Heredity (2002) 88, 250–257

www.nature.com/hdy

Genetic diversity within and divergence between rare and geographically widespread taxa of the *Acacia acuminata* Benth. (Mimosaceae) complex

L Broadhurst and D Coates
CALMScience, Western Australian Herbarium, Department of Conservation and Land Management, Locked Bag 104, Bentley Delivery Centre, Western Australia 6983

The *Acacia acuminata* complex is a group of woody shrubs and small trees comprised of three formally described taxa (*A. oldfieldii*, *A. acuminata* ssp. *acuminata* and ssp. *burkittii*) and two informal taxa ('small seed' and 'narrow phyllode') with contrasting geographical distributions within the south-west of Western Australia. In addition, a series of variant populations of possible hybrid origin exist. Population genetic structure was investigated in 25 populations representing the taxa and variants using 16 polymorphic allozyme loci. All taxa and variants exhibited relatively high levels of genetic variation compared with other woody angiosperms with similar geographic distributions. Levels of genetic diversity in the widely distributed ssp. *burkittii* and 'narrow phyllode' taxon were considerably higher (H_e, 0.311 and 0.319, respectively) than expected for widespread woody shrubs. In contrast, the rare and highly restricted *A. oldfieldii* exhibited significantly lower levels of genetic diversity (H_e, 0.173) compared with the other taxa in the complex, but higher than other rare woody shrubs. Although morphologically close, associations based on genetic distance showed *A. oldfieldii* was highly divergent from the other taxa ($D = 0.85$) while including variant populations confused systematic alliances. The unusual placement of some of these populations and high degree of population differentiation (G_{ST}, 12.7%) supports the suggestion that these may represent a series of hybridisation events between the various taxa.
Heredity (2002) **88,** 250–257. DOI: 10.1038/sj/hdy/6800036

Keywords: *Acacia*; population genetics; widespread; restricted

Introduction

In the south-west botanical region of Western Australia, isolation, climatic fluctuations and poor soils have contributed to a rich and diverse flora composed of many endemic and restricted species (Hopper, 1979). Approximately 8000 species have been identified to date, 75% of which are endemic (Hopper, 1992). Associated with this relatively high proportion of rare and geographically restricted species is a large number of taxa with historically fragmented or disjunct distributions (Hopper *et al*, 1996). Such range fragmentation would be expected to lead to strong genetic structure within species (Moran and Hopper, 1983). This is supported by studies on taxa from several different genera which suggest that unusually high levels of population differentiation are a feature of this flora (Coates, 2000).

Many genetic studies of Western Australian flora have concentrated on species which are rare or have highly restricted distributions. These studies have provided valuable insights into localised stochastic processes and life history characteristics and the importance these factors may have in the persistence of small populations in more recently fragmented landscapes. Genetic processes associated with rarity in plants have received significant attention over the last decade (see Karron *et al*, 1988; Hamrick and Godt, 1989; Barrett and Kohn, 1991; Ellstrand and Elam, 1993; Gitzendanner and Soltis, 2000). Theoretically, rare plants might be expected to show low levels of genetic variation both at the species and population levels due to selection under a narrow range of environmental conditions, and genetic drift and inbreeding in small isolated populations (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). Low genetic variation within rare species may also be due to founder events associated with recent speciation (see Young and Brown, 1996). Interestingly, while some rare taxa in south-west Australia conform with theoretically expected low levels of genetic variation (Moran and Hopper, 1983, 1987) many others do not (Moran and Hopper, 1987; Coates, 1988, 1992; Tischler, 1998), maintaining levels which are consistently higher than those exhibited by rare plants on a worldwide basis (Hamrick and Godt, 1989; Hamrick *et al*, 1992; Young and Brown, 1996).

Similar findings have been documented elsewhere (see Young and Brown, 1996) and are not necessarily unexpected given the broad range of life histories and evolutionary patterns found in the rare plants (Karron, 1987). An alternative approach to a better understanding of rarity, small population size and restricted geographic range in plants has been proposed by Karron *et al* (1988) and

Correspondence: L Broadhurst, CALMScience, Western Australian Herbarium, Department of Conservation and Land Management, Locked Bag 104, Bentley Delivery Centre, Western Australia 6983
Received 23 July 2001; accepted 14 November 2001

others (see Gitzendanner and Soltis, 2000). Comparisons of the patterns of genetic variation in closely related groups or between pairs of rare and widespread or common species should at least reduce the confounding effects of life history and evolutionary factors (Young and Brown, 1996).

In contrast to rare or restricted taxa, widespread species are predicted to exhibit higher levels of genetic diversity although both classes distribute this diversity in a similar manner (Hamrick and Godt, 1989). The large continuous populations of widespread taxa are theoretically less susceptible to genetic drift and hence loss of diversity, in contrast to the small and ecologically limited populations characteristic of many endemic species (Hamrick and Godt, 1989). As more studies have been completed, however, it has become evident that a range of values exist within each geographical class (Gitzendanner and Soltis, 2000).

While the evolutionary patterns of genetic variation observed within rare and geographically restricted southwest Australian taxa often reflect that expected of extremely fragmented and disjunct populations, patterns associated with widespread species remain largely unexplored. Widespread species tend to have more continuous population systems in a broader range of environments. One possibility is that these species might be expected to show less genetic structure due to increased influences of gene flow even though they cover a much greater geographic range. Alternatively, as pointed out by Godt and Hamrick (1999), widespread species might be expected to exchange fewer genes across their geographic range and have overall higher levels of population divergence than geographically restricted species.

The *Acacia acuminata* Benth. (Mimosaceae) complex is a group of woody shrubs and small trees comprised of three formally described taxa (*A. oldfieldii* F.Muell., *A. acuminata* Benth. ssp. *acuminata* and *A. acuminata* ssp. *burkittii* F.Muell. ex Benth. Kodela and Tindale), two informal taxa ('small seed' and 'narrow phyllode'), and three variants of uncertain status ('variant 1', 'variant 2' and 'variant 1/2') (Maslin *et al*, 1999; Maslin, 2001). In this analysis the as yet undescribed 'small seed' and 'narrow phyllode' are treated as distinct taxa rather than subgroups within the *A. acuminata* complex as has been done elsewhere (Maslin *et al*, 1999; M Byrne, unpublished). The complex is distributed across much of the south-west land division of Western Australia and extending eastwards through the arid zone into central Australia (Figure 1). Both ssp. *acuminata* and ssp. *burkittii* have relatively large geographical ranges (Figure 1). The morphologically distinct 'small seed' taxon is localised, while the 'narrow phyllode' taxon is widely distributed between ssp. *acuminata* and ssp. *burkittii*. The three variants which exhibit morphological affinities with both ssp. *acuminata* and ssp. *burkittii* are restricted to the Geraldton region (Maslin *et al*, 1999) while the rare and extremely geographically restricted *A. oldfieldii* is the fifth taxon within the complex.

The taxa within the *A. acuminata* complex exhibit varying degrees of geographic distribution providing a unique opportunity to compare population genetic patterns within and among the populations and taxa. Levels of genetic diversity within populations were also compared between the geographically restricted and more widespread taxa to assess the genetic implications of rarity in this complex, and whether the geographically restricted taxa were genetically depauperate. In addition, we sought to determine relationships between the taxa and how they may contribute to a better understanding of the complex evolutionary processes characteristic of the flora in this region.

Materials and methods

Sampling strategy

Seed was collected from populations of each taxon across the known distributions (Figure 1). Within each population several legumes were randomly collected from individual plants (10) and kept separate. Seed was later extracted and maintained as individual collections from which 10 seeds were randomly selected. Seed was surface sterilised with 1% sodium hypochlorite, rinsed three times, covered with boiling water and left to cool to room temperature. The following day any unimbibed seed were nicked to stimulate germination. Seedlings were frozen at −72°C until processed.

Isozyme electrophoresis

Seedlings (1–5) from individuals within each population were ground in extraction buffer (Broadhurst *et al*, 1999) and centrifuged for 5 mins. Allozymes were run using the cellulose acetate system (Helena Laboratories, Beaumont, TX, USA) and stained according to Richardson *et al* (1986) with modifications as described by Coates (1988) with the exception that 4 ml agar were used with all stain ingredients proportionally increased. Running times varied according to each enzyme system. Twelve enzyme systems were assayed: aspartate aminotransferase (*Aat*, EC2.6.1.1), esterase (*Est*, EC3.1.1.-), glucose-6-phosphate dehydrogenase (G-6-*pdh*, EC1.1.1.49), glutamate dehydrogenase (*Gdh*, EC1.4.1.2), glucose-6-phosphate isomerase (*Gpi*, EC5.3.1.9), isocitrate dehydrogenase (*Idh*, EC1.1.141), malate dehydrogenase (*Mdh*, EC1.1.1.37), malate dehydrogenase (oxaloacetate-decarboxylating) (*Me*, EC1.1.1.40), menandione reductase (*Mnr*, EC1.6.99.-), phosphoglucomutase (*Pgm*, EC5.4.2.2), phosphogluconate dehydrogenase (*Pgdh*, EC1.1.1.44), shikimate dehydrogenase (*Skd*, EC 1.1.1.25). Sixteen zones of activity were scored and each zone was assumed to represent a single locus.

Genetic analyses

Single locus diversity measures including the average number of alleles per polymorphic locus (A_P), percentage of polymorphic loci per population (P, criterion 99%), observed heterozygosity (H_o) and gene diversity (expected panmictic heterozygosity) (H_e) were estimated using POPGENE (Yeh and Boyle, 1997). Differences in genetic variation between species at the population level were assessed using nested analysis of variance for allelic richness and gene diversity, t-test for polymorphism and split-plot analysis of variance for heterozygosity with loci treated as split-plot treatments (Weir, 1990) using MINITAB Release 12. Fixation indices (F_{IS}) (Wright, 1978) were estimated to examine population deviation from random mating using POPGENE (Yeh and Boyle, 1997). Total genetic diversity (H_T) and its distribution within (H_S) and between (D_{ST}) populations and the proportion of inter-

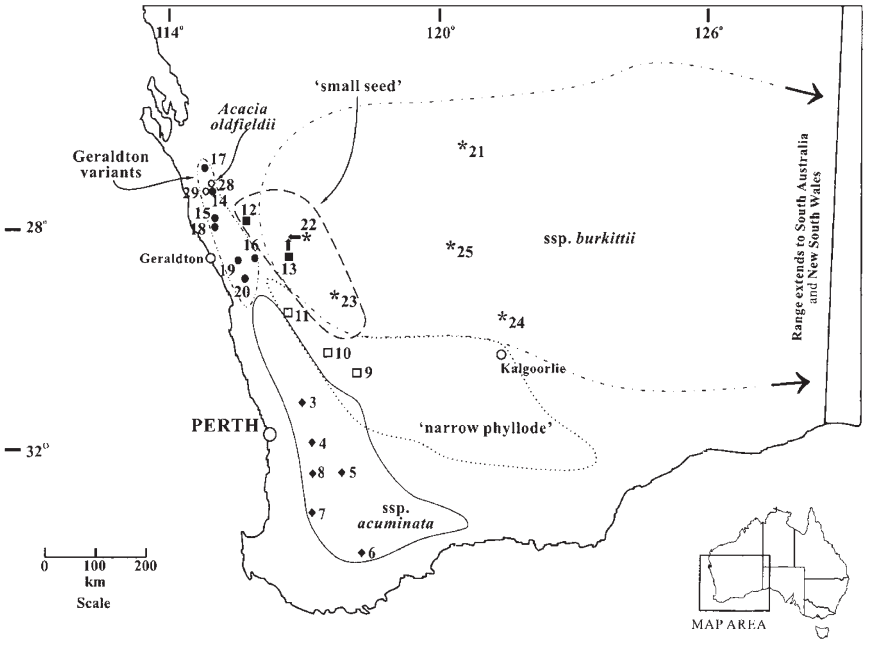

Figure 1 Distribution of taxa and study sites within the *A. acuminata* complex. Refer to Table 1 for site numbers.

populational differentiation (G_{ST}) were calculated using GENESTAT (Whitkus, 1985).

An UPGMA phenogram based on populations representing the five recognised taxa, *A. oldfieldii*, ssp. *acuminata*, ssp. *burkittii*, 'small seed' and 'narrow phyllode', was constructed using Nei's genetic distance (*D*) and standard error bars calculated with Ritland's Genetic Distance and Clustering (GD) program (Ritland, 1989). A second UPGMA was constructed using populations of the five taxa and the Geraldton variants to investigate whether any relationship(s) between these variants and the taxa that was not evident from morphological studies were present.

Results

No monomorphic loci were observed within the complex although the alternate allele(s) for loci *Gdh-1*, *Mnr-1* and *Pgdh-1* was either rare or at very low frequencies. While no fixed differences between species were observed, the most frequent allele for *Aat-2*, *Aat-3*, *Gdh-1*, *Idh-1*, *Mdh-2* and *Pgdh-1* in *A. oldfieldii* was alternate to that in the other taxa.

Variation within and among the taxa

Genetic variation within populations and population means for allelic richness, polymorphism, gene diversity and heterozygosity for all taxa and Geraldton variants are shown in Table 1. Within populations of the most widespread taxon, ssp. *burkittii*, A_P (average number of alleles per polymorphic locus) ranged from 2.4 to 2.6, *P* (percentage polymorphic loci) from 81.3% to 93.8%, H_e (gene diversity) from 0.275 to 0.313 and H_o (heterozygosity) from 0.136 to 0.194. The F_{IS} ranged from 0.263 to 0.414. In the other widespread taxon, ssp. *acuminata*, A_P varied from 1.9 to 2.6, *P* from 56.3% to 87.5%, H_e from 0.187 to 0.287 and H_o from 0.099 to 0.194. Significant differences between these two taxa were noted for both A_P ($p = 0.001$) and H_e ($p = 0.008$).

Diversity measures in populations of 'narrow phyllode', which is distributed between ssp. *acuminata* and ssp. *burkittii*, were similar to those of ssp. *burkittii* (A_P, range 2.4–2.6; *P*, 75–87.5%; H_e, 0.263–0.330; H_o, 0.133–0.154). This taxon also had high F_{IS} values ranging from 0.397–0.499. While no significant differences were observed for any measure between 'narrow phyllode' and ssp. *burkittii*, differences were noted between 'narrow phyllode' and ssp. *acuminata* for both A_P ($p = 0.007$) and H_e ($p = 0.011$).

In the highly restricted *A. oldfieldii* populations, mean values for all measures of variation were clearly lower than population means for all other taxa and Geraldton variants. The mean A_P was 2, *P* 59.4%, H_e 0.168, H_o 0.078, while F_{IS} was moderate at 0.332. All measures were significantly different between this taxon and ssp. *burkittii* (A_P, $p = 0.000$; H_e, $p = 0.000$; H_o, $p = 0.000$), ssp. *acuminata* (A_P, $p = 0.020$; H_e, $p = 0.002$; H_o, $p = 0.007$) and 'narrow phyllode' (A_P, $p = 0.001$; H_e, $p = 0.000$; H_o, $p = 0.001$). The localised 'small seed' exhibited similar mean values to those of ssp. *acuminata* (A_P, 2.3; *P*, 71.9%; H_e, 0.245; H_o, 0.155). The F_{IS} was the closest to zero of all the taxa but still exhibited homozygote excesses (0.220). This taxon did not differ significantly for any value with ssp. *acuminata* or 'narrow phyllode' but was different to ssp. *burkittii* for A_P ($p = 0.020$) and to *A. oldfieldii* for H_o ($p = 0.000$) and H_e ($p = 0.005$).

Differences at the species level

Pooled single locus diversity measures also varied between the taxa. The measures of A_P and *P* were highest in the three widespread taxa, ssp. *acuminata*, ssp. *burkittii* and 'narrow phyllode' (A_P 2.9, 3.3 and 3.1; *P* 87.5%, 100% and 87.5%, respectively) and lowest in the restricted *A. oldfieldii* and localised 'small seed' (A_P, 2.3 and 2.4; *P*, 75% and 81.3%, respectively). Values for H_e were lowest in *A. oldfieldii* (0.173) and in the other taxa ranged from 0.252 in 'small seed' to 0.319 in 'narrow phyllode'. A similar pattern was observed for H_o, while fixation indices were

Table 1 Single locus diversity measures based on 16 loci for populations in the *A. acuminata* complex. See Figure 1 for site locations

Taxon	*Site*	*N*	A_P	*P*	H_e	H_o	F_{IS}
ssp. *acuminata*	3	17.9	2.1 (0.2)	62.5	0.217 (0.065)	0.121 (0.037)	0.232 (0.118)
	4	29.9	2.6 (0.3)	87.5	0.287 (0.060)	0.172 (0.041)	0.340 (0.076)
	5	7.8	1.9 (0.2)	56.3	0.237 (0.064)	0.141 (0.060)	0.020 (0.187)
	6	24.8	2.1 (0.2)	75.0	0.241 (0.057)	0.122 (0.032)	0.371 (0.102)
	7	21.8	2.4 (0.3)	75.0	0.257 (0.057)	0.194 (0.048)	0.230 (0.087)
	8	26.0	2.3 (0.3)	75.0	0.187 (0.049)	0.099 (0.024)	0.324 (0.119)
Mean		21.4	2.2 (0.1)	71.8	0.238 (0.014)	0.142 (0.015)	0.253 (0.052)
Taxon		128.1	2.9 (0.3)	87.5	0.265 (0.058)	0.142 (0.031)	0.388 (0.055)
ssp. *burkittii*	21	38.9	2.6 (0.3)	93.8	0.277 (0.057)	0.136 (0.034)	0.393 (0.092)
	22	29.9	2.4 (0.2)	81.3	0.284 (0.050)	0.194 (0.038)	0.263 (0.092)
	23	29.4	2.5 (0.2)	87.5	0.275 (0.057)	0.168 (0.042)	0.270 (0.097)
	24	24.6	2.6 (0.3)	81.3	0.313 (0.060)	0.168 (0.041)	0.383 (0.099)
	25	31.6	2.4 (0.2)	81.3	0.286 (0.057)	0.163 (0.039)	0.414 (0.078)
Mean		30.9	2.5 (0.1)	85.0	0.287 (0.007)	0.166 (0.009)	0.345 (0.032)
Taxon		154.4	3.3 (0.2)	100.0	0.311 (0.054)	0.164 (0.030)	0.360 (0.072)
'narrow phyllode'	9	29.7	2.6 (0.4)	75.0	0.279 (0.059)	0.133 (0.036)	0.486 (0.076)
	10	30.7	2.4 (0.3)	75.0	0.330 (0.060)	0.154 (0.037)	0.499 (0.094)
	11	29.9	2.6 (0.2)	87.5	0.263 (0.049)	0.149 (0.046)	0.397 (0.092)
Mean		30.1	2.5 (0.1)	79.2	0.291 (0.020)	0.145 (0.006)	0.461 (0.032)
Taxon		90.3	3.1 (0.4)	87.5	0.319 (0.057)	0.146 (0.035)	0.495 (0.077)
A. oldfieldii	28	34.6	1.8 (0.2)	50.0	0.160 (0.055)	0.075 (0.027)	0.365 (0.146)
	29	31.0	2.1 (0.3)	68.8	0.175 (0.052)	0.081 (0.024)	0.299 (0.132)
Mean		32.8	2.0 (0.2)	59.4	0.168 (0.006)	0.078 (0.003)	0.332 (0.033)
Taxon		65.6	2.3 (0.3)	75.0	0.173 (0.054)	0.078 (0.024)	0.362 (0.118)
'small seed'	12	30.0	2.3 (0.2)	81.3	0.275 (0.057)	0.194 (0.054)	0.183 (0.104)
	13	29.9	2.2 (0.3)	62.5	0.214 (0.066)	0.117 (0.038)	0.257 (0.127)
Mean		30.0	2.3 (0.1)	71.9	0.245 (0.031)	0.155 (0.010)	0.220 (0.037)
Taxon		59.9	2.4 (0.3)	81.3	0.252 (0.060)	0.156 (0.044)	0.360 (0.093)
Geraldton variants							
'variant 1'	14	38.4	2.4 (0.3)	81.3	0.278 (0.056)	0.150 (0.035)	0.304 (0.084)
	15	28.8	2.8 (0.3)	87.5	0.320 (0.051)	0.223 (0.045)	0.286 (0.084)
	16	44.6	2.8 (0.3)	81.3	0.246 (0.054)	0.140 (0.029)	0.304 (0.079)
	17	27.9	2.3 (0.2)	75.0	0.215 (0.050)	0.142 (0.048)	0.291 (0.114)
Mean		34.9	2.6 (0.1)	81.3	0.265 (0.023)	0.164 (0.020)	0.296 (0.005)
'variant 2'	18	40.8	2.4 (0.3)	68.8	0.261 (0.060)	0.151 (0.041)	0.377 (0.087)
	19	51.8	2.3 (0.3)	75.0	0.253 (0.050)	0.113 (0.028)	0.434 (0.087)
Mean		46.3	2.4 (0.1)	71.9	0.257 (0.004)	0.132 (0.019)	0.406 (0.029)
'variant 1/2'	20	46.8	2.3 (0.2)	81.3	0.263 (0.055)	0.111 (0.029)	0.465 (0.099)

N, sample size per locus; A_P, average number of alleles per polymorphic locus; *P*, % polymorphic loci per population; H_o, observed heterozygosity; H_e, expected panmictic heterozygosity; F_{IS}, fixation index; standard errors in parentheses.

extremely high in 'narrow phyllode' (0.495) and at similar levels in all other taxa (0.360–0.388).

The taxa also exhibited differences in genetic diversity indices (Table 2). Total genetic diversity was lowest in *A. oldfieldii* (H_T, 0.178), highest in 'narrow phyllode' (0.332) and ssp. *burkittii* (0.318) with ssp. *acuminata* and 'small seed' being mid-range (0.266 and 0.259, respectively).

Table 2 Gene diversity statistics over all loci unbiased for sample size and population number for *Acacia acuminata* taxa

Taxon	H_T	H_S	D_{ST}	G_{ST}
ssp. *acuminata*	0.266	0.237	0.029	0.108
ssp. *burkittii*	0.318	0.287	0.031	0.098
'narrow phyllode'	0.332	0.290	0.042	0.125
A. *oldfieldii*	0.178	0.166	0.012	0.069
'small seed'	0.259	0.245	0.014	0.053
Geraldton variants	0.299	0.261	0.038	0.127

H_T, total genetic diversity; H_S, mean gene diversity within populations; D_{ST}, mean diversity between populations; G_{ST}, proportion of interpopulation differentiation.

Differences within and among the Geraldton variants

The Geraldton variant populations exhibited a range of single locus diversity measures both within and among the different variants. The mean A_P ranged from 2.3 in the 'variant 1/2' population to 2.6 in 'variant 1'. The mean *P* was 81.3% in 'variant 1' and 'variant 1/2' and 71.9% in 'variant 2' while H_e was similar in 'variant 1' and 'variant 1/2' (0.265 and 0.263, respectively) and marginally lower in 'variant 2' (0.257). The mean H_o was highest in 'variant 1' (0.164) and lowest in 'variant 1/2' (0.111). Fixation indices were highest in 'variant 1/2' (F_{IS}, 0.465), lower in 'variant 2' (0.406) and considerably lower again in 'variant 1' (0.296).

Genetic divergence among populations and phylogenetic relationships

All taxa strongly partitioned total genetic diversity within populations (H_S) rather than among (D_{ST}). Consequently,

the levels of interpopulational differentiation in the taxa (G_{ST}) were relatively low (Table 2). The proportion of interpopulation differentiation is highest in the three most widespread taxa, ssp. *acuminata* (G_{ST}, 10.8%), ssp. *burkittii* (G_{ST}, 9.8%) and 'narrow phyllode' (G_{ST}, 12.5%), and lowest in the two geographically restricted taxa, *A. oldfieldii* (G_{ST}, 6.9%) and 'small seed' (G_{ST}, 5.3%).

Excluding the Geraldton variants, populations clustered according to systematic boundaries (Figure 2). *Acacia oldfieldii* was clearly a discrete taxon, significantly distant from the remaining taxa (*D*, 0.85). The 'small seed' populations also grouped together as a significant cluster. While the remaining populations were not strongly differentiated, these did group within respective taxon boundaries. All ssp. *burkittii* populations were clustered together but as two discrete groups. Populations of ssp. *acuminata* were clustered together with one population of 'narrow phyllode' (10) while the remaining populations of this latter taxon (9 and 11) clustered separately.

Including the Geraldton variants in the UPGMA analysis confused some of the taxonomic alliances (Figure 3). *Acacia oldfieldii* was again significantly distant to all other taxa while 'small seed' and the 'narrow phyllode' populations 9 and 11 again remained as discrete groups. However, the ssp. *burkittii* populations 24 and 25 were separated from the remaining three populations and allied with a 'narrow phyllode' population (10) previously included within ssp. *acuminata*, and two 'variant 1' populations (15 and 17). The other ssp. *burkittii* populations were associated with another 'variant 1' population (16). The ssp. *acuminata* population 3 was removed from all other ssp. *acuminata* populations which were associated with the remaining Geraldton variant populations.

Discussion

Apart from *A. oldfieldii*, levels of genetic diversity observed within both the taxa and Geraldton variants of the *A. acuminata* complex were considerably higher than that expected of long-lived woody angiosperms (H_e 0.183, s.e. 0.011; Hamrick *et al*, 1992). Most taxa and variants exhibited levels of gene diversity consistent with that expected of widespread species (H_e 0.257, s.e. 0.039; Hamrick *et al*, 1992) with two exceptions. Gene diversity in ssp. *burkittii* and 'narrow phyllode' were at considerably higher levels than that observed in other similarly distributed species (H_e 0.311 and 0.319, respectively).

As expected of a rare and highly restricted species, gene diversity in *A. oldfieldii* was considerably lower (H_e 0.173) than that observed in any of its closely related relatives. These levels of diversity were still more than twice that exhibited by other endemic taxa on a worldwide basis (H_e 0.078, s.e. 0.016) and were more similar to narrowly distributed or regional species (Hamrick *et al*, 1992). While *A. oldfieldii* does not conform with the average expected for endemic taxa, recent studies have shown that some rare and geographically restricted species not only have higher than expected levels of genetic variation, but also have levels comparable to closely related widespread common congeners (Young and Brown, 1996; Gitzendanner and Soltis, 2000).

Examples of higher than expected genetic diversity have been observed in other restricted species of *Acacia* within Western Australia. Genetic diversity values in the rare *A. sciophanes* were higher than those for both ssp. *acuminata* and ssp. *burkittii* but significantly lower than estimates for its closely related widespread congener *A. anfractuosa* (Tischler, 1998). The sexually reproducing populations of the *A. anomala* exhibit similar *A* and *P* values (2.0 and 61.1%, respectively) to *A. oldfieldii* and a marginally higher gene diversity (H_e 0.209; Coates, 1988) while indices for the single large population of the highly localised *Acacia* sp. 'Dandaragan' are somewhat lower but still higher than expected (*A*, 1.55; *P*, 55%; H_e, 0.109) (Elliott *et al*, 2002). These studies indicate that rare and geographically restricted *Acacia* species can exhibit relatively high levels of genetic diversity although they are

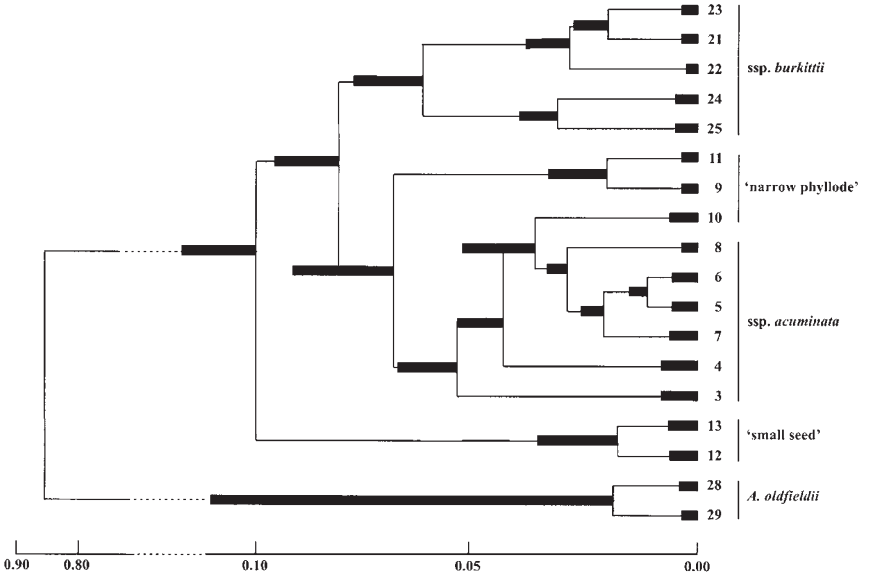

Figure 2 Phylogenetic relationships between populations within the *A. acuminata* complex excluding Geraldton variants. Branches are significant if error bar is less than half the branch length (Ritland, 1989).

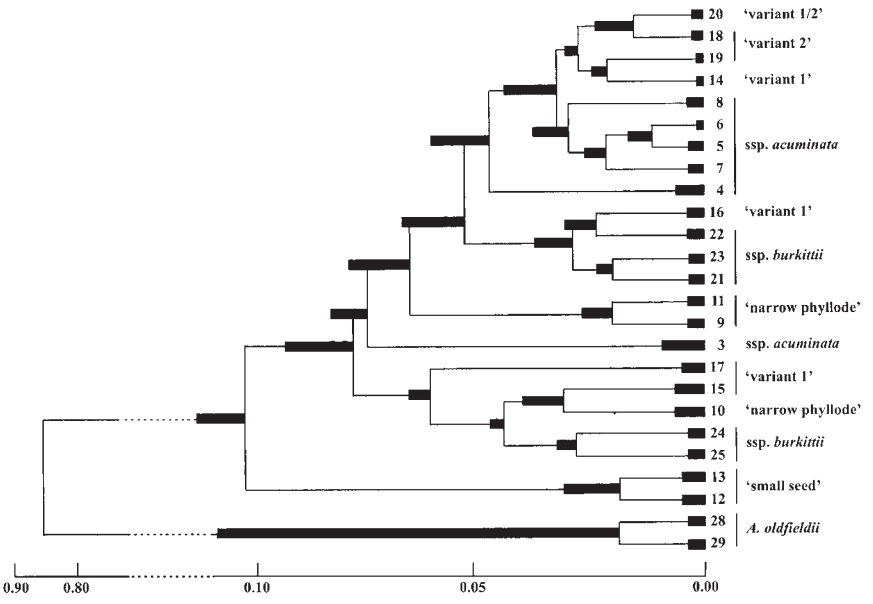

Figure 3 Phylogenetic relationships between populations within the *A. acuminata* complex including Geraldton variants. Branches are significant if error bar is less than half the branch length (Ritland, 1989).

generally lower than their widespread and common close relatives.

Of the three widespread taxa within the *A. acuminata* complex, allelic richness and gene diversity were significantly higher in ssp. *burkittii* and 'narrow phyllode' than in ssp. *acuminata*. *Acacia acuminata* ssp. *burkittii* is very widely distributed across a harsh and arid environment of inland Australia. Populations residing in challenging environments can exhibit higher genetic diversity (Brown and Schoen, 1992) and the levels of genetic variability observed in this species may serve to buffer against various environmental stresses experienced across the large geographic range. Lower levels of genetic diversity within ssp. *acuminata* may reflect its distribution in a more ecologically favourable environment where the climate is generally more mesic.

The 'narrow phyllode' taxon is geographically distributed between ssp. *acuminata* and ssp. *burkittii*. It is considered to be most closely related to ssp. *acuminata* and both taxa can difficult to distinguish when their geographic ranges overlap (Maslin *et al*, 1999). This taxon also has morphological affinities with ssp. *burkittii* but can be readily differentiated by fruit structure (Maslin *et al*, 1999). In this study ssp. *acuminata* and 'narrow phyllode' were associated together rather than with ssp. *burkittii* suggesting that these are more closely related (Figure 2). It is possible, given its geographic distribution, that 'narrow phyllode' may be the result of extensive and ongoing hybridisation between ssp. *acuminata* and ssp. *burkittii*. Hybridisation events can create new species and/or increase genetic variability (Mayr, 1970; Richards, 1997). Although levels of population differentiation within the *A. acuminata* complex are relatively low, 'narrow phyllode' exhibits one of the highest levels of population differentiation (G_{ST}, 12.5%) within the complex. Extensive and ongoing hybridisation over large temporal and geographical scales might readily produce hybrid populations with a diverse array of genetic products. Such circumstances could result in higher than usual levels of population differentiation.

The similarity of diversity levels between the 'small seed' taxon and ssp. *acuminata* would suggest that although this taxon appears to have a limited distribution (Figure 1), it was/is as widespread as ssp. *acuminata*. To date, relatively few collections of 'small seed' exist, but it might also be distributed along the south coast of Western Australia (Maslin *et al*, 1999). Unfortunately much of the land between the study sites and the south coast is now extensively cleared and it may be impossible to determine the true range of this taxon.

Within the Geraldton variants there is no clear relationship between genetic variation and geographical distribution. All these variants have narrow geographic ranges but exhibit mean diversity indices which are higher than the restricted *A. oldfieldii* and in many instances are similar to or slightly higher than levels observed in the widespread taxa. Given that phylogenetic relationships within the complex are confused when the Geraldton variants are included in the analysis (Figure 3) and their morphological delimitation is unclear, some other explanation may be more appropriate.

Taxonomically, the Geraldton variants exhibit combinations of morphological attributes associated which each of the three widespread taxa (Maslin *et al*, 1999). As such these may simply represent the extremes of variation found on the edge of the species' ranges (Maslin *et al*, 1999). The diversity indices do suggest that the Geraldton variants are not, or at least do not behave as, restricted taxa and are consistent with them being range extensions. An alternative possibility is, however, that like 'narrow phyllode', these variants are also of hybrid origin. Indeed, the Geraldton variants exhibit a similar degree of population differentiation (G_{ST}, 12.7%) to that of the 'narrow phyllode' taxon. The 'variant 1' may represent hybridisation between 'narrow phyllode' and ssp. *burkittii*, 'variant 2' may represent meetings of ssp. *acumi-*

nata and ssp. *burkittii* while the origins of 'variant 1/2' remain unclear (Maslin *et al*, 1999). The apparent breakdown of taxonomic boundaries when these variants are included in the analysis does support the notion that these have hybrid origins.

The manner in which the Geraldton variants are distributed when all populations are included in the analysis further suggests that not only are these hybrid derivatives, but that these may actually represent a series of hybridisation events. Spatially and temporally separated meetings between the various taxa might be expected to produce hybrid derivatives with a diverse array of both genetic and morphological types and which present affinities with either or both parental taxa. Indeed, repeated crossing between hybrids and one parental type can produce a population resembling the recurrent parent, but varying in the direction of the other (Grant, 1981).

The Geraldton variants, 'narrow phyllode' and 'small seed' are distributed in a region known as the Transitional Rainfall Zone which is well known for high levels of speciation, endemism and allopatric replacement of congeneric species (Hopper, 1979). Unfortunately few genetic studies of flora within this region are known although a study of two subspecies of *Geleznowia verrucosa* (Rutaceae) has suggested that a series of ancient hybrid derivatives also exist within the same area as the purported Geraldton hybrids (Broadhurst *et al*, 1999). In addition, a chloroplast study of the *A. acuminata* complex has indicated that the region where the Geraldton variants occur is an evolutionary hotspot for this complex (Byrne *et al*, in review).

Unlike studies on a number of other species in the south-west Australian flora (Coates, 2000) there is no clear geographical population genetic structure in either the geographically restricted or widespread taxa in the *A. acuminata* complex. However, the widespread taxa, ssp. *acuminata*, ssp. *burkittii* and 'narrow phyllode' have moderately higher levels of genetic differentiation among populations than the geographically restricted *A. oldfieldii* and 'small seed'. The lack of population genetic structure tends to confirm field observations that taxa in the complex generally occur as large relatively continuous population systems throughout their range, and that the moderately higher levels of among population differentiation in the more geographically widespread taxa are the result of reduced gene flow across their broader geographic range.

Although mating system studies were not carried out on the taxa in the *A. acuminata* complex, studies on other *Acacia* species indicate very high rates of outcrossing (Moran *et al*, 1989a; McGranahan *et al*, 1997). Large population size typical of taxa within this complex combined with high outcrossing rates may provide one explanation for the high levels of genetic diversity observed. However, average fixation index for all populations within taxa are positive and in many cases significantly greater than zero suggesting significant levels of inbreeding within populations. The fixation estimates for this study are based on seed progeny not maternal parents and while high levels of inbreeding may occur within populations, selection against inbred individuals may ensure these do not reach reproductive maturity. As shown in other *Acacia* species (McGranahan *et al*, 1997; Tischler, 1998) and in mating system studies on a number of other plant species there can be preferential survival of heterozygotes as plants mature from seedling to adult (Hamrick and Schnabel, 1985).

Apart from *A. oldfieldii* the relatively low level of phylogenetic separation between taxa in the complex indicates quite recent evolutionary divergence. The high degree of genetic differentiation between *A. oldfieldii* and the other *A. acuminata* taxa (D, 0.85) was somewhat unexpected given the clear morphological affinities that this species has with those taxa. Indeed, the major morphological features distinguishing *A. oldfieldii* are glabrous phyllodes rather than those with marginal hairs, and short pedunculate flower spikes (Maslin *et al*, 1999). This high degree of genetic differentiation was also reflected in the chloroplast study which placed the divergence of *A. oldfieldii* at approximately 2.5–3 My ago (M Byrne, unpublished).

The levels of genetic diversity within the *A. acuminata* complex taxa are generally much higher than expected based on geographical distribution and when compared with other woody plant species with similar life histories. Other south-west Australian *Acacia* species also show comparable levels of genetic diversity (Tischler, 1998; Elliott *et al*, 2002). These levels are noticeably higher than that observed in *Acacia* species from Eastern Australia (Moran *et al*, 1989a,b; Playford *et al*, 1993; Searle *et al*, 2000). A feature of the flora in the south-west region of Australia is its antiquity associated with prolonged geological stability and no obvious extinction episodes caused by recent glaciation. Taxa in the *A. acuminata* complex have probably persisted in relatively large continuous populations for extended periods of time. This may provide one explanation for the high levels of genetic diversity observed. In addition, hybridisation would appear to be an important component of recent evolution and may also be a contributing factor to the high levels of genetic diversity in some taxa. Hybridisation may also explain the unresolved patterns of differentiation evident in some parts of the geographical range of the complex.

References

Barrett SCH, Kohn JR (1991). Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk DA, Holsinger KE (eds) *Genetics and Conservation of Rare Plants*, Oxford University Press: New York. pp 3–30.

Broadhurst L, Coates D, Tan B. (1999). Genetic diversity in the monotypic Western Australian endemic, *Geleznowia verrucosa* Turcz. (Rutaceae). *Heredity* **82**: 292–299.

Brown AHD, Schoen DJ (1992). Plant population genetic structure and biological conservation. In: Sanderlund OT, Hindar K, Brown AHD (eds) *Conservation of Biodiversity for Sustainable Development* Scandinavian University Press: Oslo. pp 88–104.

Coates DJ (1988). Genetic diversity and population genetic structure in the rare Chittering Grass Wattle, *Acacia anomala* Court. *Aust J Bot* **36**: 273–286.

Coates DJ (1992). Genetic consequences of a bottleneck and spatial genetic structure in the triggerplant *Stylidium coroniforme* (Stylidiaceae). *Heredity* **69**: 512–520.

Coates DJ (2000). Defining conservation units in a rich and fragmented flora: implications for the management of genetic resources and evolutionary processes in south-west Australia. *Aust J Bot* **48**: 329–339.

Elliott CP, Yates CJ, Ladd PG, Coates DJ (2002). Morphometric, genetic and population studies used to clarify the conservation status of a rare *Acacia*, in Western Australia. *Aust J Bot* **50**.

Ellstrand NC, Elam DR (1993). Population genetic consequences of small population size: implications for plant conservation. *Ann Rev Ecol Syst* **24**: 217–242.

Gitzendanner MA, Soltis PS (2000). Patterns of genetic variation in rare and widespread plant congeners. *Am J Bot* **87**: 783–792.

Godt MJW, Hamrick JL (1999). Genetic divergence among intraspecific taxa of *Sarracenia purpurea*. *Syst Bot* **23**: 427–438.

Grant V (1981). *Plant Speciation*. Columbia University Press: New York.

Hamrick JL, Godt MJW (1989). Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) *Plant Population Genetics, Breeding, and Genetic Resources*, Sinauer Associates: Sunderland. pp 43–63.

Hamrick JL, Godt MJ, Sherman-Broyles SL (1992). Factors influencing levels of genetic diversity in woody plant species. *New Forests* **6**: 95–124.

Hamrick JL, Schnabel A (1985). Understanding the genetic structure of plant populations: some old problems and a new approach. In: Gregorius H-R (ed) *Population Genetics in Forestry* Springer-Verlag: Berlin. pp 50–70.

Hopper SD (1979). Biogeographic aspects of speciation in the southwestern Australian flora. *Ann Rev Ecol Syst*, **10**, 399–422.

Hopper SD (1992). Patterns of plant diversity at the population and species levels in south-west Australian mediterranean ecosystems. In: Hobbs RJ (ed) *Biodiversity of Mediterranean Ecosystems in Australia*, Surrey Beatty & Sons: Sydney. pp 27–46.

Hopper SD, Harvey MS, Chappill JA, Main AR, York Main B (1996). The Western Australian biota as Gondwanan Heritage – a review. In: Hopper SD, Chappill JA, Harvey MS, George AS (eds) *Gondwanan Heritage: Past, Present and Future of the Western Australian Biota*, Surrey Beatty & Sons: Sydney. pp 1–16.

Karron JD (1987). A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. *Evol Ecol* **1**: 47–58.

Karron JD, Linhart YB, Chaulk CA, Robertson CA (1988). Genetic structure of populations of geographically restricted and widespread species of *Astragalus* (Fabaceae). *Am J Bot* **75**: 1114–1119.

Maslin BR (2001). *Flora of Australia Volume 11B*, Mimosaceae, *Acacia* Part 2. ABRS/CSIRO Publishing: Melbourne.

Maslin B, Byrne M, Coates D, Broadhurst L, Macdonald B (1999). The *Acacia acuminata* (Jam) group: an analysis of variation to aid Sandalwood (*Santalum spicatum*) plantation research. Department of Conservation and Land Management: Perth Western Australia.

Mayr E (1970). *Populations, Species, and Evolution.* The Belknap Press: Cambridge MA.

McGranahan M, Bell JC, Moran GF, Slee M (1997). High genetic divergence between geographic regions in the highly outcrossing *Acacia aulacocarpa* (Cunn. ex Benth.). *Forest Genetics*, **4**: 1–13.

Moran GF, Hopper SD (1983). Genetic diversity and the insular population structure of the rare granite rock species, *Eucalyptus caesia* Benth. *Aust J Bot* **31**: 161–172.

Moran GF, Hopper SD (1987). Conservation of genetic resources of rare and widespread eucalypts in remnant vegetation. In: Saunders DA, Arnold GW, Burbidge AA, Hopkins AJM (eds) *Nature Conservation: The Role of Remnants of Native Vegetation*, Surrey Beatty & Sons: Chipping North. pp 151–162.

Moran GF, Muona O, Bell JC (1989a). Breeding systems and genetic diversity in *Acacia auriculiformis* and *A. crassicarpa*. *Biotropica* **21**: 250–256.

Moran GF, Muona O, Bell JC (1989b). *Acacia magnum*: a tropical tree with low genetic diversity. *Evolution* **43**: 231–235.

Playford J, Bell JC, Moran GF (1993). A major disjunction in genetic diversity over the geographic range of *Acacia melanoxylon* R.Br. *Aust J Bot* **41**: 355–368.

Richards AJ (1997). *Plant Breeding Systems.* Chapman & Hall: London.

Richardson BJ, Baverstock PR, Adams M. (1986). *Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Studies*. Academic Press: Sydney.

Ritland K (1989). Genetic differentiation, diversity and inbreeding in the mountain monkeyflower (*Mimulus caespitosus*) of the Washington Cascades. *Can J Bot* **67**: 2017–2024.

Searle SD, Bell JC, Moran GF (2000). Genetic diversity in natural populations of *Acacia mearnsii*. *Aust J Bot*, **48**: 279–286.

Tischler G (1998). *Comparison of the patterns of genetic diversity of the rare* Acacia sciophanes *and the wide spread* Acacia anfractuosa. Hons. Thesis, Murdoch University, Perth, Western Australia.

Weir BS (1990). *Genetic Data Analysis*. Sinauer Associates: Sunderland, MA.

Whitkus R (1985). FORTRAN program for computing genetic statistics from allelic frequency data. *J Hered* **76**: 142.

Wright S (1978). *Variability within and among natural populations*. The University of Chicago Press: Chicago.

Yeh FC, Boyle T (1997). POPGENE version 1.21, *Microsoft windows-based freeware for population genetic analysis*. University of Alberta: Edmonton.

Young AG, Brown AHD (1996). Comparative population genetic structure of the rare woodland shrub *Daviesia suaveolens* and its common congener *D. mimosoides*. *Cons Biol* **10**: 1220–1228.