

## GENETIC STRUCTURE AND EVOLUTION OF SPECIES IN THE MANGROVE GENUS *AVICENNIA* (AVICENNIACEAE) IN THE INDO-WEST PACIFIC

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**Abstract.** -- Allozyme variation in species of the mangrove genus *Avicennia* was screened in 25 populations collected from 22 locations in the Indo-West Pacific and eastern North America using 11 loci. Several fixed gene differences supported the specific status of *Avicennia alba*, *A. integra*, *A. marina*, and *A. rumphiana* from the Indo-West Pacific, and *A. germinans* from the Atlantic-East Pacific. The three varieties of *A. marina*, var. *marina*, var. *eucalyptifolia*, and var. *australasica*, had higher genetic similarities (Nei's *D*) and no fixed gene differences, confirming their conspecific status. Strong genetic structuring was observed in *A. marina*, with sharp changes in gene frequencies at the geographical margins of varietal distributions. The occurrence of alleles found otherwise in only one variety, in only immediately adjacent populations of another variety, provided evidence of introgression between varieties. The varieties appear to have diverged recently in the Pleistocene and are apparently not of ancient Cretaceous origin, as suggested earlier. Despite evidence of high degrees of outcrossing, gene flow among populations was relatively low ( $N_{em} < 1-2$ ), except where populations were geographically continuous, questioning assumptions that these widespread mangrove species achieve high levels of long-distance dispersal.

**Key words.**—Allozyme, *Avicennia*, biogeography, diversity, genetics, mangrove, phylogeny.

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*Avicennia* is a genus of trees which grows in the intertidal zone of coastal mangrove forests and ranges widely throughout tropical and warm temperate regions of the world (Tomlinson 1986; Duke 1991). The genus includes at least eight species, three of which occur in the Atlantic-East Pacific, and five in the Indo-West Pacific (Duke 1992). One species, *A. marina* (Forsk.) Vierh. sensu lato, has the distinction of being the most widely distributed of all mangrove tree species. It is found in the Indo-West Pacific and ranges in latitude from around 25° N to 38° S. The structure of *Avicennia* stands vary considerably from gnarled shrubbery on dry coastlines and coral atolls to closed riparian-estuarine forests up to 40 m tall within larger estuarine systems of wet coastal tropical regions.

The ability of *Avicennia* to grow and reproduce across a broad range of climatic, saline, and tidal conditions and to produce large numbers of buoyant propagules annually may explain its ubiquitous presence in mangrove habitats around the world. The widespread occurrence of *Avicennia* and its large propagule production suggest these mangroves disperse widely and are genetically uniform throughout their range. However, field experiments suggest otherwise, with propagule dispersal apparently limited by surface currents, the presence of suitable environmental conditions, and longevity of propagules during dispersal (e.g., Clarke 1992). Recent genetic studies have shown that a number of widespread shallow-water, marine animals also may not achieve great dispersal distances, because they are more genetically structured than had been assumed in the past (Knowlton and Jackson 1994; Palumbi 1994; Benzie and Williams 1995, 1997).

A recent systematic revision of *Avicennia* (Duke 1991), which was based on morphological studies in Australasia (Duke 1990a), confirmed the status of five Indo-West Pacific

species, although there was still uncertainty concerning the nature of groupings within *A. marina* complex (*A. marina* sensu lato). The uncertainty of species boundary definitions stem from the wide variation in morphological characters of *Avicennia*, which are influenced by ecological and environmental factors (Soto and Coralles 1987; Soto 1988; Duke 1990a). Three intraspecific groupings in *A. marina* have recently been attributed either varietal (Duke 1991) or subspecific (Everett 1993) status, but in the past *A. marina* sensu lato was broken up into as many as eight species. There have been serious doubts whether groupings based on morphological attributes represent cryptic species, intraspecific forms, or simply variants influenced by environmental factors.

Concerns about the reliability of morphological data for determining species boundaries are further illustrated in a study of two cryptic species in the mangrove tree *Ceriops tagal* (Perr.) C. B. Robinson (Ballment et al. 1989). Morphological attributes alone were insufficient to distinguish the two taxa (Tomlinson 1986 and references therein). However, allozyme phenotypes were distinct between taxa and there was no hybridization between sympatric individuals, thus demonstrating their reproductive isolation. Allozyme phenotypes also have been described in several mangrove genera including *Avicennia* (McMillan 1986; Baba et al. 1989), *Bruguiera* (Baba et al. 1989), *Kandelia* (Baba et al. 1989), *Rhizophora* (Baba et al. 1989; Goodall and Stoddart 1989), and *Sonneratia* (Baba et al. 1989). In none of these studies, however, were genetic models identified that might provide more powerful means of interpreting the nature and extent of gene flow among populations and the breeding systems of mangrove species.

The lack of genetic markers for which reliable interpretations exist has prevented a deeper understanding of population genetics and evolution of mangrove plants; such plants are the key structural components of these significant coastal habitats worldwide. Fundamental questions as to

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whether the broad distributional ranges and homogeneity of some species are maintained by high levels of present-day dispersal remain unresolved in the face of experimental data and observations suggesting more limited dispersal by propagules and evidence of regionally differentiated varieties with sharply delineated ranges. Given the extent to which many characters used in the past to define species appear to be under strong environmental controls, the identification of genetically differentiated units will depend largely on the results of molecular genetic analyses. *Avicennia marina* provides a particularly good model with which to investigate these issues. It is one of the most widespread of mangrove species, and it has apparent intraspecific forms over which there has been debate as to their true nature (either species or intraspecific units) and their origin (either the result of historical, biogeographic events, or the result of environmental influences).

Here we report results from the first genetic survey of a widespread and abundant mangrove group using allozymes for which genetic interpretations were possible. This study was aimed at: (1) clarifying taxonomic boundaries within *A. marina* and describing the phylogenetic relationships between these taxa and representative species of Indo-West Pacific and Atlantic-East Pacific *Avicennia*; and (2) inferring patterns of gene flow in *A. marina*. These findings are used to infer dispersal mechanisms and evolution of *Avicennia* and to add to the few available data pertinent to our understanding of the nature and dispersal range of shallow-water, marine species.

## MATERIALS AND METHODS

### *Specimen Collection*

Five species of *Avicennia* were sampled in 2.5 populations from 22 locations (Table 1). *Avicennia marina* sensu lato was sampled from 12 sites around Australia, plus one each from Lord Howe Island, New Zealand, New Caledonia, Malaysia, Thailand, and South Africa. This species was divided into three intraspecific taxa by Duke (1991) as var. *australasica* (Walp.) Moldenke ex N. C. Duke, var. *eucalyptifolia* (Val.) N. C. Duke, and var. *marina* (note that authority names for varieties given here are revised from those presented earlier by Duke [1991]). *Avicennia alba* Bl. was sampled at two sites in southeast Asia, *A. germinans* (L.) L. from two sites in Florida, and *A. integra* N. C. Duke from two sites in the Northern Territory, Australia. *Avicennia rumphianu* Hallier f. was sampled in Singapore. Species in sympatry were sampled in three locations: *A. alba* and *A. marina* in Malaysia; *A. alba* and *A. rumphiana* in Singapore; and *A. marina* and *A. integra* in the Northern Territory of Australia. In total, 328 plants were assayed, 286 of which were *A. marina*.

Fresh plant material was obtained mainly from cotyledons of the viviparous propagules, which are approximately 30 mm in diameter and 3.5 g fresh weight. These remain fresh and viable for up to 10 days when wrapped in paper, thus enabling collections to be obtained without refrigeration and simplifying sample collection from remote locations. Field sampling strategy involved collection of up to 10 progeny from each of five to 20 different trees at each site, if practicable. On several occasions sample numbers were reduced

also when propagules were lost in transit. Dates of collection varied throughout the year and followed the phenological model for *A. marina* (Duke 1990b). For *A. integra* from South Alligator River, no propagules were obtained, so leaves were sampled from transplanted seedlings grown in a tidal plant-house at the Australian Institute of Marine Science (AIMS, Townsville; 19°17'S, 147°02'E).

Propagule parts (cotyledon, plumule, and root tip) and leaves were dissected and stored at - 80°C. Following initial trials, plumules and root tips were discarded in favor of cotyledons (where available) and occasionally leaves, because these showed greater enzyme activity. When some samples, particularly leaves, were found to lose activity on refreezing, loss of activity was kept to a minimum by subsampling portions of frozen, stored tissue.

### *Analytical Technique*

Enzyme extraction and electrophoretic techniques followed Goodall and Stoddart (1989) with some modifications. Sample preparation was particularly critical in preserving activity in *Avicennia*, and extraction buffer was improved by adding Bovine Serum Albumin (50 mg) and sodium metabisulphite (190 mg) to 50 ml of the working solution (see Goodall and Stoddart 1989). Small portions of cotyledon (4 X 4 mm), or leaf (15 X 15 mm) material, were transferred to chilled depression plates where each well had been previously loaded with two drops of chilled extraction buffer and small amounts of fine-ground glass. After grinding the material to a paste, at least two further drops of chilled extraction buffer were added to the homogenate in each well.

Five enzyme systems were scored reliably for all populations and samples: aconitase (*ACO*, EC 4.2.1.3), diaphorase (*DIA*, EC 1.6.4.3) malate dehydrogenase (*MDH*, EC 1.1.1.37), phosphoglucosmutase (*PGM*, EC 2.7.5.1), and 6-phosphogluconate dehydrogenase (*PGD*, EC 1.1.1.44). Three other systems were scored in a few populations: aspartate amino transferase (*AAT*, EC 2.6.1.1), leucine amino peptidase (*LAP*, EC 3.4.1.1), and peroxidase (*PRX*, EC 1.11.1.7). Samples were run on 13% (w/v) starch gels using two buffer systems: Tris EDTA citrate pH 7.9 (TEC7.9) for *ACO*, *AAT*, *LAP*, and *PGD*; and histidine citrate pH 6.5 (HC6.5) for *DIA*, *MDH*, *PRX*, and *PGM*. Electrophoresis was carried out for 5 h at 230 V for TEC7.9 and at 425 V for HC6.5. Gel and electrode buffer recipes and enzyme staining procedures chiefly followed Soltis et al. (1983) with reference to Tanksley and Orton (1983) Richardson et al. (1986) and Shaklee and Keenan (1986). Invariant bands and banding zones were scored as monomorphic. Banding zones with variation consistent with Mendelian genetic patterns were classified into loci that were numbered within each system, starting with those migrating most anodally. Alleles at a locus were labelled according to their anodal migration distance from the origin and relative to the most common allele that was arbitrarily assigned a value of 100.

### *Data Analysis*

Gene frequencies and population genetic statistics were calculated using the BIOSYS-1 program (Swofford and Selander 1981). Cluster analysis of population data used the

TABLE 1. Species and varieties of *Avicennia* from the 25 populations in 22 locations sampled in North America, eastern Africa, Asia, and Australasia. Locations and site codes are repeated for populations of different species growing in sympatry. NT, Northern Territory; W.A., Western Australia; Qld., Queensland; N.S.W., New South Wales; Vic., Victoria; S.A., South Australia.

Species	Location	Site code	Country	Latitude	Longitude
<i>A. integrata</i>	Darwin, N.T.	DAR	Australia	12°23'S	130°51'E
	South Alligator River, N.T.	SAG	Australia	12°10'S	132°30'E
<i>A. alba</i>	Pinang, Malay Peninsula	MAL	Malaysia	5°25'N	100°20'E
	Singapore	SNG	Singapore	1°19'N	103°49'E
<i>A. rumphiana</i>	Singapore	SNG	Singapore	1°19'N	103°49'E
<i>A. germinans</i>	Coot Bay Pond, Florida	COT	U.S.A.	25°11'N	80°54'W
	Shark Point, Florida	SHK	U.S.A.	25°23'N	81°08'W
<i>A. marina</i> var. <i>marina</i>	Mgeni River	SAF	South Africa	29°53'S	31°00'E
	Pinang, Malay Peninsula	MAL	Malaysia	5°25'N	100°20'E
	Phuket	THI	Thailand	7°53'N	98°24'E
	Bunbury, W.A.	BUN	Australia	33°19'S	115°39'E
<i>A. marina</i> var. <i>eucalyptifolia</i>	Karratha, W.A.	KAR	Australia	20°44'S	116°51'E
	Darwin, N.T.	DAR	Australia	12°23'S	130°51'E
	Low Isle, Qld.	LOW	Australia	16°23'S	145°34'E
	Mowbray River, Qld.	MOW	Australia	16°33'S	145°29'E
	Bowling Green Bay, Qld.	CFE	Australia	19°17'S	147°02'E
<i>A. marina</i> var. <i>australasica</i>	Yeppoon, Qld.	ROK	Australia	23°12'S	150°48'E
	Noumea	CAL	New Caledonia	22°16'S	166°27'E
	Shorncliffe, Qld.	SHO	Australia	27°20'S	153°05'E
	Cleveland, Qld.	CLE	Australia	27°31'S	153°17'E
	Lord Howe island, N.S.W.	HOW	Australia	31°28'S	159°09'E
	Botanv Bav. N.S.W.	SYD	Australia	34°00'S	151°09'E
	Westernport Bay, Vic.	MEL	Australia	38°20'S	145°15'E
	Whangarei, North Island	NZW	New Zealand	35°43'S	174°19'E
	Adelaide, S.A.	ADL	Australia	34°45'S	138°32'E

unbiased genetic identity coefficient (Nei 1978) and unweighted pair group method. Significance levels for tests of conformance of gene frequencies to those expected under conditions of Hardy-Weinberg agreement were corrected for multiple simultaneous comparisons following Miller (1966).

Breeding within populations was quantified using an inbreeding coefficient, or Wright's (1978) fixation index,  $F = 1 - (H_o/H_e)$ , where  $H_o$  is the observed frequency of heterozygotes and  $H_e$  is that expected from Hardy-Weinberg estimates; positive values of  $F$  indicated inbreeding, negative values were indicative of outbreeding, and zero values indicated random mating. The degree of outcrossing for individual trees in some populations was estimated using allelic frequencies from single-tree progeny (Harding and Tucker 1964), calculating the outcrossing index,  $t = H_o/p$ , where  $H_o$  is the frequency of observed heterozygotes in progeny from homozygous parents and  $p$  is the frequency of nonparental alleles in the population. Distinguishing homozygous parents from progeny in a biallelic locus population was definitive only in populations with four alleles at a single locus, notably for aconitase in *A. marina*. Pooled heterozygous progeny were equated to a combination of parental and nonparental alleles.

Results from genetic analyses of the total dataset including populations with known multiple progeny per tree were no different from those based on a reduced dataset where known multiple progeny were removed. Unless otherwise stated, the analyses reported here include data for only one propagule from a tree from which several propagules had been collected.

Genetic variance was partitioned using F-statistics into that occurring within populations ( $F_{IS}$ ) and that occurring among populations ( $F_{ST}$ ), taking account of differences in sample size (Weir and Cockerham 1984). Significance of  $F_{IS}$  and  $F_{ST}$  was assessed from  $\chi^2$  estimates derived from equations in

Waples (1987). Gene flow was estimated as the average number of migrants per generation among populations,  $N_e m = [(1/F_{ST}) - 1]/4$ . Observed genotypic diversity was calculated as  $G_o = 1/[\sum f_x(x/N)^2]$ , where  $f_x$  was the number of genotypes observed  $x$  times and  $N$  was the total sample size of each population (Stoddart and Taylor 1988). Genetic diversity expected under Hardy-Weinberg equilibrium was calculated from a simple binomial expansion of the frequencies of genotype classes. The expected number of genotypes, observed diversity ( $G_o$ ) and expected diversity ( $G_e$ ) were calculated following Stoddart and Taylor (1988). An index of genetic equilibrium,  $G_o/G_e$ , quantified deviations from both Hardy-Weinberg equilibrium and multilocus linkage equilibrium. Another measure of diversity,  $D$  (the number of unique genotypes within a population per  $N$ ), was also calculated. Values of  $D$  range between zero and one, with one indicating a population where all genotypes are unique.

Because low sample sizes in some populations (see Table 4) might bias  $F_{ST}$  and  $N_e m$  estimates and affect conclusions at the intraspecific level, the following strategy was used to identify and eliminate such bias. The population (CLE) with only three individuals was not used in estimates of genetic variance. All others were checked by comparing  $F_{ST}$  estimates from respective pairwise comparisons with sample sizes of the smallest population in each pair. No significant regressions were found for the total dataset, data grouped by distance classes, or data for each variety. Five of the 18 populations of *A. marina* studied had sample sizes of fewer than 10 individuals. Estimates of  $F_{ST}$  for each of three sample size classes were comparable: sample sizes of 0-9,  $n = 58$ ,  $F_{ST} = 0.362 \pm 0.189$  (mean  $\pm 1$  SD); sample sizes of 10-19,  $n = 57$ ,  $F_{ST} = 0.418 \pm 0.166$  (mean  $\pm 1$  SD); and sample

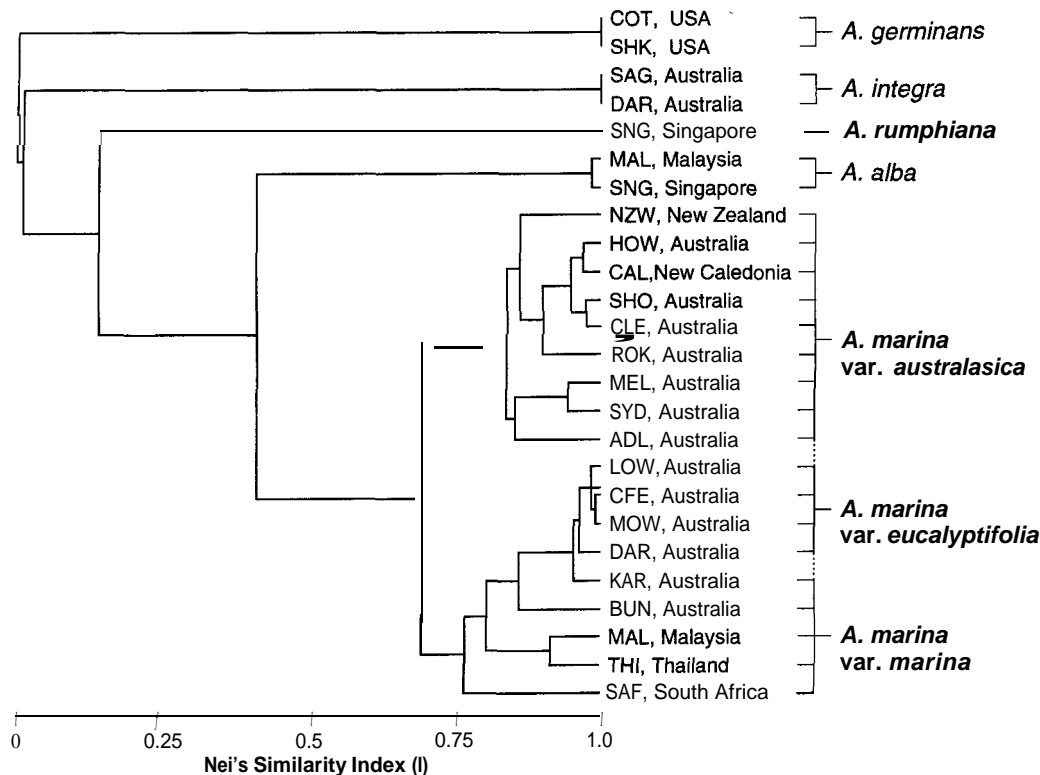


FIG. 1. Dendrogram illustrating relationships between populations, varieties, and species of *Avicennia* from the Indo-West Pacific and eastern North America. For site codes, see Table 1.

sizes of 20-29,  $n = 21$ ,  $F_{ST} = 0.333 \pm 0.220$  (mean  $\pm$  1 SD).

#### Morphological Data Assessment

Morphological data on characteristics considered important in distinguishing varieties of *A. marina* were published previously in conjunction with multivariate analyses (Duke 1991). These data were reanalyzed to display variation for each character in relation to geographic position and genetic variation (see Fig. 5).

## RESULTS

### *Species Boundaries and Phylogenetic Relationships in Indo-West Pacific Avicennia*

A cluster analysis revealed distinct groups corresponding to *A. marina* sensu lato, *A. alba*, *A. rumphiana*, *A. integra*, and *A. germinans* (Fig. 1). Fixed gene differences at most loci (Appendix) demonstrate the lack of genetic continuity among taxa particularly because a number of species had been sampled in sympatry (Table 1).

Populations of *A. marina* were divided into two main groupings, one representing var. *australasica*, and the other made up of both var. *marina* and var. *eucalyptifolia*. More importantly, however, none of these groups showed fixed gene differences, and shifts in gene frequencies seen between the three varieties were no greater than those between populations within a species, which confirms intraspecific status of the three named varieties within *A. marina* sensu lato (Ap-

pendix). The range of the genetic identities between these varieties (0.66-0.88) were similar to those between conspecifics (0.78-1.00) and higher than the range observed between recognized species (0.00-0.42) (Table 2).

### *Genetic Variation within Avicennia Species*

There were eight polymorphic loci (*ACO-2\**, *DIA-2\**, *DIA-3\**, *MDH-3\**, *MDH-5\**, *PRX-2\**, *PGM-2\**, *PGD-1\**) in *A. marina* sensu lato, two (*MDH-5\**, *PRX-2\**) in *A. alba*, and one each for *A. integra* (*PRX-2\**) and *A. rumphiana* (*PRX-2\**) (Appendix). Although marked differences in the genetic variation displayed within each species are likely to reflect differences in the intensity and range of sampling for the different taxa (Table 3), genetic diversity was greater in single *A. marina* populations than in single populations of the other taxa (Table 4).

### *Breeding System and Within-Population Variation in Avicennia marina*

*Avicennia marina* had consistently high levels of genetic diversity, measured as the number of alleles per locus, percentage of polymorphic loci, observed heterozygote frequency, expected heterozygote frequency, number of observed genotypes, number of expected genotypes, observed genetic diversity, expected genetic diversity, and the genetic equilibrium index (Table 4). High levels of cross-fertilization in *A. marina* were also demonstrated by outcrossing indices ( $t = H_o/p$ ) which, in the *ACO-2\** locus, ranged from 0.93 to

TABLE 2. Unbiased genetic identity estimates (Nei 1978) between five *Avicennia* species and three varieties of *A. marina* based on samples from 22 locations from eastern Africa, Asia, and Australasia. Data expressed as means and ranges derived from seven polymorphic loci.

Avicennia taxa	A. <i>interea</i>	A. <i>alba</i>	A. <i>rumbhiana</i>	A. <i>germinans</i>	A. <i>marina</i> sensu lato	A. <i>marina</i> varieties:		
						<i>marina</i>	<i>australifolia</i>	<i>australasica</i>
A. <i>alba</i>	0.000	0.988	—	—	—	—	—	—
A. <i>rumbhiana</i>	0.000	0.092 (0.091–0.93)	—	—	—	—	—	—
A. <i>germinans</i>	0.000	0.000	0.000	1.000	—	—	—	—
A. <i>marina</i> sensu lato	0.002 (0.000–0.034)	0.412 (0.273–0.486)	0.145 (0.091–0.199)	0.000	0.777 (0.484–0.996)	—	—	—
A. <i>marina</i> subspecific taxa:								
var. <i>marina</i>	0.000	0.400 (0.273–0.484)	0.104 (0.091–0.151)	0.000	—	0.827 (0.699–0.954)	—	—
var. <i>eucalyptifolia</i>	0.009 (0.000–0.034)	0.419 (0.389–0.439)	0.194 (0.189–0.197)	0.000	—	0.832 (0.719–0.964)	0.979 (0.948–0.996)	—
var. <i>australasica</i>	0.000	0.416 (0.277–0.486)	0.145 (0.100–0.100)	0.000	—	0.663 (0.484–0.869)	0.736 (0.550–0.833)	0.877 (0.736–0.983)

1.06 in three eastern Australian locations, CFE, ROK and SHO (Table 5). The average estimate for all locations was  $0.96 \pm 0.11$  ( $\bar{x} \pm SE$ ). The mean estimate of the inbreeding coefficient was around zero;  $F = -0.013 \pm 0.067$  ( $\bar{x} \pm SE$ ) for 11 populations, calculated using only single-progeny per tree data and excluding populations with fewer than six trees per sample.

Values of  $G_o/G_e$  were close to one in all populations except CAL (0.63), which indicates little inbreeding in all populations except CAL. The significant values of  $F_{IS}$  indicated those loci whose deviations from Hardy-Weinberg equilibrium had been significant or approached significance prior to the corrections following Miller (1966), and in some cases reflected the cumulative effect of nonsignificant deviations in several populations (Table 6). However, the more exact  $\chi^2$  tests, which pooled rare alleles, showed conformance of heterozygote frequencies to those expected under conditions of Hardy-Weinberg equilibrium in all populations, except for a heterozygote deficit at *DIA-2\** in the CFE population.

#### Between-Population Variation and Geographic Structure of *Avicennia marina*

Interpopulation differentiation measured by  $F_{ST}$  was statistically significant in all three varieties of *A. marina* (Table 6), although values for var. *eucalyptifolia* were approximately one-quarter those for the other varieties. All variable loci contributed to the significant  $F_{ST}$  for var. *marina* and var. *australasica*, but only two loci, *ACO-2\** and *MDH-5\**, made particularly large contributions in var. *eucalyptifolia*.

Estimates of  $N_e m$  for all three varieties indicated low levels of migration among populations, with all values being less than one except for var. *eucalyptifolia*, which had  $N_e m = 2.8$ . Jackknifing  $F_{ST}$  values to obtain their 95% confidence limits and calculating  $N_e m$  showed a tight range for the estimates that did not change the assessments based on the mean values of  $F_{ST}$ . Analyses were undertaken after pooling all populations within each variety to determine  $F_{ST}$ , which therefore estimate gene flow among varieties excluding any component of gene flow among populations within varieties. These data showed highly significant  $F_{ST}$  values for all loci, but  $N_e m$  values were not less than those among populations within two of the varieties (var. *marina* and var. *australasica*). Pairwise comparisons between var. *australasica* and both var. *marina* and var. *eucalyptifolia* gave  $N_e m$  values of 0.4, but gene flows between var. *marina* and var. *eucalyptifolia* were higher with an  $N_e m$  of 1.3.

Lines of best-fit through scatter plots of  $F_{ST}$  between pairs of populations as a function of geographical separation, indicated significant positive relationships for each variety and all data (var. *marina*,  $r = 0.851$ ,  $n = 10$ ,  $P < 0.001$ ; var. *eucalyptifolia*,  $r = 0.968$ ,  $n = 6$ ,  $P < 0.001$ ; var. *australasica*,  $r = 0.500$ ,  $n = 28$ ,  $P < 0.01$ ; all data,  $r = 0.518$ ,  $n = 136$ ,  $P < 0.001$ ). While maintaining an overall relationship consistent with isolation-by-distance, different varieties showed different slopes and shifts in their intercept with the y axis, indicating parallel rather than contiguous slopes. The low similarity between Bowling Green Bay (CFE) and Yeppoon (ROK) ( $I = 0.819$ ) which are only 600 km apart, contrasted with the higher similarities ( $I = 0.947-0.966$ ) between CFE

TABLE 3. Diversity indices (mean  $\pm$  1 SE) for five species of *Avicennia* and three varieties of *A. marina*.

<i>Avicennia</i> taxa	<i>F</i>	<i>D</i>	<i>G<sub>o</sub></i>	<i>G<sub>j</sub>/G<sub>i</sub></i>
<i>A. integra</i>	—	0.146	0.00	
<i>A. alba</i>		0.450	1.05	1.320
<i>A. rumphiana</i>	—	0.200	0.00	
<i>A. germinans</i>		0.163	0.00	—
<i>A. marina</i> sensu lato	0.103 $\pm$ 0.036	0.638 $\pm$ 0.076	8.088 $\pm$ 1.689	1.043 $\pm$ 0.056
<i>A. marina</i> subspecific taxa:				
var. <i>marina</i>	0.174	0.393 $\pm$ 0.129	3.894 $\pm$ 2.039	1.093 $\pm$ 0.075
var. <i>eucalyptifolia</i>	0.116 $\pm$ 0.077	0.847 $\pm$ 0.067	13.043 $\pm$ 3.026	1.250 $\pm$ 0.167
var. <i>australasica</i>	0.060 $\pm$ 0.064	0.677 $\pm$ 0.111	8.217 $\pm$ 2.617	1.070 $\pm$ 0.048

and populations much further to the north (2790–4840 km away) and higher similarities ( $I = 0.845$ ) between ROK and populations to the south (1390 km away). In addition, islands separated by more than twice the distance between CFE and ROK had greater genetic similarities to ROK: Lord Howe Island ( $I = 0.900$ ; 1390 km away) and New Caledonia ( $I = 0.831$ ; 1670 km away). These data indicate a genetic disjunction on the mainland between CFE and ROK where the ranges of two varieties meet on the eastern Australian coastline.

This indication of genetic disjunction between varieties was also supported by strong spatial patterns in the frequencies of individual alleles at *MDH-3\** and *PGD-1\** (Fig. 2). *MDH-3\*77* occurred only in the western parts of the species' range (South African, southeast Asian, Western Australian, and Adelaide populations), *MDH-3\*111* occurred only in the southeastern parts of the species' range (southeast Australia including Adelaide, New Zealand, New Caledonia, and Lord Howe Island), while *MDH-3\*100* was found only in northern and northeastern Australia (Fig. 2a). *PGD-1\*74* only occurred in the southeast Australian samples (including Adelaide), whereas *PGD-1\*100* dominated all other samples except South Africa, which had a unique allele, *PGD-1\*90* (Fig. 2b). Alleles unique to particular populations were observed in only two instances, *MDH-5\*68* in Darwin, and *PGD-1\*90* in South Africa. A plot of  $N_e m$  between neighboring populations provides a useful visual summary of genetic exchange and confirms the effects of isolation-by-distance and the relatively restricted gene flow between varieties relative to that among populations within varieties (Fig. 3). It is noteworthy that the very high gene exchange observed in northeastern Australia coincides with regions in which mangrove habitats are more or less continuous or are broken for only short distances, whereas mangrove habitats in the southeastern, southern, and southwestern coastlines are relatively infrequent and often separated by larger breaks in distribution.

When areas of equal diversity were plotted as isohyets on a map of the study area, higher diversities were revealed at the junctions of the distributions of the three varieties (Fig. 4). Although some of this variation appeared to reflect local differences among populations in their diversity, some aspects could be attributed to gene exchange between varieties. For example, the high diversity in the Darwin population of var. *eucalyptifolia* was associated, in part, with unusual variation at *MDH-5\**; however, allele *PGM-2\*163*, which is absent from the other var. *eucalyptifolia* populations and common in var. *marina* populations, was found at a frequency of 0.114 in the Darwin population of var. *eucalyptifolia* (Ap-

pendix). Other evidence of introgression between var. *eucalyptifolia* and var. *marina* was the presence of allele *MDH-3\*100*, which is found otherwise only in var. *eucalyptifolia* populations, at a frequency of 0.550 in the Karratha population of var. *marina*. Evidence for gene exchange between var. *marina* and var. *australasica* was the high frequency in the Adelaide population of var. *australasica* of *DIA-2\*93*. This allele was the dominant allele in var. *marina*, but was also found in low frequencies in var. *australasica* only in northeastern Australian populations that were separated from the Adelaide population by many populations that did not possess this allele. More striking was the high frequency (0.667) in the Adelaide population of *MDH-3\*77*, the dominant allele in var. *marina*, but absent in all other var. *australasica* populations. Evidence for gene exchange between var. *australasica* and var. *eucalyptifolia* was the presence of *PGD-1\*74* in the CFE population of var. *eucalyptifolia*. This allele is absent in other var. *eucalyptifolia* populations, but is frequent in most var. *australasica* populations. The presence of allele *DIA-3\*112*, which is common in var. *australasica* populations, in only the CFE and MOW populations of var. *eucalyptifolia* suggests gene exchange between the two varieties in northeastern Australia.

#### Geographical Variation in Morphological Characters

A plot of data on geographical variation of key morphological characters distinguishing the three varieties (Duke 1990a, 1991) indicated coincidence between the boundaries of the varieties and shifts in morphology of calyx pubescence (given by the calyx surface index, which is the ratio of the width of the glabrous margin over the length of the calyx lobe), leaf shape (given as the ratio of leaf blade length to the length from the petiole to the greatest width of the leaf), and flower size (corolla lobe width) (Fig. 5). There was little or no increase in the variability of morphological characters in populations at the periphery of the varietal ranges that might have indicated genetic exchange affecting the range of phenotypes produced. However, there were two populations which showed intermediate values for key morphological characters. Leaf shape and corolla lobe width were intermediate in the Wyndham population, for which no genetic data are available, and calyx pubescence was intermediate in the Adelaide population (ADL). No populations with intermediate values for key characters were detected at the margin of var. *eucalyptifolia* and var. *australasica* populations between Townsville (CFE) and Rockhampton (ROK).

TABLE 4. Measures of genetic variation, including multilocus genotypic diversity, in five species of *Avicennia* and three varieties of *A. marina*. Standard errors are given in parentheses where appropriate. For site codes, see Table 1.

Species/Location	Sample size	Mean no. alleles/locus	Percentage of loci polymorphic	Mean heterozygosity		Observed number of genotypes	Expected number of genotypes	Observed diversity $G_O$	Expected diversity $G_E$	$G_O/G_E$
				$H_o$	$H_e$					
<i>A. integra</i>										
DAR	11	1.0	0.0	0.000	0.000	1	1.00	0.00	0.00	—
SAG	5	1.0	0.0	0.000	0.000	1	1.00	0.00	0.00	—
<i>A. alba</i>										
MAL	5	1.1 (0.1)	9.1	0.036 (0.036)	0.048 (0.048)	3	2.40	2.78	2.10 (0.82)	1.32
SNG	3	1.0	0.0	0.000	0.000	1	1.00	0.00	0.00	—
<i>A. rumphiana</i>										
SNG	5	1.0	0.0	0.000	0.000	1	1.00	0.00	0.00	—
<i>A. germinans</i>										
COT	8	1.0	0.0	0.000	0.000	1	1.00	0.00	0.00	—
SHK	5	1.0	0.0	0.000	0.000	1	1.00	0.00	0.00	—
<i>A. marina</i> var. <i>marina</i>										
SAF	14	1.0	0.0	0.000	0.000	1	1.00	0.00	0.00	—
MAL	6	1.3 (0.2)	18.2	0.030 (0.020)	0.072 (0.057)	4	3.97	3.00	3.14 (1.30)	0.96
THI	8	1.1 (0.1)	9.1	0.023 (0.023)	0.048 (0.048)	3	2.79	2.91	2.23 (0.66)	1.30
BUN	20	1.1 (0.1)	9.1	0.023 (0.023)	0.027 (0.027)	3	2.48	1.80	1.78 (0.30)	1.01
KAR	20	1.5 (0.2)	27.3	0.109 (0.058)	0.132 (0.071)	14	13.97	11.76	10.71 (2.83)	1.10
<i>A. marina</i> var. <i>eucalyptifolia</i>										
DAR	22	1.7 (0.4)	36.4	0.136 (0.068)	0.151 (0.076)	19	17.51	16.13	15.09 (3.70)	1.07
LOW	4	1.2 (0.1)	18.2	0.045 (0.030)	0.071 (0.052)	4	2.74	4.00	2.42 (1.12)	1.65
MOW	20	1.6 (0.2)	45.5	0.136 (0.068)	0.135 (0.068)	17	14.54	15.38	11.18 (3.04)	1.38
CFE	43	1.8 (0.3)	45.5	0.104 (0.049)	0.140 (0.063)	29	28.81	16.66	18.60 (3.82)	0.90
<i>A. marina</i> var. <i>australasica</i>										
ROK	15	1.7 (0.3)	45.5	0.139 (0.064)	0.161 (0.076)	14	13.02	13.24	12.85 (3.05)	1.03
CAL	5	1.3 (0.1)	27.3	0.145 (0.086)	0.117 (0.066)	3	4.13	2.27	3.58 (1.45)	0.63
SHO	13	1.9 (0.3)	54.5	0.217 (0.067)	0.248 (0.079)	13	12.81	13.00	12.63 (1.20)	1.03
CLE	3	1.4 (0.2)	36.4	0.182 (0.094)	0.170 (0.075)	3	2.87	3.00	2.78 (0.83)	1.08
HOW	12	1.5 (0.2)	36.4	0.106 (0.046)	0.182 (0.076)	10	10.40	9.00	10.29 (2.67)	0.87
SYD	19	1.3 (0.1)	27.3	0.105 (0.069)	0.092 (0.058)	7	7.88	4.81	5.46 (1.36)	0.88
MEL	22	1.1 (0.1)	9.1	0.025 (0.025)	0.028 (0.028)	3	2.51	1.85	1.78 (0.28)	1.04
NZW	13	1.3 (0.1)	27.3	0.035 (0.019)	0.034 (0.018)	4	3.78	1.64	1.99 (0.60)	0.82
ADL	27	1.7 (0.2)	54.5	0.202 (0.065)	0.255 (0.076)	26	25.49	25.14	25.57 (3.00)	0.98

## DISCUSSION

All five *Avicennia* species included in the analysis were supported by the occurrence of several fixed gene differences between these species in sympatry, high levels of genetic similarity between populations within species, and general conformance of genotype frequencies within populations to

those expected under conditions of Hardy-Weinberg equilibrium. These species included four of the five known Indo-West Pacific species and one of the three known Atlantic-East Pacific species, each of which was first distinguished on the basis of morphological criteria. There was no evidence of hybridization among taxa, despite sampling sites where

TABLE 5. Estimates of outcrossing ( $t = H_o/p$ ) in *Avicennia marina* at aconitase (*ACO-2\**) from three locations in Queensland: Bowling Green Bay (CFE), Yeppoon (ROK), and Brisbane (SHO). Location details are listed in Table 1; numbers of progeny sampled per tree are in parentheses.

Locus	Locations (progeny sampled/tree)	$\bar{t} \pm 1 \text{ SE}$
<i>ACO-2*</i>	CFE (6,9,6,6)	1.06 $\pm$ 0.27
	ROK (6,5,5,5,4,5,5)	0.93 $\pm$ 0.18
	SHO (6,6,6,7,6,6)	0.93 $\pm$ 0.19
Total mean of sites CFE, ROK, SHO; $n = 18$		0.96 $\pm$ 0.11

more than one species was present. The lack of hybrids in *Avicennia* contrasts with those found in three other widely distributed mangrove genera (Duke 1992), namely *Lumnitzera* (Tomlinson et al. 1978), *Rhizophora* (Duke and Bunt 1979; Tomlinson 1986), and *Sonneratia* (Duke and Jackes 1987; Duke 1994). Phylogenetic relationships based on allozyme variation concur with analyses based on morphological characters (Duke 1990a) and multistate diagnostic attributes (Duke 1995). Although not all large-flowered species were included in the assessment of allozyme variation, the small-flowered species, *A. alba*, *A. rumphiana*, and *A. marina* sensu lato, were grouped together separately from the two large-flowered species, *A. integrata* and *A. germinans*.

In contrast, allelic frequencies in the three subgroups of *A. marina* were consistent with all populations belonging to a single taxon. None of the subspecific groupings showed fixed gene differences among them; the levels of genetic differentiation were the same as intraspecific variation in other taxa and were far less than those between the other, well-established species included in the study. Some characteristics of the spatial patterns of genetic variation in *A. marina* sensu lato supported the recognition of intraspecific entities. A strong geographic pattern at *MDH-3\** and *PGD-1\** suggested the occurrence of three genetic groups around the Australian coast, corresponding to the varieties *marina*, *eucalyptifolia*, and *australasica*. Populations at the margins of these groups indicated high levels of heterozygosity and the presence of specific alleles indicative of introgression between the groups at the margins of their distributions. Overseas populations of var. *marina* and var. *australasica* clustered

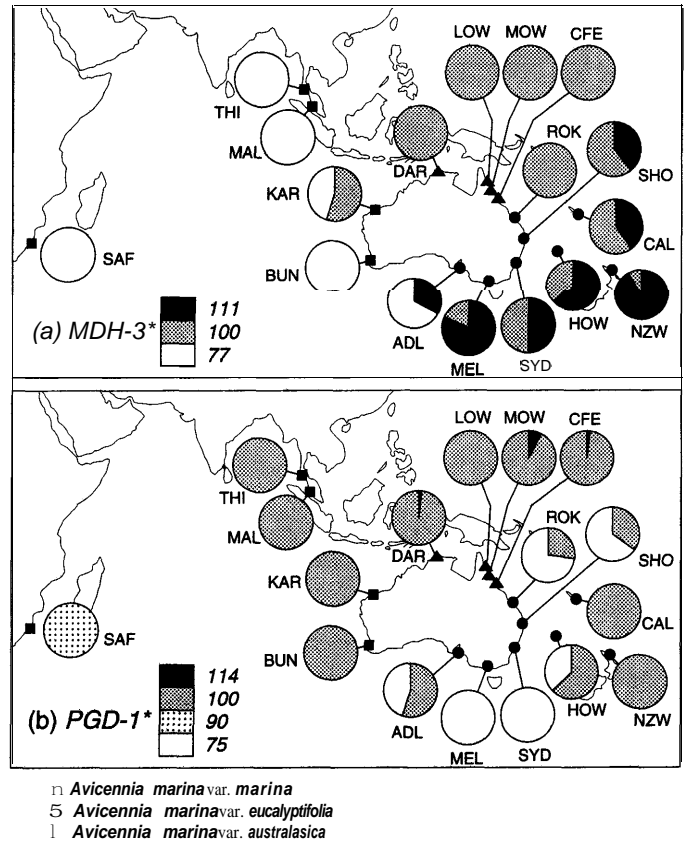


FIG. 2. Geographical variation in allele frequencies at *MDH-3\** and *PGD-1\** for *Avicennia marina* sensu lato. For site codes, see Table 1.

with their Australian counterparts even though their geographical separation was greater than that separating populations within different varieties within Australia (no overseas var. *eucalyptifolia* were sampled). However, var. *eucalyptifolia* and var. *marina* appeared to form one group in the dendrogram derived from the cluster analysis. Consistent with this result, gene flow among var. *eucalyptifolia* and var. *marina* populations (Darwin and Karratha; DAR and KAR) was as high as that among populations within varieties, which

TABLE 6. Standardized genetic variance within ( $F_{IS}$ ) and between ( $F_{ST}$ ) populations for datasets including all varieties or only one variety of *Avicennia marina* sensu lato.

Locus	All varieties		var. <i>marina</i>		var. <i>eucalyptifolia</i>		var. <i>australasica</i>	
	$F_{IS}$	$F_{ST}$	$F_{IS}$	$F_{ST}$	$F_{IS}$	$F_{ST}$	$F_{IS}$	$F_{ST}$
<i>ACO-2*</i>	0.520***	0.347***	0.402***	0*599***	0.177	0.115***	0.249***	0.263***
<i>DIA-2*</i>	0.510***	0.784***	0	0	0.558***	0.005	0	0.540***
<i>DIA-3*</i>	0.738***	0.541***	0.070	0.946***	0.425***	0.065	0.290***	0.615***
<i>MDH-3*</i>	0.427***	0.408***	0.014	0.486***	0	0	0.006	0.412***
<i>MDH-5*</i>	0	0	0	0	0.091	0.376***	0	0
<i>PGD-1*</i>	0.712***	0.393***	0	1.000***	0.027	0.004	0.292***	0.488***
<i>PGM-2*</i>	0.573***	0.214***	0.108	0.614***	0.018	0.032	0.105	0.469***
Mean	0.497***	0.384***	0.060	0.521***	0.178	0.083***	0.133***	0.398***
95% CL from jackknife	$\pm 0.001$		$\pm 0.023$		$\pm 0.001$		$\pm 0.001$	
$N_m$	0.4		0.2		2.8		0.4	
	(0.36–0.43)		(0.21–0.25)		(2.7–2.8)		(0.36–0.43)	

\*\*\*  $P < 0.001$ .

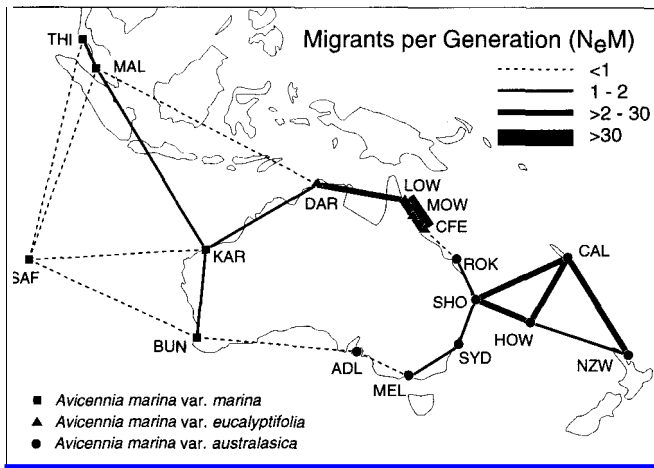


FIG 3. Patterns of gene flow within *Avicennia marina* sensu lato. For site codes, see Table 1.

suggests that perhaps only two varieties might be given taxonomic recognition. In contrast, gene flow between var. *australasica* and the two other varieties, var. *eucalyptifolia* and var. *marina*, was reduced relative to the gene flow among populations within varieties over similar geographic scales.

Many of the morphological characteristics used in the past to differentiate *Avicennia* taxa, such as leaf size and shape, have been shown to vary with estuarine location and position on a single tree and to be correlated with environmental conditions (Soto and Coralles 1987; Soto 1988; Duke 1990a). However, selected attributes of leaf shape and bark and flower characteristics can reliably distinguish varieties of *A. marina* (Duke 1991). Var. *australasica* is clearly defined in distribution and morphology and it is distinguished from the other two varieties by having grey, fissured, and pustular bark instead of the chalky green, smooth, and sometimes flaky bark of the others. The outer margin of the calyx lobe is fully pubescent and only occasionally has a very narrow (< 0.5 mm in dried specimens) glabrous, peripheral margin in var. *australasica*, whereas the other two varieties have a pronounced glabrous, peripheral margin (> 0.5 mm wide). The ratio of the width of glabrous margin to the length of calyx lobe is greater than 0.2 in var. *eucalyptifolia* and var. *marina* and less than 0.2 in var. *australasica*. Differences between var. *eucalyptifolia* and var. *marina* are more subtle, with leaf shape more lanceolate in var. *eucalyptifolia* and apiculate to subovate in var. *marina*. The ratio of leaf blade length to the length from petiole to greatest width of leaf is greater than 2.2 in var. *eucalyptifolia* and less than 2.2 in var. *marina* and var. *australasica*. In var. *eucalyptifolia* the tip of the stigma is subequal with the upper edge of the anthers, in contrast to var. *marina* where the tip of the stigma is equal with the lower edge of anthers (Duke 1991).

Geographical variation of the diagnostic characteristics of leaf shape, flower size, and bract pubescence show marked shifts between varieties with few indications of intermediate phenotypes. This was the case within the area of introgression between varieties *eucalyptifolia* and *australasica*, however, intermediate phenotypes were observed at Wyndham, the putative conjunction of varieties *eucalyptifolia* and *marina*, and

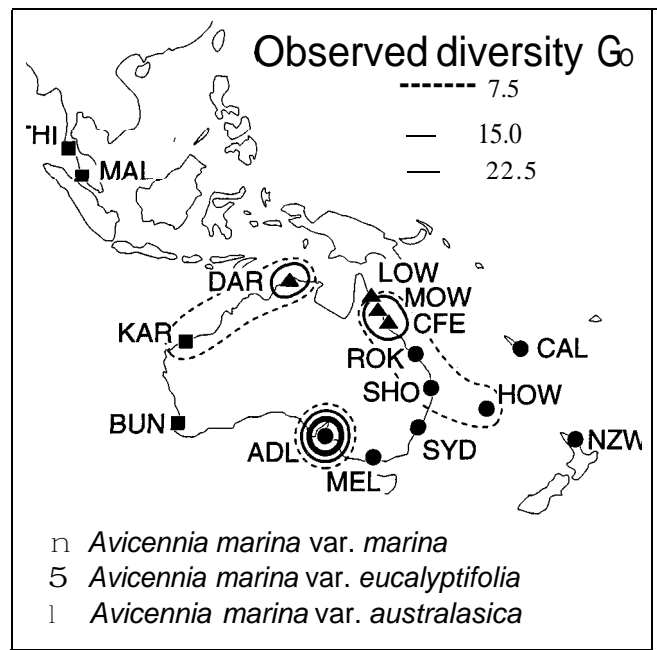


FIG. 4. Geographical patterns of genetic diversity in *Avicennia marina* sensu lato. For site codes, see Table 1.

at Adelaide (ADL), the putative conjunction of varieties *australasica* and *marina*. The presence of populations with intermediate morphological characteristics is indicative of introgression. Evidence of gene exchange between varieties was observed at several allozyme loci where alleles, found commonly in one variety were observed only in the geographically adjacent population of the other variety.

Discussion of the biogeography of Australian mangroves is notably limited (Briggs 1974; Chapman 1976; Specht 1981). However, the distribution of var. *australasica* is broadly similar to that of a range of marine taxa occurring in southeastern Australia that have collectively been recognized as having a palaeoaustral origin (Wilson and Allen 1987). A faunal grouping, recognized in fossil molluscs from the Eocene, had fluctuated in range over time from just south of Adelaide (ADL) to midway between Sydney (SYD) and Brisbane (SHO) at its greatest extent to just around Tasmania and on the mainland coast north of Tasmania at its most restricted. The size of the range depended on the northward extent of cold waters from the southern ocean. The approximate coincidence of the range of var. *australasica* with this zoogeographic region and the detection of *Avicennia* pollen in the Eocene of southern Australia (Churchill 1973) might also suggest an ancient origin for this taxon. However, the reported Eocene pollen of *Avicennia* was disputed by Muller (1981), who indicated the earliest positive identification of *Avicennia* fossils otherwise is from the Miocene in Asia. In addition, the location of the putative fossil *Avicennia* pollen in southern Australia is in the southwest, far from either the present-day range of var. *australasica* or the southeastern zoogeographic zones with palaeoaustral biotas. It has also been suggested that all three varieties had an ancient origin in the late Cretaceous, when the present Australian continent was split into

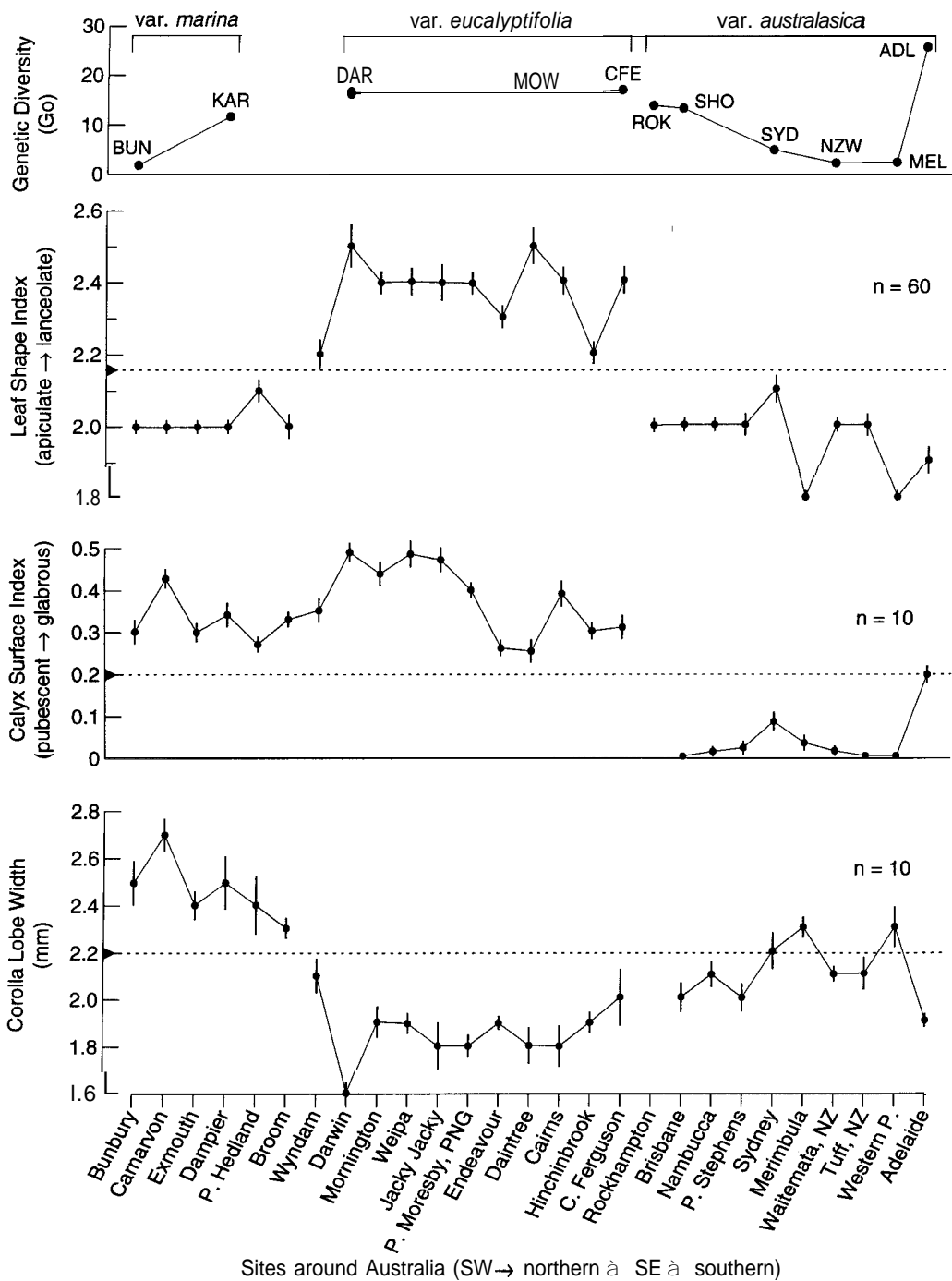


FIG. 5. Graphs illustrating the variation in genetic diversity and morphological attributes of *Avicennia marina* sensu lato throughout its range in Australia and nearby. For site codes, see Table 1.

three land areas, each of which might have allowed discrete taxa of *Avicennia* to develop (Duke 1995).

Current allozyme data and genetic findings presented in this paper do not support a hypothesis involving an ancient origin of *A. marina* varieties. The lack of genetic divergence of *A. marina* varieties suggests they are of more recent origin. Using general approximations of Nei (1987, p. 237) to estimate time of divergence using measures of genetic identity (*I*), *var. australasica* appears to have diverged from the other

varieties approximately 2 M.Y.B.P, while *var. eucalyptifolia* and *var. marina* diverged some 200,000 years ago. Molecular clocks are highly variable among lineages and among loci with rates varying as much as 20-fold (Nei 1987). However, even taking the extreme step of multiplying the observed values by 20 provides maximal estimates of divergence of *var. australasica* of approximately 40 M.Y.B.P, and that of the other varieties to possibly around 4 M.Y.B.P, which is still far younger than the late Cretaceous (65 M.Y.B.P.).

Our current findings are consistent with the origin of genetic structure in *A. marina* occurring during the last ice age, when lower sea levels apparently isolated west coast populations (var. *marina*) from those in the east (var. *eucalyptifolia*) by means of a land barrier between New Guinea and Australia. The role of this barrier in determining genetic structure of prawns (Benzie et al. 1992), starfish (Williams and Benzie 1997, 1998), and finfish (Keenan 1994) has been proposed earlier. The situation for var. *australasica* in southeastern Australia is more difficult to resolve because of a lack of information on key barriers to dispersal along the east coast. Mangroves have apparently existed in southern Australia for a considerable time, despite the more poleward position of Australia in earlier geological times. Mangrove habitat appears to have existed in Tasmania as long ago as the Lower Eocene because fossils of the mangrove palm, *Nypa*, were obtained from sediments near Strahan, western Tasmania (Pope and McPhail 1996). However, similar fossil evidence for an ancient presence of *Avicennia* in southern Australia is notably lacking.

Genetic diversity measures in *A. marina* (1.42 alleles/locus, 29.3% polymorphic loci and 9.8% observed heterozygotes) were comparable with averages for tropical trees and shrubs (Hamrick and Loveless 1986; ~1.45 alleles/locus, ~27.6% polymorphic loci and ~11.1% observed heterozygotes), and with plants in general (Hamrick and Godt 1989; ~1.69 alleles/locus, ~36.8% polymorphic loci and ~14.1% observed heterozygotes). Levels of outcrossing were high, among the highest recorded for plants (e.g., Schemske and Lande 1985), and similar to the high levels reported in *Banksia* (Carthew et al. 1988) and widespread plants in general (Hamrick and Godt 1989). *Avicennia marina* has a combination of life-history characteristics that are likely to be associated with outcrossing. These are low reproductive success (Duke 1990b) with high levels of propagule predation (Smith 1987; Smith et al. 1988), including high losses in dispersal (Steinke 1975, 1986). *Avicennia* species are heavily visited by pollinators, including honeybees, and the protandrous development of its flowers further indicate that maximization of pollen outcrossing is important to the genus (Tomlinson 1986). The high  $G_o/G_e$  ratios observed in the present study indicate that mating within local populations is almost random.

Despite the outcrossing reproductive strategy of *A. marina* sensu lato, and the species' apparent capacity for dispersal, the genetic signal of past biogeographical events has been maintained for thousands of years (at least) after the populations were reunited. This finding, together with the strong evidence for isolation-by-distance, supports the view that gene flow and dispersal of propagules is more limited than has been assumed for widespread mangrove species. High values of gene flow were only observed between sites separated by a few tens of kilometers, and where mangrove habitats were relatively continuous. Where populations were more fragmented, gene exchange was far less than that which might have been expected given their dispersal potential. Gene flow between varieties was more restricted and this may reflect barriers to reproduction, although evidence of significant levels of introgression was observed. The continuing existence of the varieties may result from unknown selective

factors preventing complete assimilation or some degree of hybrid breakdown.

### Conclusions

These first data on genetic structure of the mangrove species, *A. marina*, across much of its Indo-West Pacific range has demonstrated strong genetic structuring with possibly three intraspecific taxa. Based on these observations, we question the amount to which long-distance dispersal has influenced genetic diversification of this widespread species. High levels of outcrossing locally are not matched by high levels of gene flow between all neighboring populations. Two of the three varieties of *A. marina*, var. *eucalyptifolia* and var. *marina*, appear to have recent origins related to isolation of populations in the Indian and Pacific Oceans, respectively, possibly during periods of low sea level during the last glaciation. Significant levels of introgression are apparently occurring between them now in northern Australia. More detailed surveys of the zones of contact between varieties are required to understand the nature and extent of introgression between varieties. The origin of var. *australasica* is unclear; future work must establish whether this variety maintained its greater genetic distinction because of its adaptation to colder climates and because of its more ancient origins. The current allozyme data frame a series of important questions of fundamental interest regarding mangrove population biology and evolution.

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## APPENDIX

Allele frequencies at 11 allozyme loci for five species of *Avicennia* and three varieties of *A. marina* sampled from 25 populations from southeastern North America, eastern Africa, Asia, and Australasia (for site codes, see Table 1).

Locus		<i>A. integrata</i>		<i>A. alba</i>		<i>A. rumphiana</i>	<i>A. germinans</i>	
		DAR	SAG	MAL	SNG	SNG	COT	SHK
ACO-2*	110	—	—	—	—	—	—	—
	103	—	—	—	—	—	—	—
	100	—	—	—	—	—	—	—
	93	—	—	—	—	—	—	—
	86	—	—	—	—	1.000	—	—
	81	—	—	—	—	—	1.000	1.000
	79	—	—	1.000	1.000	—	—	—
	75	1.000	1.000	—	—	—	—	—
DIA-2*	118	—	—	—	—	—	1.000	1.000
	114	—	—	1.000	1.000	—	—	—
	110	1.000	1.000	—	—	—	—	—
	102	—	—	—	—	1.000	—	—
	100	—	—	—	—	—	—	—
DIA-3 *	93	—	—	—	—	—	—	—
	129	—	—	—	—	—	1.000	1.000
	112	—	—	—	—	—	—	—
	103	—	—	1.000	1.000	—	—	—
	101	—	—	—	—	1.000	—	—
	100	—	—	—	—	—	—	—
MDH-1 *	99	1.000	1.000	—	—	—	—	—
	87	—	—	—	—	—	—	—
	107	1.000	1.000	—	—	—	—	—
MDH-2*	100	—	—	1.000	1.000	1.000	—	—
	99	—	—	—	—	—	1.000	1.000
	112	1.000	1.000	—	—	—	—	—
MDH-3*	100	—	—	1.000	1.000	—	—	—
	98	—	—	—	—	1.000	—	—
	97	—	—	—	—	—	1.000	1.000
	141	1.000	1.000	—	—	—	—	—
	114	—	—	—	—	—	1.000	1.000
MDH-4*	111	—	—	—	—	—	—	—
	109	—	—	1.000	1.000	—	—	—
	100	—	—	—	—	1.000	—	—
	77	—	—	—	—	—	—	—
	120	1.000	1.000	—	—	—	—	—
	118	—	—	1.000	1.000	—	—	—
MDH-5 *	104	—	—	—	—	—	1.000	1.000
	100	—	—	—	—	—	—	—
	92	—	—	—	—	1.000	—	—
	100	—	—	—	—	—	—	—
	96	—	—	—	—	1.000	—	—
PGD-1 *	92	—	—	0.600	1.000	—	—	—
	68	1.000	1.000	—	—	—	—	—
	66	—	—	—	—	—	1.000	1.000
	54	—	—	0.400	—	—	—	—
	124	—	—	—	—	—	1.000	1.000
	114	—	—	—	—	—	—	—
PGD-2*	100	—	—	1.000	1.000	—	—	—
	96	1.000	1.000	—	—	—	—	—
	90	—	—	—	—	—	—	—
	88	—	—	—	—	1.000	—	—
	74	—	—	—	—	—	—	—
	149	—	—	—	—	—	1.000	1.000
PGM-2*	119	—	—	—	—	1.000	—	—
	100	—	—	1.000	1.000	—	—	—
	81	1.000	1.000	—	—	—	—	—
	291	—	—	—	—	—	1.000	1.000
	163	—	—	—	—	—	—	—
	138	—	—	—	—	—	—	—
N	100	—	—	1.000	1.000	—	—	—
	91	—	—	—	—	1.000	—	—
	88	1.000	1.000	—	—	—	—	—
	63	—	—	—	—	—	—	—
N	11	5	5	3	5	8	5	

APPENDIX. Continued.

Locus		<i>A. marina</i> var. <i>marina</i>					<i>A. marina</i> var. <i>eucalyptifolia</i>			
		SAF	MAL	THI	BUN	KAR	DAR	LOW	MOW	CFE
<i>ACO-2*</i>	110	—	—	—	—	—	0.068	—	—	0.035
	103	—	0.583	0.500	—	0.125	0.159	0.625	0.425	0.547
	100	1.000	0.250	0.500	—	0.375	0.659	—	0.400	0.256
	93	—	0.167	—	1.000	0.500	0.114	0.375	0.175	0.163
	86	—	—	—	—	—	—	—	—	—
	81	—	—	—	—	—	—	—	—	—
	79	—	—	—	—	—	—	—	—	—
<i>DIA-2*</i>	75	—	—	—	—	—	—	—	—	—
	118	—	—	—	—	—	—	—	—	—
	114	—	—	—	—	—	—	—	—	—
	110	—	—	—	—	—	—	—	—	—
	102	—	—	—	—	—	—	—	—	—
<i>DIA-3*</i>	100	—	—	—	—	—	—	—	0.025	0.070
	93	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	0.930
	129	—	—	—	—	—	—	—	—	—
	112	—	—	—	—	—	—	—	0.050	0.035
	103	—	—	—	—	—	—	—	—	—
	101	—	—	—	—	—	—	—	—	—
<i>MDH-1*</i>	100	1.000	0.917	—	1.000	1.000	1.000	1.000	0.925	0.814
	99	—	—	—	—	—	—	—	—	—
	87	—	0.083	1.000	—	—	—	—	0.025	0.151
	107	—	—	—	—	—	—	—	—	—
<i>MDH-2*</i>	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	99	—	—	—	—	—	—	—	—	—
	112	—	—	—	—	—	—	—	—	—
<i>MDH-3*</i>	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	98	—	—	—	—	—	—	—	—	—
	97	—	—	—	—	—	—	—	—	—
	141	—	—	—	—	—	—	—	—	—
	114	—	—	—	—	—	—	—	—	—
<i>MDH-4*</i>	111	—	—	—	—	—	—	—	—	—
	109	—	—	—	—	—	—	—	—	—
	100	—	—	—	—	0.550	1.000	1.000	1.000	1.000
	77	1.000	1.000	1.000	1.000	0.450	—	—	—	—
	120	—	—	—	—	—	—	—	—	—
<i>MDH-5*</i>	118	—	—	—	—	—	—	—	—	—
	104	—	—	—	—	—	—	—	—	—
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	92	—	—	—	—	—	—	—	—	—
	100	1.000	1.000	1.000	1.000	1.000	0.659	1.000	1.000	1.000
<i>PGD-1*</i>	96	—	—	—	—	—	—	—	—	—
	92	—	—	—	—	—	—	—	—	—
	68	—	—	—	—	—	0.341	—	—	—
	66	—	—	—	—	—	—	—	—	—
	54	—	—	—	—	—	—	—	—	—
	124	—	—	—	—	—	—	—	—	—
<i>PGD-2*</i>	114	—	—	—	—	—	—	—	—	—
	100	—	1.000	1.000	1.000	1.000	0.023	—	0.075	0.012
	96	—	—	—	—	—	0.977	1.000	0.925	0.953
	90	1.000	—	—	—	—	—	—	—	—
	88	—	—	—	—	—	—	—	—	—
<i>PGM-2*</i>	74	—	—	—	—	—	—	—	—	0.035
	149	—	—	—	—	—	—	—	—	—
	119	—	—	—	—	—	—	—	—	—
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
<i>N</i>	81	—	—	—	—	—	—	—	—	—
	291	—	—	—	—	—	—	—	—	—
	163	—	—	—	0.175	0.175	0.114	—	—	—
	138	1.000	—	—	0.825	0.800	0.500	0.875	0.575	0.744
	100	—	1.000	1.000	—	0.025	0.364	0.125	0.425	0.256
	91	—	—	—	—	—	—	—	—	—
88	—	—	—	—	—	—	—	—	—	
63	—	—	—	—	—	0.023	—	—	—	
<i>N</i>	14	6	8	20	20	22	4	20	43	

## APPENDIX. Continued.

		<i>A. marina var. australasica</i>								
Locus		ROK	CAL	SHO	CLE	HOW	SYD	MEL	NZW	ADL
<i>ACO-2*</i>	110	0.133	—	0.154	—	—	—	—	—	0.074
	103	0.333	<b>0.500</b>	0.423	<b>0.500</b>	<b>0.583</b>	0.974	1.000	<b>1.000</b>	0.648
	100	0.300	—	0.192	—	0.042	—	—	—	—
	93	0.233	<b>0.500</b>	0.231	<b>0.500</b>	0.375	<b>0.026</b>	—	—	<b>0.278</b>
	86	—	—	—	—	—	—	—	—	—
	81	—	—	—	—	—	—	—	—	—
	79	—	—	—	—	—	—	—	—	—
	75	—	—	—	—	—	—	—	—	—
<i>DIA-2*</i>	118	—	—	—	—	—	—	—	—	—
	114	—	—	—	—	—	—	—	—	—
	110	—	—	—	—	—	—	—	—	—
	102	—	—	—	—	—	—	—	—	—
<i>DIA-3 *</i>	100	<b>0.967</b>	1.000	<b>0.731</b>	1.000	<b>1.000</b>	1.000	<b>1.000</b>	<b>1.000</b>	<b>0.333</b>
	93	0.033	—	0.269	—	—	—	—	—	0.667
	129	—	—	—	—	—	—	—	—	—
	112	<b>0.067</b>	1.000	0.846	<b>0.500</b>	<b>0.625</b>	<b>1.000</b>	1.000	<b>0.077</b>	<b>0.815</b>
	103	—	—	—	—	—	—	—	—	—
<i>MDH-1 *</i>	101	—	—	—	—	—	—	—	—	—
	100	<b>0.933</b>	—	<b>0.154</b>	0.500	<b>0.375</b>	—	—	<b>0.923</b>	<b>0.185</b>
	99	—	—	—	—	—	—	—	—	—
	87	—	—	—	—	—	—	—	—	—
<i>MDH-2*</i>	107	—	—	—	—	—	—	—	—	—
	100	1.000	1.000	1.000	<b>1.000</b>	1.000	1.000	1.000	1.000	1.000
	99	—	—	—	—	—	—	—	—	—
<i>MDH-3 *</i>	112	—	—	—	—	—	—	—	—	—
	100	<b>1.000</b>	1.000	<b>1.000</b>	<b>1.000</b>	1.000	1.000	1.000	<b>1.000</b>	1.000
	98	—	—	—	—	—	—	—	—	—
	97	—	—	—	—	—	—	—	—	—
<i>MDH-4*</i>	141	—	—	—	—	—	—	—	—	—
	114	—	—	—	—	—	—	—	—	—
	111	—	<b>0.400</b>	0.385	0.167	<b>0.625</b>	<b>0.500</b>	<b>0.818</b>	<b>0.923</b>	<b>0.333</b>
	109	—	—	—	—	—	—	—	—	—
	100	<b>1.000</b>	0.600	<b>0.615</b>	<b>0.833</b>	<b>0.375</b>	<b>0.500</b>	<b>0.182</b>	<b>0.077</b>	—
<i>MDH-5*</i>	77	—	—	—	—	—	—	—	—	<b>0.667</b>
	120	—	—	—	—	—	—	—	—	—
	118	—	—	—	—	—	—	—	—	—
	104	—	—	—	—	—	—	—	—	—
<i>PGD-1 *</i>	100	<b>1.000</b>	<b>1.000</b>	<b>1.000</b>	1.000	1.000	<b>1.000</b>	1.000	<b>1.000</b>	<b>1.000</b>
	92	—	—	—	—	—	—	—	—	—
	100	1.000	<b>1.000</b>	<b>1.000</b>	<b>1.000</b>	1.000	1.000	<b>1.000</b>	<b>1.000</b>	1.000
	96	—	—	—	—	—	—	—	—	—
	92	—	—	—	—	—	—	—	—	—
<i>PGD-2*</i>	68	—	—	—	—	—	—	—	—	—
	66	—	—	—	—	—	—	—	—	—
	54	—	—	—	—	—	—	—	—	—
	124	—	—	—	—	—	—	—	—	—
	114	—	—	—	—	—	—	—	—	—
<i>PGM-2*</i>	100	<b>0.267</b>	<b>1.000</b>	<b>0.346</b>	<b>0.167</b>	0.625	—	—	<b>1.000</b>	<b>0.574</b>
	96	—	—	—	—	—	—	—	—	—
	90	—	—	—	—	—	—	—	—	—
	88	—	—	—	—	—	—	—	—	—
	74	<b>0.733</b>	—	<b>0.654</b>	<b>0.833</b>	<b>0.375</b>	<b>1.000</b>	<b>1.000</b>	—	<b>0.426</b>
<i>PGM-2*</i>	149	—	—	—	—	—	—	—	—	—
	119	—	—	—	—	—	—	—	—	—
	100	1.000	1.000	<b>1.000</b>	<b>1.000</b>	1.000	<b>1.000</b>	<b>1.000</b>	<b>1.000</b>	1.000
	81	—	—	—	—	—	—	—	—	—
	291	—	—	—	—	—	—	—	—	—
<i>PGM-2*</i>	163	<b>0.233</b>	0.100	<b>0.077</b>	—	—	—	—	—	<b>0.093</b>
	138	0.033	—	0.077	—	—	<b>0.316</b>	<b>1.000</b>	<b>0.038</b>	0.463
	100	0.733	<b>0.900</b>	0.808	1.000	1.000	0.684	—	0.962	0.444
	91	—	—	—	—	—	—	—	—	—
	88	—	—	—	—	—	—	—	—	—
	63	—	—	<b>0.038</b>	—	—	—	—	—	—
<i>N</i>		<b>15</b>	<b>5</b>	13	<b>3</b>	<b>12</b>	<b>19</b>	22	<b>13</b>	27