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Phenotypic leaf variation in *Avicennia marina* in tropical Queensland: Can discrete sub-populations be recognised in the field?

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Abstract.

The geographic patterns of phenotypic variation in leaf morphology traits were studied in the mangrove *Avicennia marina* (Forsk.) Vierh. in tropical Queensland to determine whether discrete sub-populations could be recognised in the field. Significant differences in the various leaf characters occurred among the sites, which were not explained by longitude or latitude, nor by intersite distances. Hierarchical cluster analysis of the estimated site means showed no coherent geographical groupings of the sites, suggesting that site populations do not follow a differentiation by distance model. Principal component analysis showed that site groupings with consistent leaf morphological characteristics could be identified, suggesting the *A.marina* occurs as widely scattered discrete sub-populations, and that phenotypic structuring occurs over quite short (< 100 km) distances. If, as seems increasingly likely, this phenotypic structuring is at least partly reflecting the genetic diversity, it

implies that a conservative approach to sourcing plant material for mangrove restoration projects would be appropriate.

Introduction

The mangrove *Avicennia marina* (Forsk.) Vierh. shows considerable variability in form across its entire distribution range, and genetic studies (using allozymes, microsatellites and AFLP) have shown that many populations of *A. marina* have unique genetic characters, and a population structure consistent with the discrete sub-populations model (Duke *et al.* 1998; Maguire *et al.* 2000; 2002; Melville and Burchett 2002; Giang *et al.* 2003). Some of this genetic diversity seems to be expressed by the leaf morphology of this species, as leaves displayed no significant differences in morphology within sites, but showed marked differences between sites (Melville and Burchett 2002). These authors further concluded that leaf morphology was not highly affected by sediment characteristics, that genetic factors have the largest influence on leaf morphology, and that leaf morphology is of some use as a genetic marker of population differentiation. Moreover, based on provenance glasshouse trials (unpublished data), clear and consistent associations were seen between provenances and particular growth characters, including leaf length and width of the most fully expanded leaf pair and these phenotypic differences in *A. marina* populations from around Australia appear to be largely due to genetic rather than environmental factors.

In view of these findings, this study aimed to quantify this observed phenotypic variation in leaf dimensions using simple field measurements, and to estimate the geographical extent over which discrete sub-populations can be recognised in the

field. The results of this study will facilitate germplasm selection for mangrove restoration projects, while ensuring genetic diversity is conserved. This is of particular concern in restoration studies, where adaptive genetic variation within and among populations must be preserved, if evolutionary potential is to be maintained (McKay et al. 2005).

Materials and Methods

Field Measurements

Leaf dimensions (leaf area, length and maximal width, and the ratio of length:width, L/W) were measured in the field on one leaf of the most fully expanded leaf pairs. At each locality (Table 1), ten such leaves were measured on each of ten trees, using a Li-Cor (Model LI-3000A) portable leaf area meter. Only leaves without obvious damage or malformation were included.

Sites were selected on the basis of accessibility and geographic spread, so as to provide a network of sites separated by distances, ranging from approximately 1 km (Sites 2 and 3), through various intermediate distances up to 2,000 km (Sites 4 and 16).

The sampling design was based on a pilot study, which had shown that ten leaves per tree and ten trees per site provided sample means of leaf length, maximal width and L/W within 10% of the population mean at 95% confidence levels. For leaf area, this sampling strategy yielded sample means within 15% of the population mean at 95% confidence levels.

Initial data analysis

All field data were initially downloaded into a spreadsheet and, unless otherwise indicated, were $\log_e$-transformed to normalise their distributions. Using package nlme in R (Pinheiro and Bates 2000), linear mixed effects models with latitude and longitude as fixed effects and the variances of intercept residuals of sites, trees within sites, and leaves within trees within sites were used to identify any geographical trends in any of the leaf dimensions.

One-way ANOVAs, using $\log_e$-transformed data were calculated using SPSS™. Tukey's post-hoc Honestly Significant Difference (HSD) test (Byrkit 1987) was then applied to identify significant differences between the sites.

Intersite distances between all sites were calculated using a macro in ArcGIS™, and differences in non-transformed site means were regressed with intersite distances to examine whether differences were related to geographical distances or whether they represent discrete sub-populations.

Cluster analysis of sites by leaf character

Estimated site means from the analysis of leaf characters were re-scaled (0-1) and a hierarchical cluster analysis was performed (squared Euclidean pairwise distances clustered by average linkage between groups) to determine whether there were any geographical groupings in the sites.

Principal components analysis and bi-plot

The $\log_e$-transformed variables leaf area, length, maximal width and L/W ratio were averaged over leaves within each tree and then over trees within each site. The correlation matrix of the resultant site means was submitted to principal components analysis. Two components with eigenvalues >1 were extracted which between them mapped 99.8% of the variance among the four variables. The solution was rotated by the varimax method and scores for the sites on the two components were obtained by the regression method. The variable loadings and the site scores were jointly plotted in component space to produce a bi-plot in which the variables are represented as vectors through the origin and the sites as points. The relationships among the variables are represented by the angles among their vectors, the relationships among the sites are represented by their inter-point Euclidean distances, and the relationships between the sites and the variables are represented by the orthogonal projections of the site points on the variable vectors (Jackson 1991).

Analysis of components of variation

Multi-level analysis using MLwiN (Centre for Multilevel Modelling 2001) was used to examine components of variation for the $\log_e$-transformed data variables (leaf area, leaf length, maximal leaf width and L/W) among replicate leaves within each tree, among trees within each site, and among sites. The values obtained were compared to those found in the genetic analysis of *A. marina* by Maguire *et al.* (2000; 2002), as well as the phenotypic data from the glasshouse trial (unpublished data).

Results

Leaf dimensional data for the 16 sites are summarised in Table 2 and show that significant differences in various leaf characters occur among the sites. Neither

longitude nor latitude (which were more or less correlated in this sampling design of this study) significantly explain these leaf dimensional differences (Table 3), suggesting that broad-scale factors, correlated with longitude or latitude (such as temperature, rainfall and light intensity) are not directly involved. When differences in mean site leaf length are plotted against the distance between site pairs (Fig. 1), no significant trends with increasing or decreasing distances were found, suggesting that the site populations do not follow a differentiation by distance model. Identical relationships with the other leaf dimensions (data not shown) were also found.

Hierarchical cluster analysis of the estimated site means from the analysis of leaf characters showed that there were no coherent geographical groupings of the sites (Fig. 2), further indicating that the site populations do not follow a differentiation by distance model.

The bi-plot (Fig. 3) resulting from principal components analysis shows the relative positions of the 16 sites to the four leaf dimensional variables, and indicates a number of groupings of the sites: sites 1 (Cooktown), 13 (Port Alma) and 9 (Alva Beach) are grouped by their small leaf area; sites 15 (Boyne River) and 16 (Burrum Heads) by their large leaf width; site 4 (Cape York) by its extremely narrow leaves; site 8 (Taylor's Beach) by its extreme leaf length; sites 11 (Midge Point), 10 (Point Adelaide) and 2 (Weipa A) by their high L/W ratios; and sites 3 (Weipa B), 6 (Cairns), 5 (Port Stewart), 7 (Cardwell), 12 (Illowong Beach) and 14 (Auckland Creek) by their intermediate position in terms of all leaf dimensions.

The components of variation for the data variables among replicate leaves within each tree, among trees within each site, and among sites (Table 4) show that most variation (39-46%) occurs between replicate leaves among trees, followed by trees among sites (30-41%), with least variation among sites (18-24%).

Discussion

The non-significant relationships with longitude and latitude (Table 3) suggest that broad-scale factors such as temperature, rainfall and light intensity are not the causal factors of the significant differences in the various leaf characters among the sixteen populations. As suggested by Melville and Burchett (2002) and by glasshouse trials (unpublished data), these phenotypic differences in leaf variables were consistently expressed within estuarine neighbourhoods and under uniform glasshouse conditions and appear to be largely due to genetic rather than environmental factors. This is supported by the finding that no significant trends in the various leaf variables were found with increasing or decreasing distances between the sixteen populations (Fig. 1). In turn, it suggests that *Avicennia marina* occurs as discrete sub-populations, a finding consistent with the microsatellite data (Maguire *et al.* 2000; 2002; Giang *et al.* 2003), and with the results of the hierarchical clustering (Fig. 2) of the site means of the various leaf variables.

No geographic groupings of sites were indicated in Fig. 2, and this was also apparent in the principal components analysis (Fig. 3). Principal components analysis also showed that the various sub-populations can be characterised by their small leaf area (sites 1, 9 and 13), large leaf width (sites 15 and 16), narrow leaves (site 4), extreme leaf length (site 8), high L/W ratios (sites 2, 10 and 11) and the intermediate position

in terms of all leaf dimensions (sites 3, 5, 6, 7, 12 and 14). These scattered geographic groupings with distinct leaf dimensions over 2000 km of tropical coastline suggest that considerable genetic structuring occurs more or less randomly in this species over quite short distances (< 100 km, see Fig. 1), and it seems likely that each estuarine population has been subject to random accumulation or loss of alleles. The causes of such genetic structuring can only be speculated on at this stage, but includes founder and bottleneck effects (Saenger 1998), exacerbated by limited gene flow between sub-populations, together with some adaptive genetic variation among sub-populations (Arnaud-Haond *et al.* 2006).

The distribution of variation between the present phenotypic field data, and the phenotypic glasshouse data (unpublished data) is similar, but differs from the genotypic data (Maguire *et al.* 2000). This suggests that similar structuring processes are acting at the genetic and phenotypic levels, both in seedlings under uniform glasshouse conditions and in adult trees in the field, but that the slightly increased level of variation for trees within sites in the phenotypic data may be accounted for by minor, localised environmental influences in the field.

Given these findings, what are the implications for selection of plant materials of *Avicennia marina* for mangrove restoration projects? Apart from any private alleles a sub-population might contain, each sub-population reflects some local adaptive genetic variation, and to ensure that the entire genetic diversity of a species is conserved while its evolutionary potential is maintained, restoration projects should utilise local plant material. In view of the finding that genetic structuring occurs in

this species over quite short distances (< 100 km, see Fig. 1), a conservative approach to sourcing plant material would seem to be appropriate.

Finally, genetic structuring similar to *Avicennia marina* is being increasingly reported from other mangrove species, including *Avicennia germinans* (Dodd and Rafii 2002), *Aegiceras corniculatum* (Ge and Sun 1999), *Hibiscus tiliaceus* (Tang *et al.* 2003), *Heritiera littoralis* (Jian *et al.* 2004), *Ceriops decandra* (Tan *et al.* 2005), *C. tagal* (Ge and Sun 2001), *Bruguiera gymnorhiza* (Sugaya *et al.* 2003) and *Kandelia obovata* (Sun *et al.* 1998). Thus, the conservative approach to sourcing plant material, as suggested for *Avicennia marina,* may be applicable to most mangrove species, if local adaptive genetic variation is to be conserved.

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Table 1: Localities from which leaf dimensional data were collected in August 2005.

Site number	Locality	Longitude	Latitude
1	Cooktown	145.2476°E	15.4717°S
2	Weipa A	141.8600°E	12.6383°S
3	Weipa B	141.8650°E	12.6300°S
4	Cape York	142.5317°E	10.6917°S
5	Port Stewart	143.6950°E	14.0700°S
6	Cairns	145.7583°E	16.8800°S
7	Cardwell	146.0483°E	18.2800°S
8	Taylor's Beach	146.3317°E	18.6234°S
9	Alva Beach	147.4733°E	19.4650°S
10	Point Adeldaide	148.2683°E	20.0717°S
11	Midge Point	148.6933°E	20.6333°S
12	Illowong Beach	149.1917°E	21.1767°S
13	Port Alma	150.8117°E	23.5950°S
14	Auckland Creek	151.2300°E	23.8467°S
15	Boyne River	151.2900°E	23.9367°S
16	Burrum Heads	152.6033°E	25.1867°S

Table 2: Mean (and standard deviation) of non-transformed leaf area (cm^2), length (cm), width (cm) and length:width ratio (L/W) of *A. marina* populations. Values in columns followed by the same letters are not significantly different ($p < 0.05$ n = 100) using Tukey's HSD test.

Site number	Area	Length	Max. width	L/W ratio
1	11.95 ± 3.05^{a}	8.97 ± 1.18^{a}	2.44 ± 0.44^{a}	3.77 ± 0.73^{bcd}
2	19.28 ± 5.26^{cd}	11.64 ± 1.63^{d}	3.00 ± 0.50^{abcd}	3.94 ± 0.58^{cd}
3	17.28 ± 4.68^{bcd}	10.86 ± 1.49^{cd}	3.01 ± 0.57^{abcd}	3.72 ± 0.74^{abcd}
4	16.13 ± 4.02^{bc}	11.28 ± 1.67^{d}	2.64 ± 0.47^{a}	4.39 ± 0.93^{d}
5	18.30 ± 4.66^{cd}	11.16 ± 2.08^{cd}	3.13 ± 0.52^{bcd}	3.67 ± 0.88^{abcd}
6	15.92 ± 4.12^{abc}	10.63 ± 1.67^{bcd}	2.81 ± 0.49^{abc}	3.89 ± 0.91^{cd}
7	19.20 ± 5.29^{cd}	10.88 ± 1.59^{cd}	3.20 ± 0.65^{bcd}	3.54 ± 0.85^{abcd}
8	22.39 ± 7.30^{d}	11.81 ± 2.35^{d}	3.46 ± 0.69^{cd}	3.50 ± 0.79^{abcd}
9	15.30 ± 6.46^{abc}	9.25 ± 1.63^{ab}	2.95 ± 0.62^{abcd}	3.30 ± 0.53^{abc}
10	19.64 ± 5.22^{cd}	11.94 ± 1.62^{d}	3.04 ± 0.62^{bcd}	4.09 ± 0.96^{cd}
11	18.30 ± 4.91^{cd}	11.84 ± 2.05^{d}	2.92 ± 0.47^{abc}	4.14 ± 0.88^{cd}
12	16.03 ± 3.76^{bc}	10.21 ± 1.42^{abcd}	2.89 ± 0.50^{abc}	3.65 ± 0.84^{abcd}
13	13.24 ± 3.03^{ab}	9.05 ± 1.18^{ab}	2.77 ± 0.48^{ab}	3.36 ± 0.74^{abc}
14	17.84 ± 4.91^{bcd}	10.43 ± 1.76^{abcd}	3.21 ± 0.60^{bcd}	3.38 ± 0.89^{abc}
15	16.25 ± 3.93^{bc}	9.53 ± 1.35^{abc}	3.35 ± 0.50^{bcd}	2.90 ± 0.53^{a}
16	19.54 ± 5.21^{cd}	10.32 ± 1.32^{abcd}	3.61 ± 0.58^{d}	2.90 ± 0.41^{ab}

Table 3: Results of linear mixed effects models with longitude and latuitude and $\log_e$-transformed leaf dimensions from 16 sites.

Leaf character	**Values**	**Std. error**	**Df**	**t-value**	**P**
Area					
Intercept	16.354	9.122	1440	1.793	0.073
Longitude	-0.102	0.069	13	-1.487	0.161
Latitude	0.078	0.053	13	1.479	0.163
Length					
Intercept	6.502	5.562	1440	1.169	0.243
Longitude	-0.030	0.042	13	-0.722	0.483
Latitude	0.015	0.032	13	0.474	0.644
Width					
Intercept	9.472	5.082	1440	1.864	0.063
Longitude	-0.065	0.038	13	-1.688	0.115
Latitude	0.059	0.029	13	2.019	0.065
L/W Ratio					
Intercept	-2.964	5.158	1440	-0.575	0.566
Longitude	0.034	0.039	13	0.884	0.393
Latitude	-0.044	0.030	13	-1.447	0.164

Table 4: Comparison of the distribution of variation between levels in phenotypic and genotypic analyses of *Avicennia marina.* (Genotypic data from Maguire et al., 2000; seed and seedling growth data from glasshouse trials as yet unpublished).

Data Source	**% Distribution of variation between levels**		
	Among sites	**Trees within sites**	**Leaves within trees**
Genetic data			
Microsatellites	41-71	0-10	31-49
Phenotypic data			
Seed morphology	25-62	7-19	7-27
Seedling growth	13-60	0.2-23	33-84
Leaf length	24.2	30.3	45.5
Leaf width	19.0	38.1	42.9
L/W ratio	18.9	41.3	39.6
Leaf area	21.0	32.6	46.3

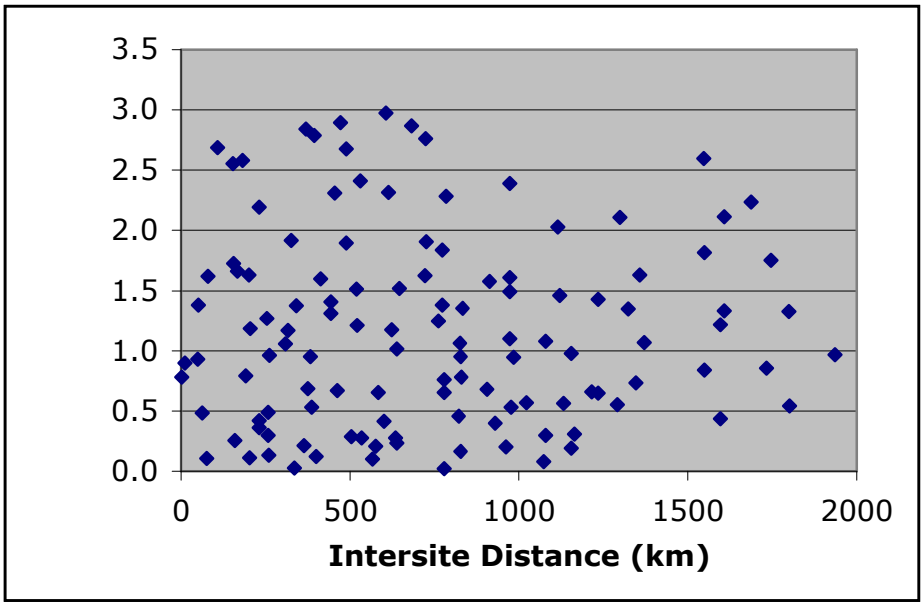

Figure 1: Plot of differences in non-transformed mean leaf length between sites against distances between site pairs.

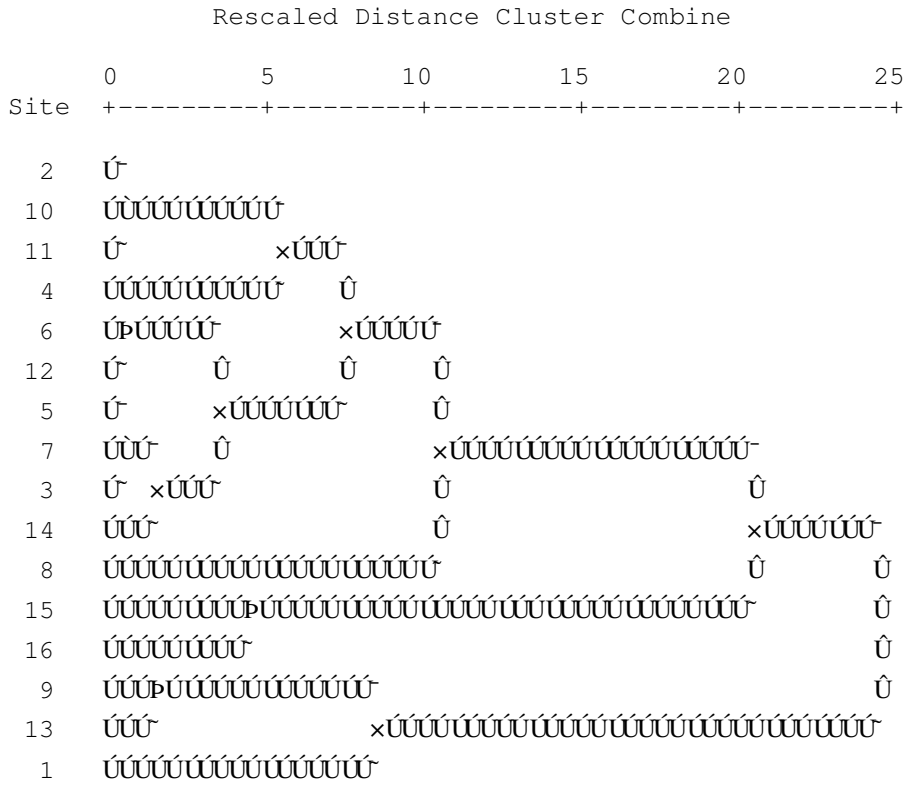

Figure 2: Output from hierarchical cluster analysis, using squared Euclidean pairwise distances between sites.

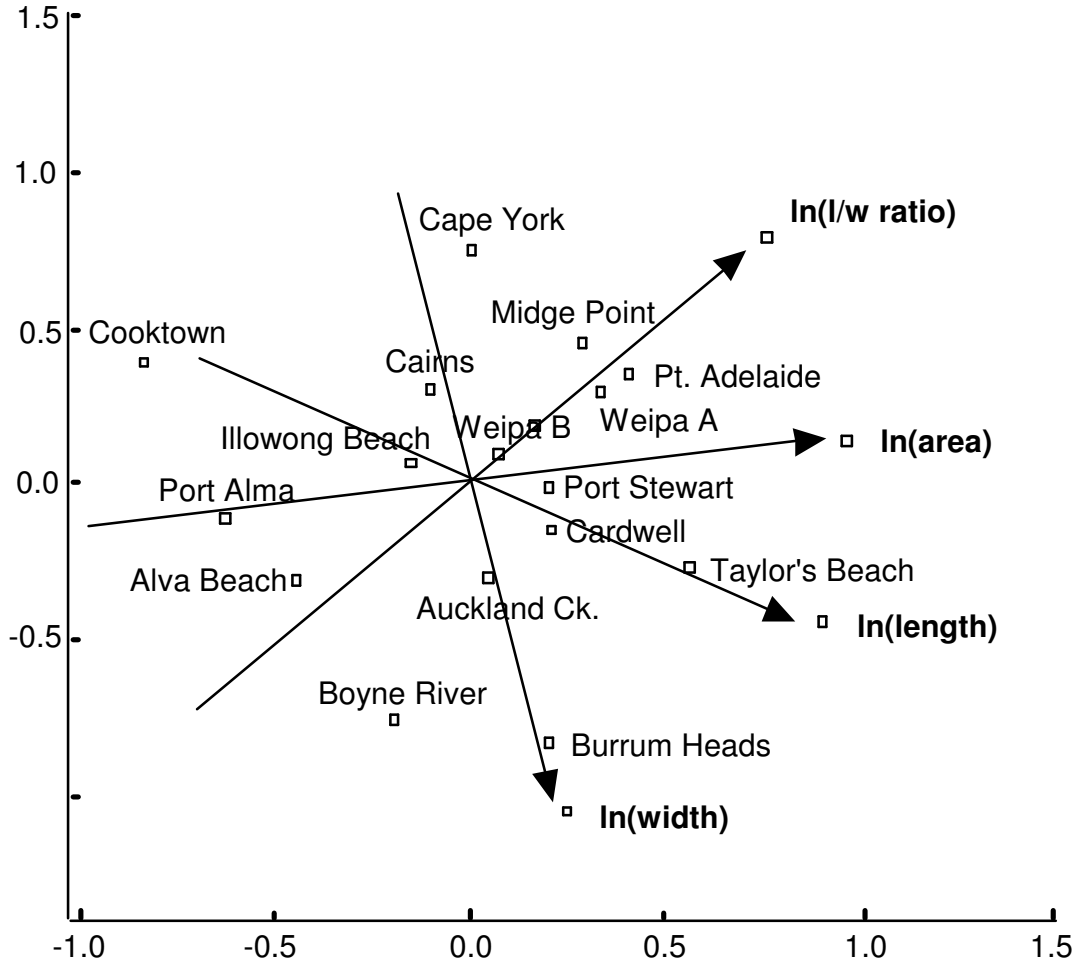

Figure 3: Bi-plot of leaf dimensional variables, represented as vectors through the origin, and the relative positions of the 16 sites to the four vectors following principal components analyses.