H_o	H_s[b]	*F*
NG	1 Erambu-Bupul	1.60	36.7	0.055	0.056	–0.030
	2 Makapa	1.80	43.3	0.093	0.098	0.020
	3 Wasua Pedeya	1.90	56.7	0.076	0.102	0.119
	4 Dimisisi	1.73	56.7	0.063	0.081	0.139
	5 Pongaki	2.03	53.3	0.058	0.073	0.099
	6 Bensbach-Balamuk	1.63	43.3	0.060	0.070	0.083
	7 Oriomo Sawmill	1.37	20.0	0.049	0.062	0.095
	Mean	**1.72**	**42.3**	**0.065**	**0.077**	**0.075**
NT/WA	8 Maningrida	2.13	63.3	0.105	0.121	0.035
	9 Blackmore River	2.07	73.3	0.134	0.148	0.064
	10 Doongan	1.67	53.3	0.075	0.078	0.017
	11 Kununurra	1.63	46.7	0.093	0.100	0.061
	12 West Salt Creek	2.07	56.7	0.123	0.135	0.084
	13 Boroloola	1.63	43.3	0.087	0.088	–0.027
	Mean	**1.86**	**56.1**	**0.103**	**0.112**	**0.037**
North	14 Lockhart	2.23	70.0	0.179	0.185	0.053
Qld	15 Kuranda	1.73	53.3	0.114	0.134	0.081
	16 Buckley LA	1.90	56.7	0.119	0.148	0.196
	Mean	**1.95**	**60.0**	**0.137**	**0.156**	**0.110**
Cairns	17 Mt Molloy	1.93	56.7	0.078	0.107	0.139
	18 Garioch	1.77	53.3	0.102	0.126	0.116
	Mean	**1.85**	**55.0**	**0.090**	**0.117**	**0.127**
South	19 Mt. Larcom	1.93	66.7	0.110	0.149	0.220
Qld	20 Biloela	1.67	46.7	0.111	0.123	0.048
/North	21 Samford	2.17	66.7	0.129	0.143	0.051
NSW	22 MacLean	1.73	50.0	0.096	0.125	0.158
	Mean	**1.87**	**57.3**	**0.122**	**0.135**	**0.119**
Total mean		1.83	53.08	0.104	0.112	0.084
SE		0.21	11.23	0.056	0.033	0.060

[a] 0.99 criterion
[b] unbiased estimate (NEI 1978)
A mean number of alleles per locus
P mean percentage of polymorphic loci
H_o observed heterozygosity
H_e expected heterozygosity
F Wright's fixation index

(Figure 2) divided populations into five distinct groups which correspond to the biological groups. NG populations were significantly separated from the Australian populations (mean genetic distance = 0.365), with the geographically close populations in NG being genetically similar. Within Australia the NT and WA populations separate from those of Queensland/ NSW. Within Queensland/NSW, there was a significant split into two distinct areas, with the four southern populations, Mt Larcom, Biloela, Samford and MacLean (northern NSW) separate from the northern populations, Lockhart, Buckley L.A. and Kuranda. Two northern Queensland populations, Mt Molloy and Garioch were significantly separated from both Queensland and the NT.

Estimates of total genetic diversity (H_T) and the distribution of genetic diversity within (H_P) and between (D_{PT}) populations of *A. aulacocarpa* are presented in Table 4. At each of eighteen of the loci the genetic differentiation among populations is more than 40%. The total genetic diversity for *A. aulacocarpa* is 0.298, with 37.4% of this residing within populations and a very large portion (62.6%) apportioned between populations. However when this very high level of 62.6% of genetic diversity apportioned between the 22 populations is partitioned into within and between biological groups, 57.2% can be apportioned to differences between biological groups and only 5.4% to differences between populations within biological

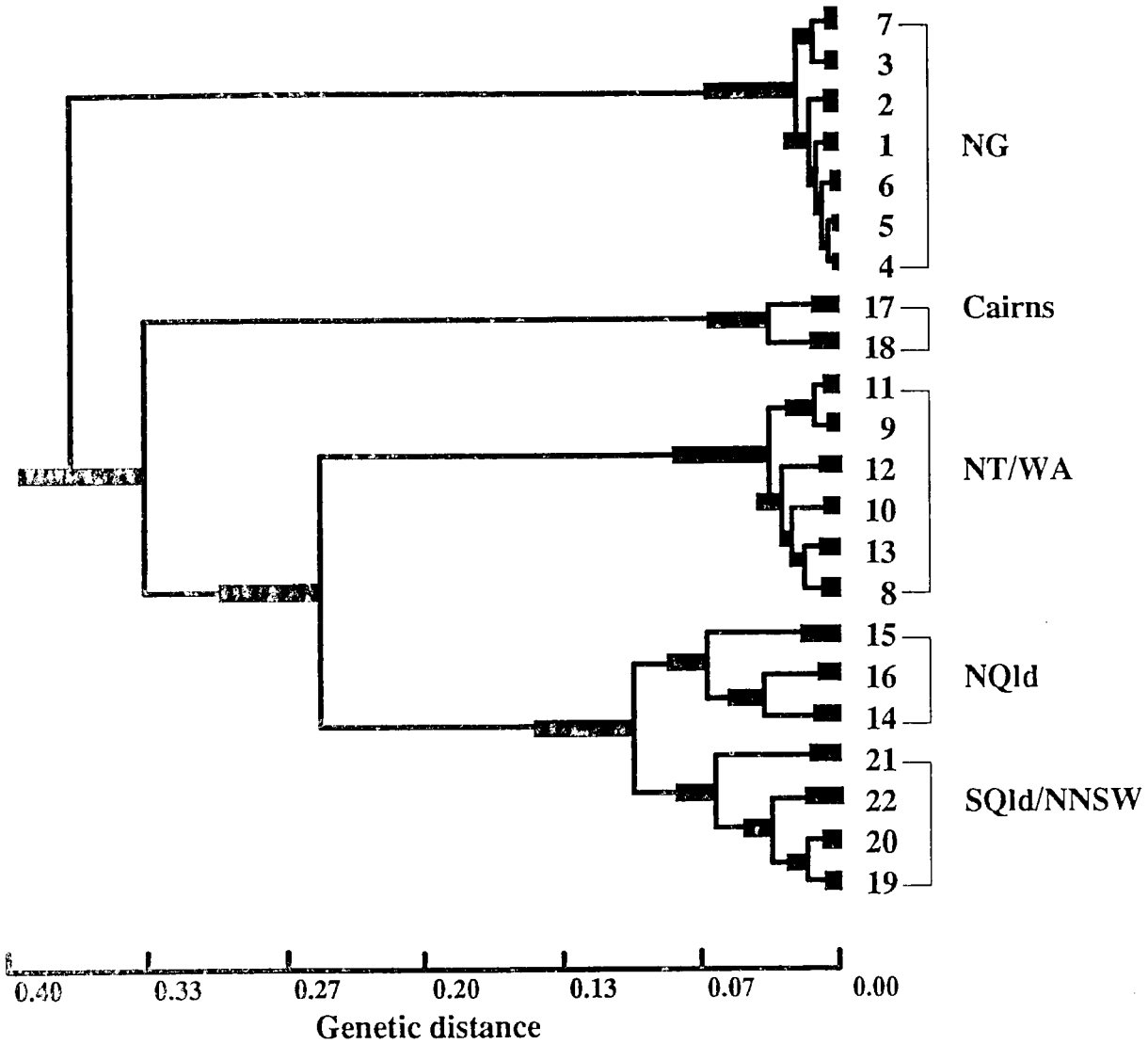

Figure 2 Dendrogram of the UPGMA cluster analysis based on Nei's (1978) unbiased genetic distances between 22 populations of *Acacia aulacocarpa*. Cluster analysis is statistically significant if the standard error bar is less than half the branch length.

groups.

The diversity within each biological group was also examined by analysing the groups separately (Table 5). Each biological group showed low levels of differentiation between populations, indicating that the populations within groups are genetically similar to each other. This was particularly apparent in the NG and Cairns biological groups which had only 7.3% and 8.1%, respectively, of the diversity apportioned between populations.

The overall mean estimate of migrants per generation (N_m) for the species using the private alleles method (1.67) is ten fold greater than the estimate using Wright's F_{ST} (0.15) (Table 6). The large differentiation between biological groups may be confounding the results obtained using F_{ST}. The number of migrants was highest in the NG biological group and lowest in the northern Queensland and southern Queensland /northern NSW biological groups using both methods. Further, gene flow for the Cairns biological group is the second highest estimate using the F_{ST} method but quite low using the private alleles method but only two populations were sampled in this group.

Mating System

Most single-locus estimates of outcrossing for the four populations of *A. aulacocarpa* were very high with little evidence of inbreeding (Table 7). Chi square tests revealed that there was no significant heterogeneity between single locus estimates for each population. The average outcrossing rates for Makapa, Blackmore River

Table 4 Distribution of genetic diversity within and between populations and zones of *Acacia aulacocarpa*

Locus	H_T	H_Z	H_P	G_{ZT}	G_{PZ}	G_{PT}
Ac	0.603	0.129	0.116	0.786	0.022	0.807
Alt	0.093	0.090	0.082	0.030	0.087	0.117
Adh	0.035	0.034	0.330	0.044	0.019	0.062
Aat–1	0.480	0.078	0.071	0.836	0.014	0.851
Aat–2	0.039	0.038	0.035	0.023	0.084	0.106
Aat–3	0.472	0.129	0.120	0.727	0.018	0.745
Aat–4	0.446	0.118	0.109	0.735	0.022	0.756
Cat	0.081	0.078	0.073	0.036	0.064	0.100
Est–1	0.296	0.231	0.169	0.220	0.210	0.430
Gpi–2	0.582	0.320	0.298	0.450	0.053	0.503
Gly–2	0.035	0.034	0.029	0.017	0.137	0.154
Idh–1	0.090	0.089	0.084	0.051	0.017	0.068
Idh–2	0.496	0.215	0.120	0.542	0.202	0.744
Mdh–2	0.494	0.088	0.072	0.821	0.033	0.854
Mdh–3	0.231	0.211	0.184	0.084	0.119	0.203
Me	0.487	0.217	0.189	0.554	0.057	0.612
Pep–1	0.007	0.007	0.007	0.000	0.000	0.000
Pep–2	0.165	0.000	0.000	1.000	0.000	1.000
Per–1	0.672	0.358	0.307	0.464	0.076	0.542
Pgm–1	0.637	0.546	0.375	0.143	0.268	0.411
Pgm–2	0.058	0.058	0.055	0.011	0.053	0.065
Pgd–3	0.304	0.236	0.136	0.226	0.327	0.553
Sdh	0.216	0.140	0.118	0.353	0.104	0.457
Sod	0.434	0.000	0.000	1.000	0.000	1.000
Ugp–1	0.754	0.435	0.362	0.424	0.097	0.520
Ugp–2	0.158	0.144	0.140	0.083	0.027	0.110
Ugp–3	0.162	0.060	0.054	0.633	0.033	0.666
Ugp–4	0.437	0.012	0.012	0.973	0.000	0.973
Mean	0.298	0.136	0.111	0.542	0.084	0.626
SE	0.040	0.024	0.018	0.060	0.020	0.046

H_T – total gene diversity
H_Z – mean gene diversity within zones
H_P – mean gene diversity within populations

$G_{ZT} = (H_T–H_Z) / H_T$
$G_{PZ} = (H_Z–H_P) / H_T$
$G_{PT} = (H_T–H_P) / H_T$

Table 5 Distribution of genetic diversity within and between five biological groups of *Acacia aulacocarpa*

Biological group	H_Z	D_{PZ}	G_{PZ}
NG	0.109	0.008	0.073
NT/WA	0.152	0.023	0.149
North Qld	0.221	0.034	0.154
South Qld / North NSW	0.188	0.027	0.143
Cairns	0.180	0.015	0.081

Table 6 Estimates of gene flow (Nm) between 22 populations and 5 biological groups of *Acacia aulacocarpa* using private alleles ($p(1)$) and Wright's F_{ST}

Species / Biological group	$N(m)$ est	
	p (1)	F_{ST}
Acacia aulacocarpa	1.67	0.15
NG	5.04	3.04
NT/WA	2.57	1.43
North Queensland	0.88	1.37
South Queensland / North NSW	1.03	1.51
Cairns	1.12	2.84

and Garioch did not differ significantly from 100%, indicating very strong outcrossing as measured at the viable seedling stage. However a statistically significant level of inbreeding (p = 0.05) was detected in the Mt Larcom population with a reduced outcrossing rate of 0.802. Multilocus estimates of outcrossing rates were also very high (Table 7) and only the outcrossing rate for Mt Larcom was significantly less than 100%. There

Table 7 Single locus (SL) and multilocus (ML) estimates of outcrossing rate (*t*) and inbreeding coefficients for four populations of *Acacia aulacocarpa* (standard errors in parentheses).

Locus	Population			
	Makapa	Blackmore R.	Garioch	Mt. Larcom
Ac	0.87 (0.25)	–[a]	–	–
Aat–2	–	–	–	0.99 (0.22)
Aat–3	–	–	1.34 (0.21)	0.81 (0.18)
Est–1	0.96 (0.18)	0.55 (0.17)	–	0.85 (0.13)
Gpi–2	0.89 (0.15)	1.04 (0.18)	–	–
Mdh–3	0.79 (0.28)	0.88 (0.10)	–	–
Me	–	–	–	0.82 (0.23)
Per–1	–	1.35 (0.18)	0.83 (0.26)	0.54 (0.10)
Pgd–3	–	0.86 (0.11)	–	–
Ugp–1	0.97 (0.16)	–	0.97 (0.14)	–
Mean SL	0.90 (0.09)	0.94 (0.07)	1.05 (0.12)	0.80* (0.08)
ML	0.94 (0.03)	0.93 (0.03)	0.99 (0.07)	0.89* (0.04)
F_e [b]	0.03	0.04	0.01	0.06
F_s [c]	0.02	0.06	0.12	0.22

[a] Dash indicates locus not variable in population
[b] $F_e = (1-t) / (1+t)$, the inbreeding coefficient
[c] F_e = means fixation index among seedlings
* rejection of the null hypothesis that $t = 1$

were no significant differences between the multilocus outcrossing rates for the four populations. When the mean single locus and multilocus estimates are compared, there was no significant difference between estimates for each of the four populations. All four populations had small but statistically nonsignificant, positive average fixation indices indicating no significant departure from Hardy-Weinberg equilibrium.

DISCUSSION

The genetic diversity at the species level for *A. aulacocarpa* (H_T = 0.298, A_S = 4.40, P_s = 93%) was high compared not only to estimates available for other tropical acacia species which co-occur in northern Australia and New Guinea but plants generally. In addition the mean genetic diversity in populations for *A. aulacocarpa* (0.111) is generally higher than most intercontinental Australian acacia species. However this value is lower than the mean estimates for tropical trees (0.160, Loveless 1992), animal/insect pollinated eucalypts (0.174, MORAN 1992) and wind-pollinated conifers (0.173, HAMRICK & GODT 1990). The pattern of genetic differentiation in *A. aulacocarpa* is very unusual in that most of the variation (62.6%) is distributed between rather than within populations. In fact the proportion of genetic diversity between populations is the highest reported for a tree species. Generally woody plants have most of their genetic diversity within populations (HAMRICK & GODT 1990). For instance, mean estimates of genetic differentiation are 26.4% for eucalypts (MORAN 1992), 6.8% for wind-pollinated conifers (HAMRICK & GODT 1990), 7.5% for northern hemisphere wind-pollinated angiosperms (MUONA 1990) and 9.6% for tropical tree species (LOVELESS 1992).

A. aulacocarpa could be divided into five biological groups with differing genetic composition(Figure 2) and these groups largely correspond to geographic zones of the distribution. Two of the three Queensland (QLD) groups correspond to the northern and southern sections of its range. Field surveys will be required to determine whether the third QLD group (Cairns group, Mt Molloy (17) and Garioch (18)) is sympatric with the NQLD group or whether they occupy different ecological niches. Both populations have a distinct allozyme profile compared to the other QLD populations. They may correspond to narrow-phylloded forms which PEDLEY (1978) suggested could be an intraspecific taxon. Seed collections were not available from any of the disjunct populations of central Queensland and analysis of these populations may clarify the extent of differentiation between populations in Queensland. A variety, *A. aulacocarpa* var.*fruticosa* occurs on mountain tops in the Glasshouse Mountains in south-eastern Queensland (WHITE 1946) and was not studied.

Most of the variation between the 22 populations can be ascribed to differences between the five biological groups, and genetic distances between these biological groups were very large. In particular that between the NG group and all the Australian groups was excep-

tionally high (0.365). This is comparable to the distance between northern and southern populations in *A. melanoxylon* (0.344) suggested as indicative of subspecies status by PLAYFORD *et al.* (1993), and to that of congeneric species (0.4, GOTTLIEB 1977). THOMSON (1994) from a taxonomic examination of seed characteristics, seedling morphology and botanical specimens proposed that five groups occurred within the species closely corresponding to the major geographic groups. These groups are essentially the same as found in this study. He further suggested *A. aulacocarpa* could be separated into five subspecies and the isozyme data are consistent with this proposal.

There has probably been an underestimation of numbers of rare alleles especially in populations sampled from small numbers of maternal parents. At only a limited number of loci in some populations could pollen gene frequencies be estimated with any certainty. These frequencies could give population estimates more independent of the number of maternal trees sampled but previous studies indicate that results in terms of genetic structure of populations do not change from that based on total progeny data (BROWN *et al.* 1975, MORAN & BELL 1983, MORAN 1992).

The high rates of outcrossing in *A. aulacocarpa* are similar to estimates reported for other Australian *Acacia* species (MORAN 1992). These high estimates suggest that environmental factors may cause only minor variation in outcrossing rates in *Acacia* species, unlike other animal-pollinated species such as eucalypts (MORAN & BELL 1983). It also suggests a strong self-incompatability system operating in the life cycle of these species between pollination and the viable seedling stage. All four populations of *A. aulacocarpa* were highly outcrossing despite the fact that each population came from a different biological group and are representative of the wide range of environmental conditions in which *A. aulacocarpa* grows (BOLAND *et al.* 1984). It would therefore appear that the levels of outcrossing are under strong genetic control.

It is clear that there is genetic differentiation into five biological groups allied with comparatively low variation within groups. Outcrossing mating systems promote the flow of genes thereby causing less intra- and inter-population differentiation than inbreeders (BROWN 1979). The high outcrossing rates in *A. aulacocarpa* contribute to the low levels of genetic differentiation between populations at least within geographic zones (groups). The large genetic distances between the groups indicate that long term geographic isolation and genetic drift could be significant factors causing genetic differentiation over the species distribution. The distribution of genetic diversity in three intercontinental acacias, *A. mangium, A. auriculiformis*, and *A. crassicarpa*, has been suggested by MORAN *et al.* (1989b) to be due to bottlenecks during the Pleistocene glaciations. Similarly the present range of *A. aulacocarpa* could be a reflection of the wider distribution of it or its progenitor on the Australian geological plate in the Tertiary. The low genetic diversity and high similarities between populations in NG apparent from this study, could be attributed to geographic separation of these populations from the main part of the distribution in the Quaternary period and subsequent contractions of populations during cycles of aridity.

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Table 2 Allelic frequencies for 15 loci in 22 populations of *Acacia aulacocarpa*

Locus	Allele	Population																				
		New Guinea							Northern Territory / Western Australia						Queensland / North South Wales							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Ac	1									0.12	0.01		0.21										
	2								0.01	0.03		0.17	0.19				0.10			0.07	0.01	0.02	0.17
	3			0.01	0.02	0.01			0.99	0.84	0.99	0.83	0.60	1.00	0.99	0.79	0.90	0.01		0.93	0.95	0.98	0.83
	4	1.00	0.91	0.99	0.98	0.98	1.00	1.00		0.01					0.01	0.21					0.04		
	5		0.09			0.01												0.99	1.00				
Aat-1	1												0.14										
	2	1.00	1.00	0.99	0.98	1.00	1.00	1.00	0.98	1.00	1.00	1.00	0.86	1.00		0.27	0.22			0.04	0.11		0.08
	3			0.01	0.02				0.01						1.00	0.73	0.78	0.99	0.99	0.96	0.89	1.00	0.92
	4																	0.01	0.01				
Aat-3	1									0.14	0.01		0.01										
	2	0.03	0.01	0.11	0.03	0.01	0.03		0.98	0.86	0.99	1.00	0.85	1.00	0.05			0.01		0.06		0.06	
	3	0.97	0.99	0.88	0.97	0.99	0.97	1.00	0.01				0.14		0.94	1.00	1.00	0.64	0.63	0.94	1.00	0.69	1.00
	4								0.01						0.01			0.35	0.37			0.25	
Aat-4	1									0.14	0.01		0.01										
	2	0.03	0.01	0.11	0.03	0.01	0.03		0.98	0.86	0.99	1.00	0.85	1.00	0.05			0.10	0.25	0.06		0.06	
	3	0.97	0.99	0.88	0.97	0.98	0.97	1.00	0.01				0.14		0.94	1.00	1.00	0.84	0.73	0.94	1.00	0.69	1.00
	4			0.01		0.01			0.01						0.01			0.06	0.02			0.25	
Gpi-2	1								0.01	0.06			0.01		0.01	0.44	0.18			0.02		0.06	
	2												0.05									0.01	
	3	0.01				0.01			0.90	0.75	1.00	0.81	0.85	0.98	0.59	0.56	0.77	1.00	0.99	0.97	1.00	0.90	1.00
	4			0.01		0.01			0.03	0.04			0.03							0.01		0.03	
	5	0.28	0.44	0.49	0.56	0.61	0.38	0.44	0.04	0.15		0.16	0.03	0.02	0.40		0.05		0.01				
	6	0.71	0.54	0.46	0.44	0.36	0.62	0.55	0.02			0.03	0.03										
	7		0.02	0.04		0.01		0.01															
Idh-2	1											0.36											
	2	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	0.97	0.98	0.64	1.00	0.97	0.64	0.63	0.99			0.01			
	3										0.02												
	4			0.01						0.03				0.03	0.25	0.37	0.01	1.00	1.00	0.80	0.81	1.00	0.82
	5						0.01								0.11					0.19	0.19		0.18
Mdh-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00					0.01	0.01		0.07						0.04	
	2								0.99	0.99	1.00	1.00	0.94	0.99	1.00	0.93	1.00	1.00	0.98	0.53	0.84	0.95	0.76
	3												0.05										
	4								0.01										0.02	0.47	0.16	0.01	0.24
	5									0.01													

Table 2 (continued)

Locus	Allele	Population																				
		New Guinea							Northern Territories / Western Australia						Queensland / North South Wales							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Me	1															0.05	0.09						
	2				0.01										0.44	0.93	0.91	0.04		0.74	0.95	0.80	0.92
	3									0.01						0.02							0.02
	4	0.98	0.80	0.98	0.94	0.98	0.91	0.98	0.70	0.97	1.00	0.96	0.94	0.67	0.46			0.86	0.93	0.26	0.05	0.20	0.06
	5		0.08	0.02	0.05	0.02	0.02	0.02	0.29	0.02		0.02	0.05	0.32	0.01			0.08	0.06				
	6	0.02	0.12				0.07		0.01			0.02	0.01	0.01	0.09			0.02	0.01				
Pep-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			1.00	1.00	1.00	1.00
	2																	1.00	1.00				
Per-1	1		0.02			0.02			0.34	0.54	0.06	0.45	0.69	0.40	0.20	0.06	0.08						
	2	1.00	0.92	0.96	0.95	0.98	0.96	0.97	0.66	0.46	0.94	0.55	0.31	0.60	0.15		0.07	0.07		0.14	0.04	0.26	0.30
	3				0.02										0.65	0.89	0.85		0.14	0.44	0.50	0.15	0.40
	4		0.01	0.04	0.03		0.04	0.03								0.05				0.42	0.46	0.58	0.30
	5		0.05																			0.01	
	6																	0.93	0.86				
Sdh	1								0.01														
	2	0.01	0.01	0.01								0.02			0.01	0.11	0.23						
	3					0.01						0.01								0.02			
	4	0.99	0.95	0.98	0.93	0.98	0.97	0.98	0.79	0.91	0.90	0.97	0.96	1.00	0.99	0.89	0.77	0.59		0.98	1.00	0.88	0.99
	5																	0.40	1.00				
	6										0.01												
	7		0.04	0.01	0.07	0.01	0.03	0.02	0.20	0.09	0.09		0.04					0.01				0.11	0.01
	8																					0.01	
Sod	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00															
	2								1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ugp-1	1															0.48	0.37			0.96	0.92	0.93	0.88
	2	0.66	0.23	0.40	0.49	0.37	0.28	0.53	0.06				0.01		0.44	0.15	0.23	0.06	0.16			0.01	0.09
	3	0.01				0.01			0.03		0.01		0.04					0.89	0.83			0.01	0.03
	4	0.06	0.46	0.39	0.19	0.10	0.12	0.26	0.02	0.01			0.01		0.24	0.06	0.04	0.04		0.04	0.01	0.05	
	5	0.26	0.03	0.11	0.28	0.48	0.30	0.18	0.89	0.99	0.99	1.00	0.94	1.00	0.32	0.31	0.35	0.01	0.01		0.07		
	6	0.01	0.28	0.10	0.04	0.04	0.23	0.03									0.01						
	7						0.07																
Ugp-3	1											0.01			0.10	0.06	0.07						
	2	1.00	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.90	0.94	0.86	0.05	0.36	1.00	1.00	0.99	1.00
	3			0.01													0.07	0.95	0.64				
	4					0.01																0.01	

Table 2 (continued)

Locus	Allele	Population																				
		New Guinea							Northern Territories / Western Australia						Queensland / North South Wales							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Ugp–3	1	0.02							1.00	1.00	0.96	1.00	1.00	1.00	0.97	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00
	2	0.98	1.00	1.00	1.00	0.98	1.00	1.00			0.03				0.03			0.02					
	3					0.02					0.01												