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Geographic Variation in *Eucalyptus diversifolia* (Myrtaceae) and the Recognition of New Subspecies *E. diversifolia* subsp. *hesperia* and *E. diversifolia* subsp. *megacarpa*

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Abstract

Patterns of geographic variation in morphological and chemical characters are documented in *Eucalyptus diversifolia* Bonpl. (soap mallee, white coastal mallee). This species is found in coastal and subcoastal Australia from southern Western Australia to Cape Nelson (western Victoria), with a number of disjunctions in the intervening region. Morphological data from adult plants collected at field localities and seedlings grown under uniform conditions were analysed using univariate and multivariate methods, including oneway ANOVA, multiple comparison tests, non-metric multidimensional scaling (NMDS), nearest neighbour networks, and minimum spanning trees. Seedling material was tested for isozyme polymorphism, and adult leaf flavonoids were analysed using liquid chromatography. Morphological and chemical characters are also documented in *E.* aff. *diversifolia*, a closely related but unnamed taxon restricted to ironstone outcrops near Norseman (WA), and putative *E. diversifolia*–*E. baxteri* hybrids from Cape Nelson. Congruent patterns in data sets distinguish three groups of *E. diversifolia* adults and progeny: (1) those to the west of the Nullarbor disjunction; (2) South Australian populations to the east of this disjunction; and (3) those from Cape Nelson. Formal taxonomic recognition of the three forms at subspecific level is established, namely *E. diversifolia* subsp. *diversifolia*, *E. diversifolia* subsp. *hesperia*, and *E. diversifolia* subsp. *megacarpa*. Patterns of geographic affinity between populations are consistent with a hypothesis of genetic exchange between normally disjunct regional populations of *E. diversifolia* via coastal land-bridges exposed during periodic times of low sea level since the mid Tertiary.

Introduction

Eucalyptus diversifolia Bonpl. (informal subgenus *Monocalyptus* Pryor & Johnson) grows as a multistemmed mallee or small tree to approximately 6 m in height. It is found in coastal and subcoastal regions from Point Culver (south-west of Madura, Western Australia) to Cape Nelson (western Victoria; Fig. 1), with a number of major disjunctions in the intervening region. In the eastern part of its range, it is found mostly on consolidated calcareous sands deposited during the Pleistocene (and on derived soils, such as terra rossa), although it also extends locally onto other surrounding soil types (Parsons 1966; Parsons and Specht 1967). In the west it occupies a narrow band some 50 m broad along the limestone scarp of the Hampton Range (Miocene), from the South Australia–Western Australia border to west of Madura. This scarp separates the Hampton Tableland (the southern part of the Nullarbor Plain) from the low lying, coastal Roe Plain. In addition, it has been reported from four small, disjunct regions of cliff-top dunes further to the south-west (the 'Culver System' of Beard 1975).

The bark of *E. diversifolia* is commonly smooth and grey, and is shed to reveal new cream bark underneath (hence the common names 'soap mallee' and 'white coastal mallee'). Leaves are held rather erect, and flowers are white. Fruit are variable in size, but typically have broad discs and four locules. The species was first described by Bonpland (1814) from specimens growing in the Malmaison Botanical Gardens in France, the seed originating from Kangaroo Island, South Australia (Bentham 1867). The specific epithet refers to the markedly different

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form of juvenile and adult leaves, although this is not uncommon for eucalypts. Specimens have been referred to by a number of names, Maiden (1903, Vol. 1) recording the following: *E. santalifolia* F.v.M., *E. dumosa* Benth. non. A. Cunn., *E. cneorifolia* DC., *E. connata* Dum.-Cours, *E. santalifolia* F.v.M. var. *firma* Miq., *E. firma* F.v.M. herb. ex Miq., *E. cuspidata* Tausch, *E. viminalis* Labill. var. *diversifolia* Benth. and *E. pachyloma* Benth. *Eucalyptus diversifolia* Woolls is an illegitimate name, referring to *E. macarthurii* Deane & Maiden (Chapman 1991).

Eucalyptus diversifolia Bonpl. was classified in series *Diversiformes* Blakely in Chippendale (1988) along with nine Western Australian species. It is the only monocalypt to occur in both eastern and western Australia. Its closest allies are *E. pachyloma*, found on siliceous sands in the Stirling Ranges, and an unnamed taxon (*E.* species C: Brooker and Kleinig 1990, henceforth referred to as *E.* aff. *diversifolia*), which is restricted to two ironstone outcrops near Norseman. This latter taxon has distinctive fruit with very broad discs. The relationship between these three taxa is unclear. Ladiges *et al.* (1987) place *E. pachyloma* and *E.* aff. *diversifolia* as sister taxa, and *E. diversifolia* as sister to these two. However, while *E. pachyloma* is distinct from *E. diversifolia* in both adult and juvenile morphology (Maiden 1920), the seedlings of *E.* aff. *diversifolia* are rather similar to those of *E. diversifolia*, especially those from Western Australia.

The aim of this study was to document patterns of variation in *E. diversifolia* in adult and seedling morphology, adult leaf flavonoids, and seedling isozymes, and to relate any patterns found to geographical, environmental, and/or historical factors. Material from *E.* aff. *diversifolia* was also examined for its similarity to Western Australian *E. diversifolia*.

Methods

Sampling Regime

Eucalyptus diversifolia was collected from 19 locations across its range in December 1994 and January 1995 (Fig. 1; site details in Appendix 2). At nine sites, five individuals (Whiffin 1982) were sampled, whereas larger collections were made at sites where populations were more variable (Madura, Eucla, Lake George and Cape Nelson). At the remaining seven locations, two or three plants were

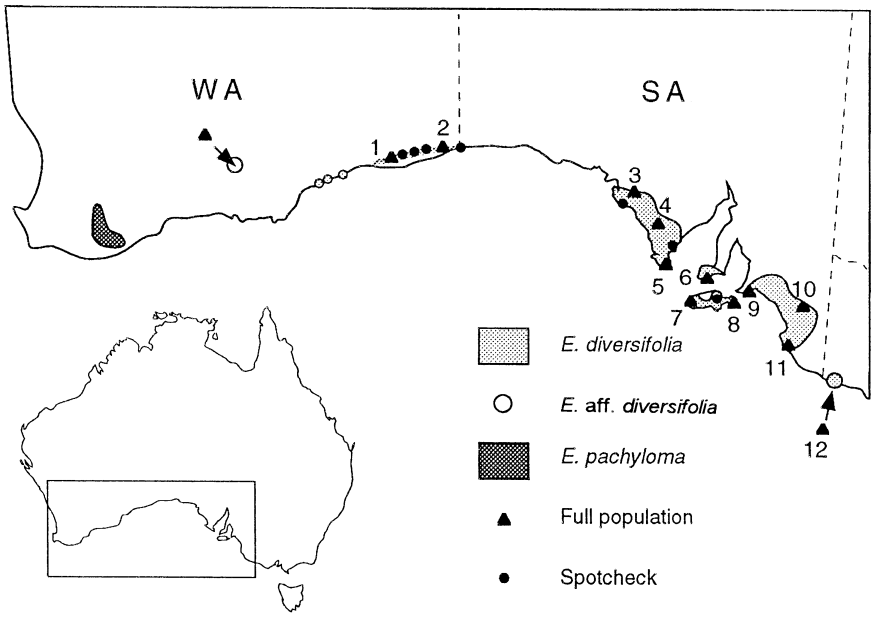


Fig. 1. Distribution and collection localities for *E. diversifolia* and related taxa. Full population localities for *E. diversifolia* referred to in text: 1. Madura; 2. Eucla; 3. Minnipa; 4. Lock; 5. Proper Bay; 6. Yorke Peninsula; 7. West Bay; 8. American River; 9. Parson's Beach; 10. Keith; 11. Lake George; 12. Cape Nelson.

sampled ('spot-checks'), to increase the geographic range of sampling. Herbarium specimens and additional mature leaves and fruit were collected from 87 trees. Mature buds and flowers were only found at two locations. In addition, *E.* aff. *diversifolia* was collected from Jimberlana Hill, near Norseman. Spot-checks were included in multivariate pattern analyses but not in univariate statistical analyses due to their small sample size. Pooling of spot-checks with full populations for statistical analyses was avoided since this could result in unintentionally grouping two 'forms' together (Thorpe 1976).

Seedling Trial

Seed from 72 *E. diversifolia* and 5 *E.* aff. *diversifolia* individuals was sown directly into 3 inch plastic tubes containing a soil mixture in the proportions: 4 parts composted pine bark (> 6 mm) : 2 parts red mountain soil : 1 part coarse sand. Dolomite, macro- and micro-nutrient preparations were added, and the soil mixture was steam pasteurised for 2 h at 67°C–71°C. Additional seed was germinated on moist filter paper in Petri dishes to supplement those pots with poor germination rates. Seedlings were thinned out to six per parent tree (three pots of two) and pots were placed in a random block design. Day and night temperatures in the glasshouse were maintained at 25° C and 18° C. Additional nutrients were supplied after 3, 7 and 8 months of growth in the form of slow-release and liquid fertilisers. Seedlings were thinned out to one per pot after 7 months when measurements had been made of most characters. Both soil and foliar fungal attacks were problematic ('damping-off'—*Pythium* spp.; 'powdery mildew'—*Peronospora* spp.), and appropriate fungicides were applied.

Morphological Characters

Nineteen quantitative characters were scored on five mature leaves and fruit from each adult tree (Table 1). For each character, a mean value was calculated from the five measurements. Size variables were measured using Vernier callipers; leaf thickness with a screw-gauge micrometer; intramarginal vein distance with a calibrated eye-piece graticule on a binocular dissecting microscope. Additional characters were measured but removed when found to be highly correlated (> 0.70) with another character and apparently measured the same feature. For example, of the character pair leaf width (w) and width 10 mm from the base (wb10), the latter was deleted although a ratio of the two, w:wb10, was included since it approximates the shape of the leaf base and was not highly correlated with any other variable.

The 15 seedling characters scored (Table 1) include measurements at three developmental stages: cotyledons (node 0), seedling leaves (node 2), and juvenile leaves (node 6). In *E. diversifolia*, seedling leaves are round to elliptical and develop only on plants grown from seed. Juvenile leaves are lanceolate to orbicular and are also produced during coppice growth. Mean values were calculated from the progeny of each parent tree, resulting in 72 *E. diversifolia* and 5 *E.* aff. *diversifolia* 'individuals'.

Use of Ratios in Morphometric Analyses

Both adult and seedling morphological data sets include a mixture of size characters and ratios that describe shape characters and/or standardise for size (e.g. leaf w:wb10, fruit disc width:width). Use of such ratios has been criticised, for example by Atchley *et al.* (1976) and Phillips (1983). The main problems identified have been: (1) non-linear change of ratios with growth (allometric growth); (2) non-normal distribution (ratios are generally right-skewed and leptokurtic); (3) high correlation with size measurements, indicating a lack of size-independence. The first problem can be avoided if only mature organs are sampled. The second problem relates specifically to analyses of variance, which require normally distributed variables and homogeneity of variance between groups (Day and Quinn 1989). Non-normality can often be reduced by use of a log transformation (e.g. Hopper *et al.* 1984; Chappill and Ladiges 1992), although this diminishes the interpretability of the results. Another method is to create each ratio in its more normal form, usually that for which the ratio of coefficients of variation is greater than unity (Frampton and Ward 1990). Ratios used in this study were created in this manner. Still, log transformation of some variables was necessary prior to ANOVA (see univariate statistics section).

The correlation of ratios with their constituent variables is more problematic. A number of mathematical approaches have been used to tackle this problem. For example, a new variable can be created that theoretically contains the size-independent 'shape' portion of a ratio (e.g. Phillips 1983; Hopper *et al.* 1984). Other methods identify the most 'redundant' of the three variables (ratio, and two constituent variables), so that it can be removed (e.g. Frampton and Ward 1983; Passioura and Ash 1993). However, approaches such as these may lead to informative variables being discarded. Accordingly, in this study when a ratio was found to be highly correlated with one of its constituent variables the variable that was felt to be least biologically informative was generally removed, with the

proviso that if the ratio were to be retained, its degree of non-normality would have to be within the range exhibited by its constituent variables.

Table 1. Characters measured for morphological analyses

Adult leaf characters	Cotyledon characters
1. length (mm)	20. length (mm)
2. width (mm)	21. width (mm)
3. distance to widest point (dwp, mm)	22. width : length
4. intramarginal vein-margin thickness (mm)	23. abaxial anthocyanins (absent or venous or abundant)
5. lamina thickness (mm)	
6. petiole length (mm)	
7. length of acuminate tip (mm)	Node 2 characters
8. width : length	
9. distance to widest point : length	24. length (mm)
10. width : width 10 mm from base	25. width (mm)
	26. width 5 mm from apex
	27. width : length
Adult fruit characters	28. dwp : length
11. length (mm)	
12. width (mm)	Node 6 characters
13. distance to widest point (dwp, mm)	29. length (mm)
14. pedicel length (mm)	30. width (mm)
15. peduncle length (mm)	31. width 5 mm from apex
16. disc width	32. lamina thickness (mm)
17. length : width	33. width : length
18. distance to widest point : length	34. dwp : length
19. disc width : width	

Isozymes

In September 1995, seed from *E.* aff. *diversifolia* and a number of populations of *E. diversifolia* was placed on moist filter paper in Petri dishes to germinate. Germination was poor, especially in seed from eastern populations. The isozyme methods used were based on the Helena Laboratories cellulose acetate gel electrophoresis system. Newly expanded cotyledons were ground in buffer and loaded onto gels with the applicator. Preparation and analysis of 13 enzyme systems followed the methods of Richardson *et al.* (1986) and Coates (1988), with the exception that the grinding buffer consisted of 0.05 M pH 9 borate, containing 1 mg ml^{-1} dithiothreitol (Sigma) and 1 mg ml^{-1} polyvinylpyrrolidone (Sigma 40T). Enzymes tested were alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT) (also called glutamate-oxaloacetate transaminase, GOT), esterase (EST), glucose phosphate isomerase (GPI), glutamate dehydrogenase (GDH), glycerate dehydrogenase (GLY), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), menadione reductase (MR), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM) and shikimate dehydrogenase (SDH).

Alleles were designated numerically with the most mobile being '1' and all others numbered in order of decreasing electrophoretic mobility. Eleven of the 13 systems exhibited no difference between samples and were deemed monomorphic. While both ADH and LAP systems were polymorphic, only the former was further investigated since this enzyme can be tested on imbibed embryos and could be examined without delay. Additional Petri dishes were set up to supplement supply of eastern seeds particularly but, once again, only a small proportion of viable seed was found. As a result, the ADH system was tested on seed from eight locations, consisting of 130 samples from 22 mother trees.

Flavonoids

Adult leaf flavonoids were analysed in both *E. diversifolia* and *E.* aff. *diversifolia*. Dried leaves from 38 adult trees, sampled from 10 localities, were finely ground. Extraction and separation of flavonoids followed the techniques of Mabry *et al.* (1970) and Markham (1982). Subsamples of 2 g were immersed in 15 mL of 80% aqueous methanol and kept in the dark for 24 h. Supernatant extracts were applied to sheets of Whatman 3MM (46 × 57 cm) chromatography paper and run in two dimensions, the first using tertiary butanol (TBA) : glacial acetic acid : distilled water (3:1:1 v/v), and the second dimension 15% aqueous glacial acetic acid. Dried chromatograms were viewed over long-wavelength UV light alone, and in the presence of ammonia vapour. Compounds in the chromatograms were considered the same if they exhibited the same colours under both viewing conditions and if their relative positions were the same.

The possibility of hybridism between *E. diversifolia* and brown stringybark, *E. baxteri*, in certain Cape Nelson individuals was investigated by running chromatograms of *E. baxteri* (from the Otways—no material was available from the Cape Nelson population), and samples containing equal parts of extracts from *E. baxteri* and the putative hybrids. Flavonoids have a reasonably simple pattern of genetic inheritance; thus, if species-specific flavonoids occur in each parent species, chemical complementation can be expected in hybrid individuals (Whiffin 1981). The *E. baxteri* samples, and those of *E.* aff. *diversifolia*, were loaded at two different levels to facilitate more accurate comparison between compounds in these taxa and *E. diversifolia*.

Multivariate Pattern Analyses

A dissimilarity matrix was generated using the Gower Metric dissimilarity index (Gower 1966). The data were represented graphically using Non-metric Multidimensional Scaling (NMDS), which has been found to be the most robust ordination method in several empirical and simulation studies (Pimentel 1981; Minchin 1987; Crisp and Weston 1993). Since NMDS is an iterative approach, convergence to the best solution does not always occur (Kent and Coker 1992). Up to 20 runs were performed to identify the best possible solution, designated by the lowest stress value (i.e. badness of fit). Patterns of inter-population affinity were clearer in three dimensions for both adult and seedling morphological data sets, but summarised sufficiently in two. Characters were regressed back into the ordination space so that linear character trends could be associated with patterns in the ordinations.

Minimum spanning trees (MSTs) were superimposed upon ordinations to identify apparent misplacements created by using a low number of dimensions (Sneath and Sokal 1973; Whiffin 1982). In addition, population mean values were ordinated and *k*-nearest neighbour networks ($k = 2$; Belbin 1987) were superimposed to further identify groups of similar populations. Data sets were also subdivided, for instance into east and west subsets, and size and shape character subsets, so as to better understand the phenetic relationship between groups.

All analyses were performed with the PATN pattern analysis package (Belbin 1987), on The University of Melbourne VAX computer.

Univariate Statistical Analyses

The Kolmogorov–Smirnov normality test (Sokal and Rohlf 1969) and Levene's homogeneity of variance test (Levene 1960) were applied to all variables prior to analysis of variance (ANOVA). Any character that failed both tests at the $\alpha = 0.05$ level was $\log_{10}$ transformed, and retested. Variables failing these tests again were not analysed further. Characters exhibiting significant differences between populations at the $\alpha = 0.05$ level were submitted to Tukey's Honestly Significant Difference (HSD) multiple comparison test (Tukey 1953), a powerful and robust test when unplanned, pairwise comparisons with unequal sample sizes are required (Day and Quinn 1989).

All statistical analyses were performed on an IBM compatible PC, using SPSS for Windows Student Version 6.1.

Results

Adult Morphology: *Multivariate Analyses*

Initially, *E.* aff. *diversifolia* was included in the ordinations, but these individuals clustered well away from *E. diversifolia*, thus compressing the region of primary interest. The data were run again using *E. diversifolia* only, using the complete data set, or size variables only, or 'shape' characters only. In all ordinations, a continuum of points resulted without sharp discontinuities; nonetheless, clusters of like individuals are evident amongst the scatter of points. Western Australian individuals cluster together, and to one side, in all ordinations,

with those from Minnipa (northern Eyre Peninsula) their nearest neighbours. Ordinations based on all characters (Fig. 2*a*), or on size characters alone (not shown), indicate a clear west–east trend, with Cape Nelson (Victoria) at the opposite side to Western Australia, and the South Australian populations in between. Amongst South Australian populations, the Lake George (south-eastern SA), Proper Bay (southern Eyre Peninsula) and West Bay (western Kangaroo Island) populations are closely associated, with most other South Australian individuals forming a dispersed group to the other side. The affinity of Yorke Peninsula individuals (y, Fig. 2*a*) is equivocal.

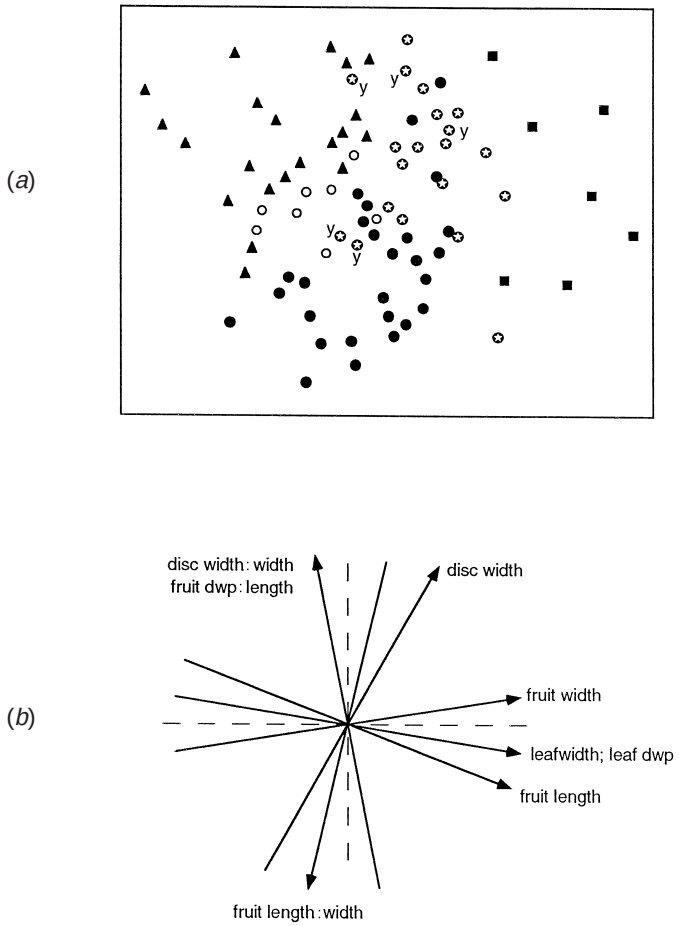


Fig. 2. (*a*) Two-dimensional NMDS ordination of 'complete' adult morphological data. ▲ Western Australia; ○ South Australia 1; ● South Australia 2; ✪ South Australia 3, including y, Yorke Peninsula; ■ Cape Nelson, Victoria. (*b*) Character vectors associated with ordination in Fig. 2*a*. Only vectors with correlation coefficients greater than 0.7 are indicated.

Population means for each character were calculated, ordinated, and a nearest-neighbour network superimposed (Fig. 3). On the bases of common patterns in the ordination of individual plants and the ordination of population means, five broad groups are suggested: (i) Western Australia: Madura, Eucla, plus spot-checks; (ii) South Australia 1: Minnipa; (iii) South Australia 2: central Eyre Peninsula (Lock, plus two spot-checks), eastern Kangaroo Island (American River plus spot-check), south-eastern SA, except Lake George (i.e. Parson's Beach and Keith); (iv) South Australia 3: southern Eyre Peninsula (Proper Bay), Yorke Peninsula, western Kangaroo Island (West Bay), Lake George; (v) Cape Nelson.

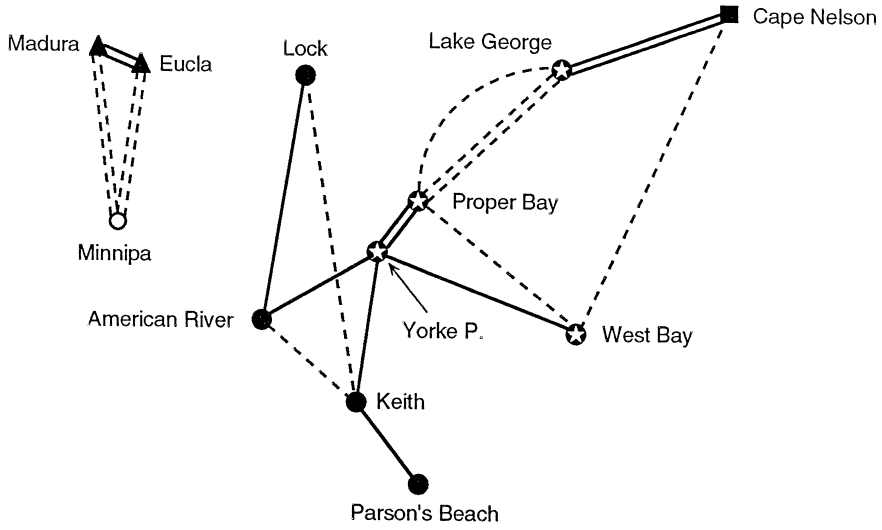


Fig. 3. K-nearest neighbour network (k = 2) superimposed upon two dimensional ordination of adult population data. Symbols indicate population affinity: ▲ Western Australia; ○ South Australia 1; ● South Australia 2; ✪ South Australia 3; ■ Cape Nelson. Each point has two lines connecting it to its two nearest neighbours. Solid lines indicate first order bonds, dashed lines indicate second order bonds. The similarity of populations can be judged by their spatial relationship as well as by the strength of bonding between them. Thus, the similarity of the two Western Australian populations is emphasised by their close proximity and the two first order bonds which connect the points. Similarly, Yorke Peninsula and Proper Bay are phenetically close.

Eight of the 19 characters exhibit high linear regression coefficients (> 0.7) with the two dimensional ordination, based on the complete data set (Table 2, Fig. 2*b*). Four characters (fruit width and length, and leaf width and distance to widest point) are associated with the west–east geographic trend in the ordination. Four other fruit characters (disc width, disc width/width, length:width, and dwp:length) are associated with the distinction of the three South Australian groups. The west–east trend of increasing fruit width and length is illustrated in Fig. 4.

Table 2. Linear regression coefficients (*r*) between adult morphological characters and fitted vectors

Leaf characters	*r*	Fruit characters	*r*
1. length (l)	0.34	11. length (l)	0.71
2. width (w)	0.71	12. width (w)	0.84
3. distance to widest point (dwp)	0.74	13. distance to widest point (dwp)	0.61
4. intramarginal vein distance (intra)	0.69	14. disc width (dw)	0.87
5. thickness (thick)	0.15	15. pedicel length (pedic)	0.19
6. petiole length (peti)	0.59	16. peduncle length (pedun)	0.19
7. length of acuminate tip (acum)	0.28	17. length:width (l:w)	0.80
8. width:length (w:l)	0.53	18. dwp:length (dwp:l)	0.77
9. dwp:length (dwp:l)	0.53	19. disc width:width (dw:w)	0.83
10. width:wb10 (w:w10)	0.65		

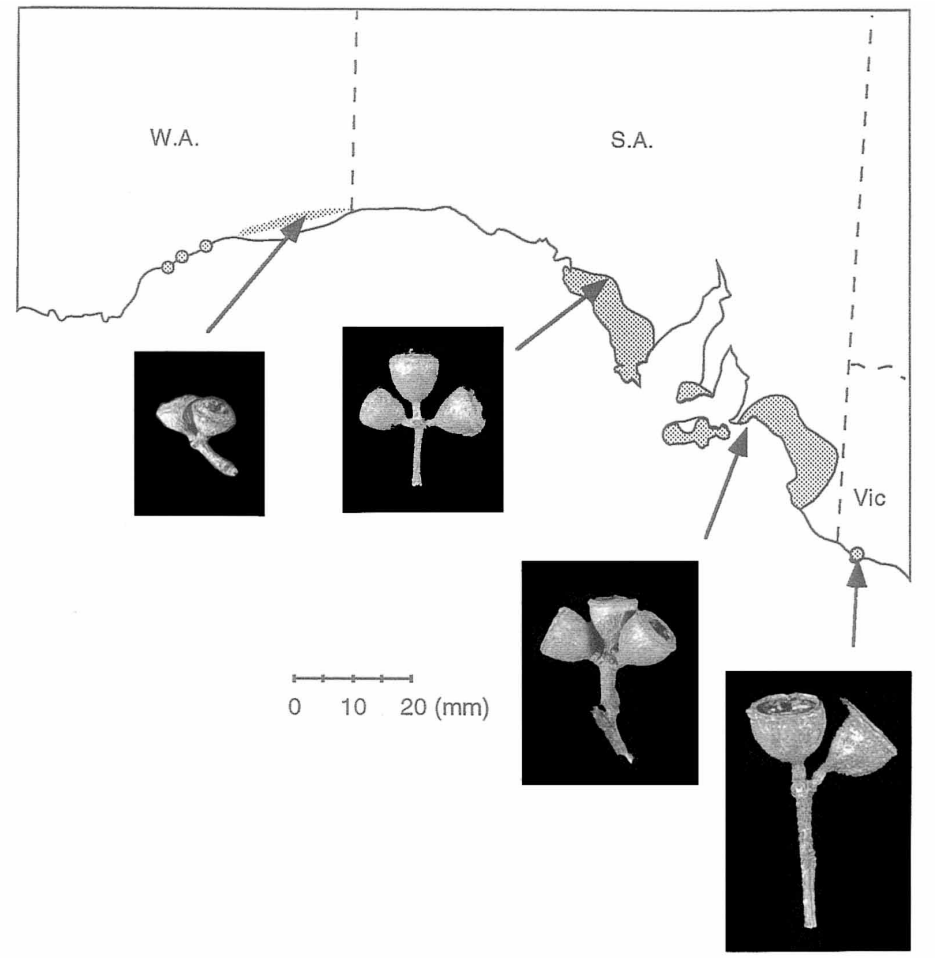


Fig. 4. *Eucalyptus diversifolia*: west–east trends in fruit length and width. Illustrated, from left to right, are fruit from East Madura spot-check 2, Minnipa (SA 1), Parson's Beach (SA 2), and Cape Nelson. Fruit from Western Australian individuals, particularly those occurring on the scarp of the Hampton Range between Madura and Eucla, tend to be smaller than those from eastern regions. Fruit from Cape Nelson individuals are the largest observed for this species.

Adult Morphology: *Univariate Analyses*

For each character, population means, standard deviations and significant differences are given in Tables 3 and 4. In general, fruit characters show more significant differences between populations than leaf characters. Characters exhibiting the greatest difference between all populations (i.e. highest *F*-values) are fruit length and fruit width. A number of characters show significant differences between WA populations and those to the east, especially Cape Nelson and Lake George. Most noticeably, Cape Nelson individuals have significantly longer and wider fruit than individuals from all other populations, albeit with one exception (Parson's Beach).

Seedling Morphology: *Multivariate Analyses*

Initial ordinations included *E*. aff. *diversifolia* progeny because a marked similarity was evident between these and certain Western Australian seedlings. However, while this similarity was evident in the ordinations, their inclusion compressed the space occupied by the bulk of *E. diversifolia* seedlings. Accordingly, two data sets were analysed separately:

Table 3. Adult leaf morphology: population mean values (*x*) and sample standard deviation (s.d.), by character

Character definitions are given in Table 1. Population abbreviations: Amer Rv = American River; Parson's = Parson's Beach; Prop Bay = Proper Bay; Yorke P = Yorke Peninsula; L.George = Lake George; C.Nelson = Cape Nelson. Group affinity, defined from multivariate analyses of adult morphological characters, indicated in parentheses; *n* = number of individuals sampled. Letters denote significant difference between upper case symbol and matching lower cases (Tukey's HSD multiple comparison; family error rate = 0.05). All size measures in mm; ratios unitless. 1: Not including putative *E. diversifolia–E. baxteri* hybrids

Population		l	w	dwp	acum	thick	peti	intra	w:l	dwp:l	w:w10
Madura (WA)	*x*	92.4	15.8	28.2a	2.3a	0.41	13.1a	0.12b	0.17a	0.31abc	1.45ab
n = 9	s.d.	14.3	1.9	3.7	0.8	0.04	2.9	0.03	0.02	0.03	0.20
Eucla (WA)	*x*	90.1	15.8	27.3a	2.3a	0.38	13.8	0.12	0.17	0.30abc	1.49
n = 6	s.d.	8.5	2.5	1.9	1.0	0.05	2.2	0.03	0.03	0.03	0.10
Minnipa (SA1)	*x*	82.2	14.4A	28.2a	3.1a	0.44	15.7	0.12ab	0.18	0.34	1.48ab
n = 8	s.d.	6.2	1.1	2.8	0.8	0.02	1.3	0.02	0.02	0.03	0.10
Lock (SA2)	*x*	95.9	16.1	34.0	2.4a	0.37	14.3	0.16	0.17	0.35	1.62
n = 5	s.d.	10.6	1.4	4.0	1.7	0.04	2.4	0.02	0.02	0.04	0.10
Amer Rv. (SA2)	*x*	89.3	15.1	32.8	2.6a	0.40	14.1	0.12	0.17	0.37	1.76
n = 5	s.d.	4.2	1.6	3.1	0.7	0.03	1.4	0.02	0.02	0.03	0.10
Parson's (SA2)	*x*	92.4	17.8	32.7	2.0a	0.42	15.3	0.14	0.19	0.36	1.7
n = 5	s.d.	10.5	1.5	3.4	0.7	0.06	1.7	0.03	0.02	0.05	0.20
Keith (SA2)	*x*	83.2	16.7a	31.4	2.7a	0.36	15.3	0.14	0.2	0.38B	1.84A
n = 5	s.d.	6.9	1.2	4.4	1.4	0.04	2.7	0.01	0.02	0.03	0.30
Prop Bay (SA3)	*x*	88.5	17.3	31.5	5.0A	0.41	16.1	0.14	0.2	0.36	1.74
n = 5	s.d.	9.6	2.2	2.5	0.8	0.02	1.4	0.03	0.03	0.04	0.10
Yorke P. (SA3)	*x*	84.4	15.6	32.1	4.0	0.41	16.4	0.13	0.18	0.38A	1.71
n = 5	s.d.	3.9	3.1	3.2	1.3	0.04	3.0	0.02	0.03	0.03	0.10
West Bay (SA3)	*x*	87.3	18.3a	32.5	2.6a	0.41	15.0	0.17A	0.21	0.37	1.72
n = 5	s.d.	10.4	0.8	4.1	1.1	0.04	1.5	0.03	0.03	0.03	0.10
L.George (SA3)	*x*	101.7	18.8a	35.0A	3.3	0.4	17.3A	0.15	0.19	0.35	1.78B
n = 6	s.d.	3.7	2.9	3.2	0.9	0.05	1.7	0.02	0.02	0.03	0.10
C. Nelson (Vic.)	*x*	88.7	18.4	33.1	3.1	0.4	16.7	0.17B	0.21A	0.38C	1.75
n = 5[1]	s.d.	7.5	2.9	2.2	1.0	0.06	1.3	0.05	0.04	0.05	0.20

Table 4. Adult fruit morphology: population mean values (*x*) and sample standard deviation (s.d.), by character

Character definitions are given in Table 1. Population abbreviations as in previous table. Group affinity indicated in parentheses; *n* = number of individuals sampled. Letters denote significant difference between upper case symbol and matching lower cases (Tukey's HSD multiple comparison; family error rate = 0.05). All size measures in mm; ratios unitless. *Failed Levene's Test in both raw, and log transformed, states

Population		l	w	dwp	dw	pedic	pedun	dw:w	l:w*	dwp:l*
Madura (WA)	*x*	7.0abc	9.3ab	6.8a	2.3	2.2	10.4	0.24A	0.77	0.97
n = 9	s.d.	0.8	1.1	0.9	0.5	0.5	2.4	0.03	0.1	0.08
Eucla (WA)	*x*	7.0abc	9.4ab	7.0a	2.2	3.2A	10.9	0.23	0.75	1.00
n = 6	s.d.	0.4	0.7	0.4	0.3	0.9	1.8	0.02	0.04	0.00
Minnipa (SA1)	*x*	8.1c	9.5ab	7.7aB	2.0a	2.4B	11.3a	0.21	0.85	0.96
n = 8	s.d.	0.7	0.4	0.8	0.2	0.8	1.3	0.01	0.07	0.08
Lock (SA2)	*x*	8.3c	9.7b	7.0a	2.0a	2.3	11.5a	0.21	0.86	0.85
n = 5	s.d.	0.9	0.8	0.8	0.4	0.3	1.2	0.03	0.08	0.11
Amer Rv. (SA2)	*x*	7.3c	9.5ab	5.8ab	1.9a	1.6a	8.4	0.2	0.77	0.79
n = 5	s.d.	0.7	0.8	0.7	0.3	0.2	1.3	0.02	0.03	0.06
Parson's (SA2)	*x*	9.3A	10.1b	6.5a	1.9a	1.3a	8.9	0.19ab	0.93	0.70
n = 5	s.d.	0.4	0.8	1.1	0.2	0.6	1.3	0.02	0.1	0.10
Keith (SA2)	*x*	8.0c	10.1b	6.7a	2.0a	1.1a	9.8	0.20b	0.8	0.84
n = 5	s.d.	0.7	1.0	1.1	0.4	0.6	2.1	0.03	0.09	0.15
Prop Bay (SA3)	*x*	7.8c	9.8b	6.9a	2.5	1.9	11.7a	0.26B	0.81	0.88
n = 5	s.d.	0.7	0.8	1.1	0.2	0.4	1.8	0.03	0.06	0.10
Yorke P. (SA3)	*x*	7.4c	10.0b	7.1a	2.3	1.5a	9.0	0.23	0.75	0.96
n = 5	s.d.	0.8	1.4	0.8	0.5	0.8	1.6	0.03	0.1	0.06
West Bay (SA3)	*x*	7.3c	10.7b	7.1a	2.6	0.9abc	7.1A	0.24	0.69	0.98
n = 5	s.d.	0.7	0.8	0.5	0.4	0.7	0.4	0.02	0.08	0.04
L.George (SA3)	*x*	8.5Bc	11.5Ab	7.5a	2.5	2.1	11.0a	0.22	0.73	0.89
n = 6	s.d.	1.3	1.3	1.6	0.5	0.8	1.7	0.03	0.05	0.12
C. Nelson (Vic.)	*x*	10.5C	13.7B	9.8A	2.9A	2.7C	11.3a	0.21	0.76	0.94
n = 5	s.d.	0.7	0.8	1.2	0.4	1.6	3.2	0.04	0.03	0.1

E. diversifolia only, and *E.* aff. *diversifolia* plus Western Australian *E. diversifolia* only. In the ordination of *E. diversifolia* only, populations cluster according to their broad geographic affinity: Victorian, South Australian and Western Australian plants occupy reasonably discrete regions of the ordination space (Fig. 5*a*). A congruent pattern of population affinity is evident in the ordination of the complete data set and in an ordination of population means with superimposed nearest neighbour network (Fig. 6). This pattern suggests the definition of four groups: (i) Western Australia (Madura, Eucla, plus two spot-checks); (ii) South Australia 1: Eyre Peninsula, minus Proper Bay (i.e. Minnipa, Lock, plus two spot-checks); Yorke Peninsula; Kangaroo Island (West Bay and American River); (iii) SA 2: Fleurieu Peninsula (Parson's Beach); south-eastern South Australia (Lake George and Keith); Proper Bay; (iv) Cape Nelson (Victoria). The affinity of West Bay seedlings (w, Fig. 5*a*) is split between the two South Australian groups, which are different from those defined on the basis of adult morphology. With the exception of Proper Bay being in a group of otherwise south-eastern South Australian populations (SA2), the groups based on ordinations of seedling morphology correspond more closely to the geographic proximity of their constituent populations.

Characters with the highest regression coefficients in the ordination of *E. diversifolia* only (Fig. 5*a*) are node 2 width and node 2 width:length (Table 5). Those with correlation coefficients greater than 0.7 are illustrated in Fig. 5*b*. Character vectors suggest that, while

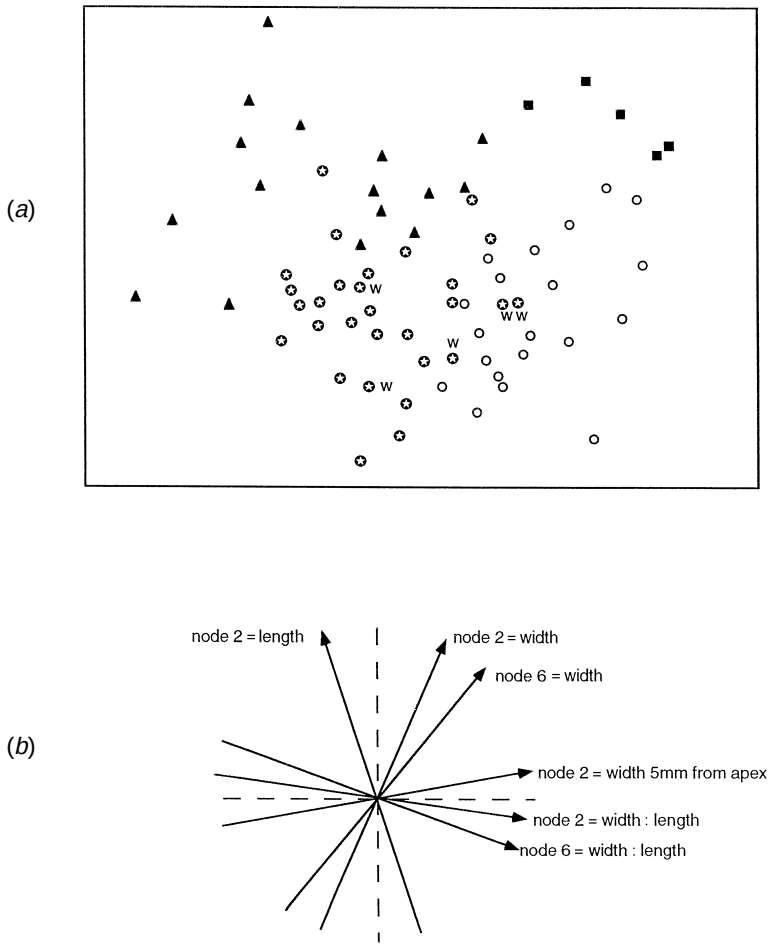


Fig. 5. (*a*) Two-dimensional NMDS ordination of seedling morphological data. ▲ Western Australia; ✪ South Australia 1 (Yorke Peninsula, Kangaroo Island, Eyre Peninsula minus Proper Bay), including w, West Bay; ○ South Australia 2 (Parson's Beach, Keith, Lake George, Proper Bay); ■ Cape Nelson. (*b*) Character vectors associated with 5*a*. Only vectors with correlation coefficients greater than 0.7 are indicated.

both Cape Nelson and Western Australian seedlings have large seedling and juvenile leaves, they are distinct from each other in shape. South Australian seedlings are mostly separated from Cape Nelson and Western Australian seedlings by having smaller seedling and juvenile leaves, while the differentiation into two South Australian subgroups is correlated with their position along the shape vectors node 2 width:length and node 6 width:length. The four groups of *E. diversifolia* seedlings were also evident in ordinations based only on size or shape variables (not shown), although less clearly. This could suggest that congruent geographic patterns exist in size and shape variables, or that the division of characters into the two subsets is somewhat false (i.e. shape variables still contain a significant proportion of size information, and vice versa).

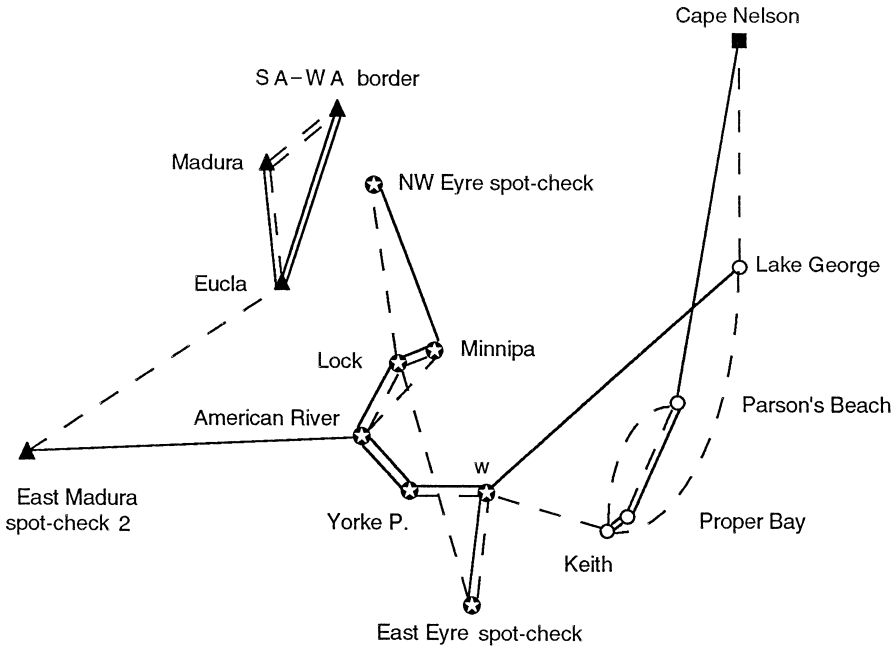


Fig. 6. K-nearest neighbour network superimposed upon two dimensional ordination of seedling population mean data. Symbols indicate population affinity (see text): ▲ Western Australia; ✪ South Australia 1; ○ South Australia 2; ■ Cape Nelson. Solid lines indicate first-order bonds, dashed lines second-order bonds. The unity of three of four Western Australian populations is evident from the strong bonding between them and their close proximity. Similarly, populations in South Australia 1 exhibit strong within-group ties. The intermediate position of West Bay (w) between the two South Australian groups can also be seen. Note: South Australian groups are different from those suggested by adult morphological data.

Table 5. Linear correlation (*r*) between seedling morphological characters and fitted vectors

Cotyledons	*r*	Node 2	*r*	Node 6	*r*
1. length (l)	0.43	5. length (l)	0.87	10. length (l)	0.68
2. width (w)	0.61	6. width (w)	0.92	11. width (w)	0.77
3. width:length (w:l)	0.57	7. width 5 mm from apex (wa5)	0.75	12. wa5	0.5
4. anthocyanins	0.43	8. width:length (w:l)	0.9	13. thickness	0.58
		9. distance to widest point:length (dwp:l)	0.51	14. width:length (w:l)	0.80
				15. dwp:l	0.66

Seedling Morphology: *Univariate Analyses*

Population means, standard deviations and significant differences for the quantitative seedling characters are listed in Tables 6 and 7. Values for *E.* aff. *diversifolia* are provided for comparison. Abundance of cotyledon anthocyanin is a multistate character, and thus not suitable for analysis with parametric methods. Three characters (cotyledon length, node 2 width and node 6 width:length) exhibited heterogeneous variance between populations and thus were not suitable for analysis; however, cotyledon length was suitable after log transformation. Characters providing most discrimination between populations are cotyledon width and width:length, node 2 length and width:length, and node 6 width. Thus, a mixture of size and shape characters vary between populations over all three developmental stages. As in analyses of adult morphology, the greatest number of significant differences occur between populations at either extreme of the species range. Although the character node 6 width:length was not able to be assessed statistically, it was evident from the seedling trial that shape of juvenile leaves differed between populations. The shape difference between western and eastern *E. diversifolia* juvenile leaves, and the similarity of *E.* aff. *diversifolia* to many western *E. diversifolia* seedlings, was obvious. Some of the more extreme forms are illustrated in Fig. 7.

Seedling Morphology: *Similarity of Western Australian* E. diversifolia *and* E. *aff.* diversifolia

Seedlings from different *E.* aff. *diversifolia* mother trees exhibited a considerable degree of morphological diversity, as did Western Australian *E. diversifolia* progeny. During early development it was often not possible to distinguish the two groups of seedlings apart. As growth proceeded differences emerged, with *E.* aff. *diversifolia* seedlings developing narrower juvenile foliage with more prominent venation. Intranode and petiole development also occurred earlier in *E.* aff. *diversifolia* seedlings than in progeny of Western Australian *E. diversifolia* (around nodes 12 and 13 in *E.* aff. *diversifolia*, but at nodes greater than 17 in Western Australian *E. diversifolia*; Table 9). An ordination of the two groups of seedlings was undertaken using the same seedling characters as used previously. Whilst *E.* aff. *diversifolia* progeny are placed away from *E. diversifolia* in the ordination space, a minimum spanning tree emphasises the fact that some *E.* aff. *diversifolia* seedlings are less similar to each other than they are to *E. diversifolia* (Fig. 8*a*). The characters that discriminate most between Western Australian *E. diversifolia* and *E.* aff. *diversifolia* are node 2 width and dwp:length, and node 6 width (Fig. 8*b*). A mixture of additional size and shape variables discriminates *E.* aff. *diversifolia* seedlings from each other.

Isozymes

Of the six putative alleles identified in the ADH system, two (alleles 1 and 6) were found only rarely (Table 8). Allele 1 was found in only two samples, both heterozygotes: one from Madura, and the other from *E.* aff. *diversifolia*; however, it is possible that these represent smudged 2-2 heterozygotes. Allele 2 was common in Western Australian *E. diversifolia* (0.40), but only found in one individual from the east (Lock, Eyre Peninsula). This allele had a frequency of 0.97 in *E.* aff. *diversifolia*. Allele 3 was the most common allele in Western Australian *E. diversifolia*, with a frequency of 0.53. It was also the most frequent allele (0.58) in individuals from West Bay (Kangaroo Island), but less common, or absent, in other eastern populations. Allele 4 was rare in Western Australian samples, but quite common in the east. Allele 5 was not found in Western Australian samples, but was present in all populations from the east, being most abundant at Lock (0.85). Allele 6 was only found in three samples, all from the Yorke Peninsula, and all from the one mother tree. Further testing is required since it is possible that allele '6' was really allele 5 misinterpreted.

Although sample sizes for eastern populations were small, a basic division between western and eastern *E. diversifolia* is suggested. In addition, Western Australian individuals are somewhat intermediate between those to the east and *E.* aff. *diversifolia* (Fig. 9).

Table 6. ***Eucalyptus diversifolia*** **and** ***E.*** **aff.** ***diversifolia*** **cotyledon and node 2 characters: population mean values (*x*) and sample standard deviations (s.d.), with significant differences between** ***E. diversifolia*** **populations indicated**

Population abbreviations as for Table 2. Group affinity indicated in parentheses; character abbreviations given in Table 4. *n* = number of parent trees (generally six seedlings per parent). Letters denote significant difference between upper case symbol and matching lower cases (Tukey's HSD multiple comparison; family error rate = 0.05). All size variables in mm; ratios and log transformed variable unitless

Population		Cotyledon characters				Node 2 characters				
		length	log (l)	width	w:l	length	width	wa5	w:l	dwp:l
Madura (WA)	*x*	15.3	1.18	10.3ab	0.68abc	56.6A	33	13.1A	0.58abe	0.40A
n = 8	s.d.	1.1	0.03	1.5	0.1	5.8	4.1	2.7	0.05	0.03
Eucla (WA)	*x*	14.3	1.15	10.9	0.77	59.6B	32	14.2	0.54abcde	0.45
n = 4	s.d.	1.3	0.04	1.1	0.03	8.6	0.9	0.1	0.05	0.04
Minnipa (SA1)	*x*	14.5	1.16	10.7a	0.75c	48.1b	28.2	13.8	0.59abe	0.42
n = 5	s.d.	0.3	0.01	0.6	0.06	4.4	3.5	2.5	0.03	0.04
Lock (SA1)	*x*	13.8	1.14	9.8ab	0.71c	45.8ab	27.1	13.8	0.59abe	0.45
n = 5	s.d.	0.5	0.02	0.4	0.03	2.4	3.7	1.5	0.07	0.03
Yorke P. (SA1)	*x*	12.8	1.10a	9.2ab	0.73c	45.1ab	27.6	15.7	0.61	0.47a
n = 5	s.d.	1.6	0.06	1.2	0.07	5.3	3.8	2.7	0.07	0.02
West Bay (SA1)	*x*	12.7	1.10a	9.5ab	0.75c	45.8ab	28.2	14.2	0.62	0.49a
n = 5	s.d.	0.8	0.03	0.9	0.04	4.2	3.2	2.1	0.07	0.02
Amer. Rv. (SA1)	*x*	14	1.14	10.0ab	0.72c	45.5ab	26.9	13.6B	0.60be	0.47a
n = 5	s.d.	2.1	0.06	2.1	0.06	5	0.8	1.4	0.06	0.02
Prop Bay (SA2)	*x*	14.1	1.14	10.5a	0.75c	41.0ab	29.4	17.7a	0.73A	0.48a
n = 4	s.d.	1.7	0.05	1.1	0.02	4	2.2	1.5	0.06	0.04
Parson's (SA2)	*x*	13.1	1.11a	10.5ab	0.81A	45.5ab	33.4	18.3ab	0.74B	0.44
n = 5	s.d.	1.9	0.06	1.1	0.04	2	2.9	2.9	0.06	0.02
Keith (SA2)	*x*	14.1	1.15	10.7a	0.77	41.3ab	28.2	16.8	0.68C	0.49ab
n = 5	s.d.	1.5	0.05	0.8	0.05	2.6	1.7	1.4	0.02	0.03
L.George (SA2)	*x*	16.6	1.22A	13.5A	0.82B	46.4ab	31.6	15.4	0.68D	0.46
n = 6	s.d.	1.7	0.04	1.2	0.05	3.7	3	1.1	0.06	0.03
C. Nelson (Vic.)	*x*	15.3	1.18	13.3B	0.88C	51.5	37.8	17.8a	0.74E	0.42B
n = 5	s.d.	2.4	0.07	1.7	0.08	3.4	1	3	0.06	0.06
E. aff. *diversifolia*	*x*	14.4	1.2	11.8	0.81	54.4	27.4	13.6	0.51	0.50
n = 5	s.d.	2.0	0.1	3.0	0.11	5.7	5.0	3.8	0.12	0.03

Eucalyptus diversifolia–E. baxteri *hybrids from Cape Nelson*

The progeny of two Cape Nelson individuals, IW7 and IW8, developed prominent papillate oil glands on their leaf margins, stem and abaxial midrib surfaces, and markedly apiculate apices on juvenile leaves. Seedlings of IW8 were otherwise similar to other Cape Nelson *E. diversifolia* progeny up to, and including, the development of juvenile leaves. However, after this stage the foliage of IW8 grew markedly larger. By contrast, IW7 progeny were always distinct, with bright green, glossy foliage. Seed from these trees exhibited a low germination rate and produced a high proportion of seedlings with reduced fitness ('runts'). Intranode and petiole development occurred earlier in the single surviving IW7 seedling than

Table 7. Node 6 characters: population mean values (*x*) and sample standard deviations (*s*), with significant differences between *E. diversifolia* populations

Group affinity, defined from multivariate analyses of seedling morphological characters, indicated in parentheses. *n* = number of parent trees (generally six seedlings per parent); l = length; w = width; dwp = distance to widest point. Letters denote significant difference between upper case symbol and matching lower cases (Tukey's HSD multiple comparison; family error rate = 0.05). All size variable in mm; ratios unitless

Population		Character					
		length	width	wa5	thickness	w:l	dwp:l
Madura (WA)	*x*	75.1A	36.2b	6.9a	20.0abc	0.49	0.27
n = 8	s.d.	15.4	4.6	1.4	1.7	0.07	0.03
Eucla (WA)	*x*	75.7	34.8b	7.9	18.1	0.49	0.27
n = 4	s.d.	21.7	1.9	0.6	0.7	0.13	0.03
Minnipa (SA1)	*x*	53.7a	30.8ab	8.5	19.2a	0.58	0.29
n = 5	s.d.	6.6	4.2	1.4	0.5	0.04	0.04
Lock (SA1)	*x*	62.3	36.0b	7.9	19.5a	0.57	0.27
n = 5	s.d.	6.0	4.0	0.5	2.1	0.06	0.02
Yorke P. (SA1)	*x*	64.1	32.1ab	6.9	17.8	0.50	0.29
n = 5	s.d.	5.1	2.2	1.8	2.1	0.03	0.04
West Bay (SA1)	*x*	61.7	36.5b	7.5	17.8	0.60	0.30
n = 5	s.d.	12.8	4.9	0.8	0.7	0.08	0.02
Amer. Rv. (SA1)	*x*	62	30.6ab	6.6a	18.8a	0.50	0.31
n = 5	s.d.	7.1	1.9	1.6	1.0	0.05	0.02
Prop Bay (SA2)	*x*	54.3	33.9b	9.6A	17.5	0.63	0.31
n = 4	s.d.	3.6	3.7	1.8	1.0	0.11	0.04
Parson's (SA2)	*x*	64.4	40.4A	9.3	17.6	0.63	0.29
n = 5	s.d.	6.3	5.2	0.8	1.0	0.05	0.01
Keith (SA2)	*x*	56.5	35.3b	9.1	16.9A	0.63	0.33
n = 5	s.d.	5.0	2.3	0.9	1.1	0.03	0.04
L. George (SA2)	*x*	60.7	35.9b	7.4	15.9B	0.59	0.31
n = 6	s.d.	6.3	3.2	1.4	1.2	0.03	0.03
C. Nelson (Vic.)	*x*	71.2	45.6B	7.4	17.3C	0.64	0.27
n = 5	s.d.	5.8	3.4	0.8	1.6	0.03	0.03
E. aff. *diversifolia*	*x*	71.1	25.0	6.6	16.0	0.36	0.32
n = 5	s.d.	7.6	s6.6	3.1	4.8	0.10	0.04

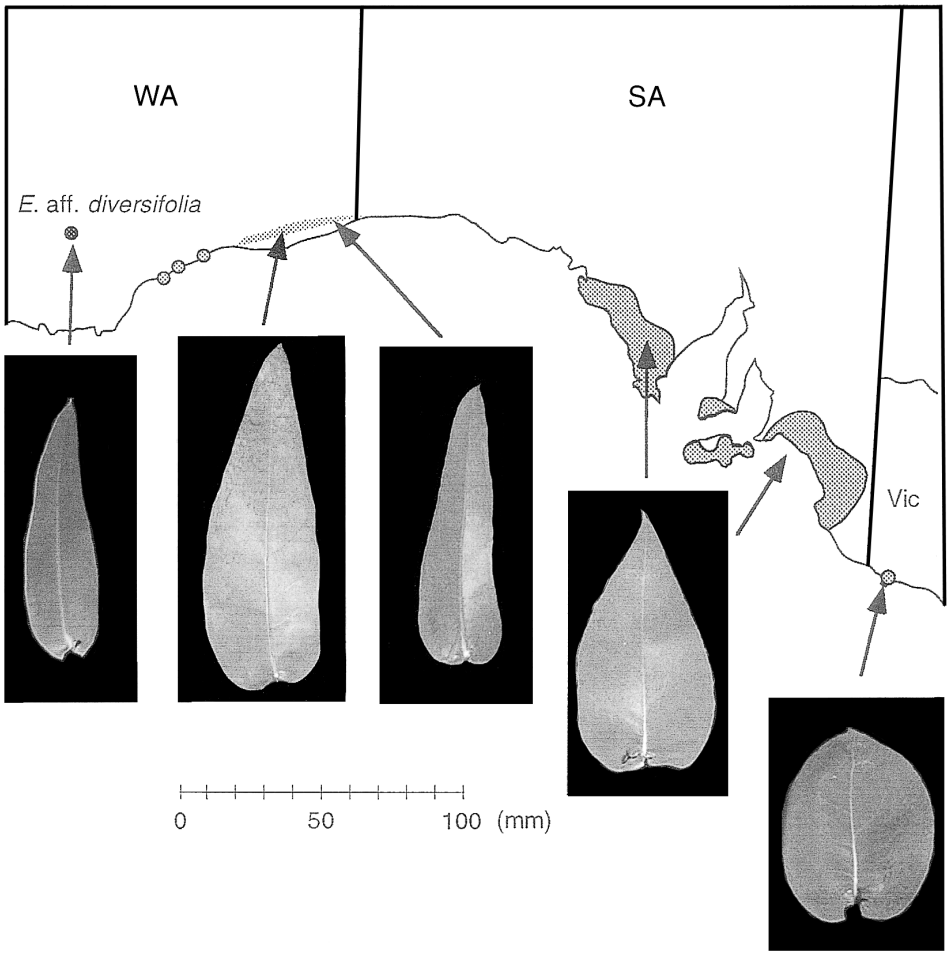


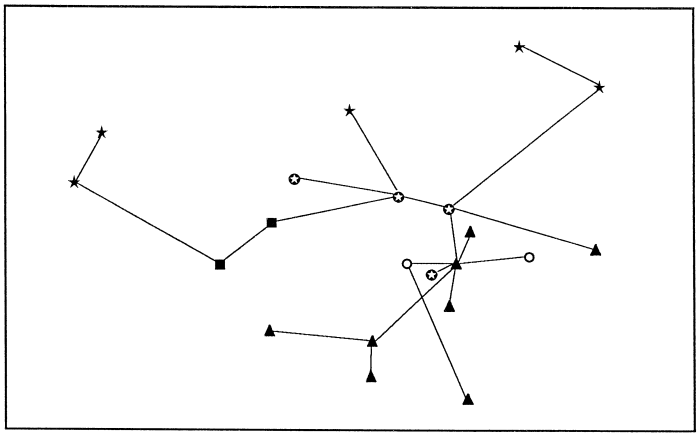
Fig. 7. West–east trend of increasing juvenile leaf width:length in *E. diversifolia*. From left to right: *E.* aff. *diversifolia, E. diversifolia* (Madura, East Madura spot-check 2, Lock, Cape Nelson). Juvenile leaves of Western Australian seedlings, although variable in size, are similar to those of *E.* aff. *diversifolia* in shape. Seedlings from South Australian populations have lanceolate to broad-lanceolate juvenile leaves. Leaves of Cape Nelson progeny are commonly wider than those of South Australian seedlings, and may be broad-lanceolate to orbicular in shape.

in 'normal' Cape Nelson seedlings, although IW8 seedlings may have been normal in this respect (Table 9).

Adult leaves of IW7 were relatively long (mean = 123.8 cm, cf. 88.7 cm for 'normal' Cape Nelson trees), with long petioles (22.2 cm, cf. 16.7 cm), although other leaf characters, including shape ratios, were not atypical. The fruit of both putative hybrids were dull green in colour and covered with warty protrusions, but not unusual in shape or size. Those of typical Cape Nelson *E. diversifolia* are brown and lack these protrusions.

The general appearance of brown stringybark (*E. baxteri*) seedlings is similar to that of IW7. *Eucalyptus baxteri* seedlings, and those of all stringybarks (series *Pachyphloiae* Blakely), also develop papillate, emergent oil glands (Ladiges 1984). Juvenile leaves of *E. baxteri* also develop apiculate apices. IW7 and IW8 were collected at a site with grey, siliceous sand, where *E. baxteri* was also found. Other Cape Nelson individuals sampled grow on yellow, calcareous sand. Taken together, the evidence is consistent with hybridisation between the two taxa at this location. This hypothesis was further investigated in the analysis of adult leaf flavonoids.

(*a*)



(*b*)

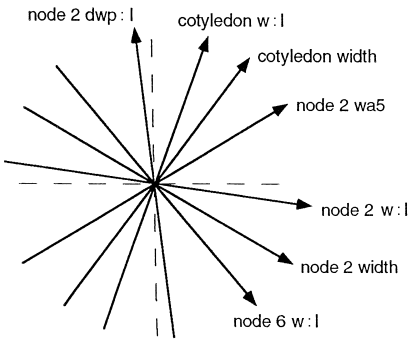


Fig. 8. (*a*) Two-dimensional ordination of Western Australian *E. diversifolia* and *E.* aff. *diversifolia* seedlings, with superimposed minimum spanning tree. Symbols indicate population from which seed was obtained. ▲ Madura; ✪ Eucla; ■ East Madura spot-check 2; O WA–SA. border spot-check. ★ *E.* aff. *diversifolia.* Points represent mean value of 4–6 seedlings per mother tree. Minimum spanning trees (MSTs) connect each point with its nearest neighbour, such that no closed loops are formed, and can be used to identify distortion associated with the portrayal of data points in only two dimensions. The MST indicates that seedlings of *E.* aff. *diversifolia* may be less similar to each other than they are to Western Australian seedlings of *E. diversifolia*. (*b*) Character vectors exhibiting highest correlation with ordination in 8.

Adult Leaf Flavonoids

Eleven flavonoid compounds were present in *E. diversifolia*, of which two were found only in putative *E. diversifolia–E. baxteri* hybrids from Cape Nelson (Table 10). Compound 9 was found only in four populations, those constituting the third South Australian group in the analysis of adult morphology. Qualitative differences between samples are easier to interpret than quantitative differences; nonetheless, variability in the amount of compounds 7 and 8 generally distinguish Western Australian samples from those of South Australia. Similarly, the absence, or only faint presence, of compounds 4 and 8 in Cape Nelson individuals distinguish them from South Australian populations. Within *E. diversifolia* then, there are possibly four flavonoid forms: Western Australia; Cape Nelson; and two from South Australia.

Seven flavonoids were present in *E.* aff. *diversifolia*, of which only one was restricted to this taxon. Compounds 4, 6 and 8, widely present in *E. diversifolia*, were not found. Compound 9, the compound common to four of five South Australian *E. diversifolia* populations, was also present in *E.* aff. *diversifolia*. Overall, the data suggest that *E.* aff. *diversifolia* is chemically distinct from all *E. diversifolia* populations sampled.

Table 8. ADH allele frequency in *E. diversifolia* and *E.* aff. *diversifolia*
Populations are listed from west to east; n1 = number of mother trees tested; *n*2 = total number of samples (seeds and cotyledons) per population

Population	Allele							
	*n*1	*n*2	1	2	3	4	5	6
E. aff. *diversifolia*	4	49	0.01	0.97	0.02	–	–	–
Madura (WA)	6	38	0.01	0.41	0.50	0.08	–	–
East Madura (WA)	2	10	–	0.35	0.65	–	–	–
Lock (Eyre P.)	2	13	–	0.04	0.12	–	0.85	–
Yorke Peninsula	2	3	–	–	–	0.17	0.33	0.50
West Bay (K.I.)	2	6	–	–	0.58	0.08	0.33	–
Lake George (SE SA)	3	9	–	–	0.17	0.33	0.50	–
Cape Nelson (Vic.)	1	1	–	–	–	0.50	0.50	–

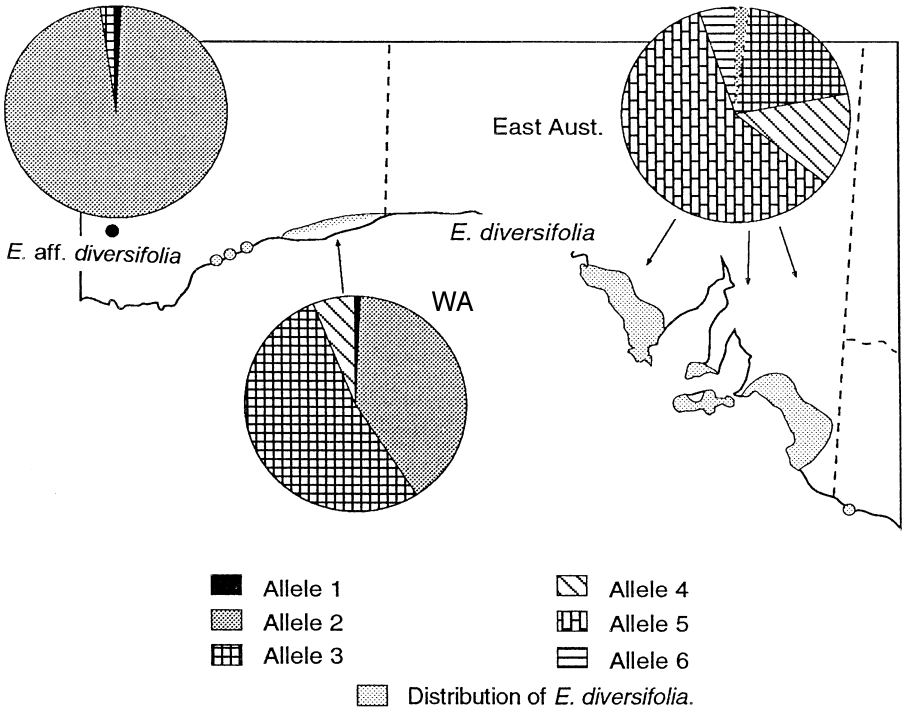


Fig. 9. ADH allele frequency in eastern and western populations of *E. diversifolia* and *E.* aff. *diversifolia* embryos and cotyledons. Allele 1 is found only in Western Australian *E. diversifolia* and *E.* aff. *diversifolia*. Conversely, alleles 5 and 6 are found only in eastern populations of *E. diversifolia*. Thus, it appears that western populations of *E. diversifolia* are intermediate between those from the east and *E.* aff. *diversifolia*.

Eucalyptus baxteri material was not available from Cape Nelson for the investigation of hybridisation between this species and *E. diversifolia*, so chromatograms were run with flavonoid extracts made from the nearest available locality (Otways National Park, Victoria). Only one of the two novel compounds in the putative hybrids was found in *E. baxteri*. This was confirmed by running chromatograms loaded with extracts made from equal parts v/v *E. baxteri* and putative hybrid extracts. Unexpectedly, neither of compounds 13 (found in

Table 9. Typical nodes at which intranode and petiole development first occur in populations of *E.* aff. *diversifolia*, *E. diversifolia* and putative *E. diversifolia–E. baxteri* hybrids from Cape Nelson, Victoria

Population	Node	
	Intranode	Petiole
E. aff. *diversifolia*	12–13	12–13
E. diversifolia		
Western Australian populations	> 17	> 17
Yorke Peninsula, West Bay, American River	14–15	14–15
Remaining South Australian populations	> 17	> 17
Cape Nelson (excluding putative hybrids)	13–14	13–14
Cape Nelson putative hybrid IW7	10	8
Cape Nelson putative hybrid IW8	10–12	14

E. baxteri) or 3 (from *E. diversifolia*) were evident in these extract mixtures; presumably this is because they were too dilute. It is unknown whether compound 12 (only found in the hybrid individuals) is a novel compound or inherited from *E. baxteri* at Cape Nelson. Nonetheless, the common occurrence of compound 11 in both *E. baxteri* and IW7 and IW8, and the commonality of several other flavonoids in these individuals and in *E. diversifolia*, is consistent with hybridisation between *E. diversifolia* and *E. baxteri* at Cape Nelson. Hybridisation between these taxa has been reported previously by Griffin *et al*. (1988; although the location is not given), and from Kangaroo Island, where the two taxa occasionally occur sympatrically on laterite soils (D. Nicolle, pers. comm.).

Discussion

Congruence of Patterns

Eucalyptus diversifolia is broadly distinguishable as three 'forms', each associated with a geographic region. One form is represented by populations to the west of the Nullarbor disjunction (hereafter, the 'western' form), one is comprised of South Australian populations to the east of the Nullarbor ('South Australian' form), while the population at Cape Nelson (not including hybrid individuals) constitutes the third form ('Cape Nelson' form). Mean values for characters such as adult leaf width, fruit size, and seedling and juvenile leaf width:length ratio increase between the western and Cape Nelson forms. In leaf flavonoid composition, Western Australian samples are mostly different from South Australian samples, while those from Cape Nelson are different again, or more similar to western, than to South Australian, samples. The western and South Australian forms differ also in ADH allele composition and frequency, while the affinity of Cape Nelson individuals in this respect is unclear since only a single sample was tested. In ADH composition, as in seedling morphology, the western form is somewhat intermediate between the eastern forms and *E.* aff. *diversifolia*.

Affinity between South Australian populations is unclear. On the basis of seedling morphology, there are two South Australian groups which correspond to broad geographic regions (south-eastern South Australia, and western plus central South Australia), with the exception of Proper Bay (southern Eyre Peninsula) being placed in the south-eastern group. By contrast, ordinations based on adult morphology suggest three South Australian groups: one has only a single population (Minnipa), while the other two groups contain populations with mixed geographic affinity. Members of South Australia Group 3 (Proper Bay, Yorke Peninsula, West Bay and Lake George) are also the only populations to exhibit flavonoid compound 9. With reference to geographic proximity to other populations, four populations

Table 10. Flavonoid compounds identified in *E. diversifolia*, *E.* aff. *diversifolia*, putative *E. diversifolia–E. baxteri* hybrids from Cape Nelson, and *E. baxteri*

+ = compound present; ? = compound present very faintly; * = tested at two different loading levels; h = putative hybrid; ID# 133, 134: *E. baxteri* from Otways National Park, Victoria; *in vitro* hybrids' contain equal parts v/v of each 'parent' extract. Compounds 1–8 were present, at least faintly, in all *E. diversifolia* populations. Compound 9 was found in *E.* aff. *diversifolia* and four *E. diversifolia* populations. Of two novel compounds (11 and 12) present in *E. diversifolia–E. baxteri* hybrids, only one was evident in *E. baxteri*

		Compound #												
Locality	ID#	1	2	3	4	5	6	7	8	9	10	11	12	13
Jimberlana Hill	30*	+		+	+	+		+		+				
(*E.* aff. *diversifolia*)	31*	+		+	+	+		+		+	+			
	264*	+		+	+	+		?		?				
	438*	+		+	+	+		+		+	+			
	441*	+		+	+	+		+		+	+			
Eucla	20	+	+	+	+	+	+	?						
	22	+	+	+	+	+	+							
	24	+	+	+		+	+	?						
	44	+	+	+	+	+	+	+	+					
	26	+	+	+	+	+	+	+	+					
Madura	32	+	+	+	+	+	+	+	?					
	34	+	+	+	+	+	+	?						
	35	+	+	+	+	+	+	+	+					
	36	+	+	+	+	?	?							
East Madura 1	42	+	+	+	?	+	+	?						
	43*	+	+	+		+	+	+						
Lock	48	+	+	+	+	+	+	+	+					
	50	+	+	+	+	+	+	+	+					
	51	+	+	+	+	+	+	+	+					
	52	+	+	+	+	+	+	+	+					

Proper Bay	54	+	+	+	+	+	+	+	+	+					
	55	+	+	+	+	+	+	+	+	+					
	56	+	+	+	+	+	+	+	+	+					
Yorke Peninsula	61	+	+	+	+	+	+	+	+	+					
	62	+	+	+	+	+	+	+	+	+					
	65	+	+	+	+	+	+	+	+	+					
West Bay	76	+	+	+	+	+	+	+	+						
	77	+	+	+	+	+	+	+	+						
	78	+	+	+	+	+	+	+		+					
	79	+	+	+	+	+	+	+	+						
Lake George	90	+	+	+		+	+	+	+	+					
	91	+	+	+	?	+	+	+	+	+					
	92	+	+	+	+	+	+	+	+	+					
Cape Nelson	1	+	+	+	?	+	+	+							
	2	+	+	+	?	+	+	+							
	6	+	+	+		+	+	+	?						
	7(h)	+	+	+		+	+	+				+	+		
	8(h)	+	+	+		+	+	+				+	+		
Otways N.P.	133*						+						+	+	
(E. baxteri)	134*						+						+	+	
Nelson–Otways	7/133	+	+			+	+	+				+	+		
(‘in vitro hybrids’)	8/133	+	+			+	+	+				+	+		
	7/134	+	+			+	+	+				+	+		
	8/134	+	+			+	+	+				+	+		

exhibit mixed, or unexpected, affinity. In adult morphology, Yorke Peninsula has affinities with both the second and third South Australian groups. West Bay (western Kangaroo Island) is similarly problematic in the circumscription of groups based on seedling morphology. In addition, West Bay associates more with south-eastern South Australian populations than with American River (eastern Kangaroo Island), which, in turn, is more closely associated with western South Australian populations. By contrast, progeny of individuals from Yorke Peninsula, West Bay and American River develop intermediate foliage earlier than those from other South Australian populations. Proper Bay individuals are distinct from other Eyre Peninsula populations on the basis of adult morphology, seedling morphology, and flavonoid composition; they also appeared to have a lower growth rate. In adult morphology, these individuals are most similar to those from Yorke Peninsula and Lake George; in seedling morphology, they are most similar to progeny of individuals from Keith and Parson's Beach.

Correlation of Patterns with Environmental Factors

In general, precipitation increases from west to east throughout the region where *E. diversifolia* occurs (Fig. 10; Specht 1972). Western Australian individuals and South Australian Group 1 (Minnipa), which clusters next to Western Australian populations in the adult ordination, occur in the driest part of this region, receiving approximately 250 mm of annual rainfall. Cape Nelson, at the opposite side of the ordination space, receives over 750 mm of rain per year. The remaining South Australian populations (i.e. adult SA groups 2 and 3) receive between 350 mm and 500 mm annual rainfall, with the exception of Parson's Beach (Fleurieu Peninsula). The west–east trends of increasing adult and juvenile leaf width, fruit size and decreasing juvenile leaf thickness may well be related to this rainfall gradient. Since seedlings were grown under identical conditions, patterns of difference in seedling morphology (or, equally, in ADH allele composition and frequency) must have underlying genetic bases; by contrast, the same cannot necessarily be claimed for trends in adult morphology.

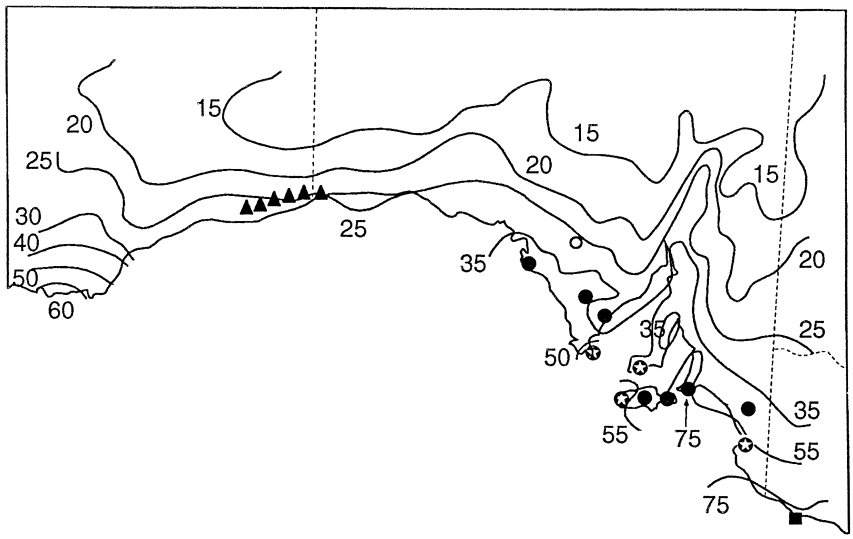


Fig. 10. Correlation of the five adult morphological groups with isohyets of annual precipitation (cm). ▲ Western Australia; ○ South Australia 1; ● South Australia 2; ✪ South Australia 3; ■ Cape Nelson. Populations from Western Australia and Minnipa (SA 1), nearest neighbours in ordinations, occur in the driest part of the region, receiving around 25 cm annual rainfall. Cape Nelson, occurring at the opposite side of ordinations, receives 78.6 cm annual rainfall. Four of seven SA 2 populations receive approximately 35 cm, while those in SA 3 receive over 50 cm annual rainfall. Thus, there is a reasonable correlation between groups defined from ordinations of adult morphology and this general index of soil moisture.

Biogeographical Interpretation

Two biogeographic hypotheses have been suggested to explain the distribution pattern of *E. diversifolia*: (1) contraction of a continuous inland distribution to disjunct, coastal refugia in response to increasing aridity throughout the Quaternary (Herbert 1928); and (2) a continuous distribution on exposed coastal plains during Quaternary glacial periods was subsequently fragmented by rising seas during interglacials (Parsons 1966, 1969; Nelson 1974). As corollaries to the latter hypothesis, Parsons (1969) suggests the Pliocene as the likely time of the species origin, while Nelson (1974) contends that it was originally geographically restricted and that it dispersed from this restricted site, during the Quaternary, to where it is found today. Distance dispersal of seed between currently disjunct areas, for example by wind or water, is improbable (Parsons 1969).

Oxygen isotopes from marine sediments indicate an approximately 100 000 year cycle of climatic change throughout the Quaternary, with extreme glacials, and interglacial periods for about 20% of the interval, and cool interstadial periods for the remainder (Chappell and Shackleton 1986). In the last two glacials, maxima sea level dropped by about 130 m, whereas in the warmest interglacials it was 5–8 m higher than present (Hope 1994). As sea level dropped, previously submerged sands were revealed. Presumably, following sufficient rainfall which leached out salt, these areas were colonised by land plants, as indicated by peat on the sea bed off south-eastern South Australia (Blackburn *et al.* 1965). Thus, land-bridges, acting as corridors for the interchange of biota, were formed between formerly disjunct areas (Green 1964; Littlejohn 1967; Martin 1972; Hopper 1979; Weston *et al*. 1984). These corridors may have been most suitable for colonisation by calcicole taxa, such as *E. diversifolia*; thus, periodic, but relatively short, 'windows of opportunity' may have existed for gene flow between otherwise disjunct, and reproductively isolated, populations of this species. Calcifuge taxa may have been precluded from colonising the exposed sediments until sufficient leaching resulted in the presence of acidic sands (Parsons 1969, 1970; Nelson 1974). The occurrence of plant taxa on the Culver dunes (probably formed within the last 10 000 years) is suggestive of a means by which taxa could survive when rising sea levels once again submerged coastal plains where they may have formerly occurred (Nelson 1974).

With a drop of 50 m in sea level, a potential migratory corridor would have been created which would join all currently disjunct areas in which *E. diversifolia* occurs (Fig. 11). By contrast, a sea level drop of only 25 m would not be sufficient to create a coastal corridor between the west and the Eyre Peninsula, the south-east to Cape Nelson, nor between Kangaroo Island and the mainland. It follows, then, that gene flow may have been possible both more frequently, and for longer periods, amongst South Australian regions than was possible between these and the west, or between Cape Nelson and south-eastern South Australia. If a greater degree of genetic mixing results in greater overall similarity, the results of this study are consistent with this biogeographic hypothesis. Perhaps, the mixed affinity of populations from Proper Bay, Yorke Peninsula, West Bay and American River has resulted from their central position in the distribution: genetic input may have occurred from all directions.

A climate significantly wetter than that at present would be required for *E. diversifolia* to occupy a continuous, inland distribution, as proposed by Herbert (1928). Although the patterns of population affinity could also be interpreted in terms of this hypothesis, there is no evidence for such a climate existing in Late Tertiary or Quaternary times. Nonetheless, Parsons (1966) proposed that the population at Cape Nelson may be a relic of a continuous, inland distribution occurring in south-eastern South Australia and western Victoria, possible only under dry conditions (e.g. glacial maxima). It is claimed that this is likely since the limiting factor to the distribution of *E. diversifolia* in this region appears to be intolerance to substrates that are not freely drained.

The hypothesis of sporadic migration across coastal land-bridges is equally applicable to *E. diversifolia* as it is to a taxon ancestral to *E. diversifolia* and *E.* aff. *diversifolia*. Whether *E. diversifolia* (or an ancestral taxon) was widespread or geographically restricted cannot be distinguished on the basis of patterns of geographic affinity in extant populations of *E. diversifolia* and *E.* aff. *diversifolia*. Furthermore, if dispersal from a formerly restricted area occurred (Nelson 1974), the direction of this dispersal cannot be determined.

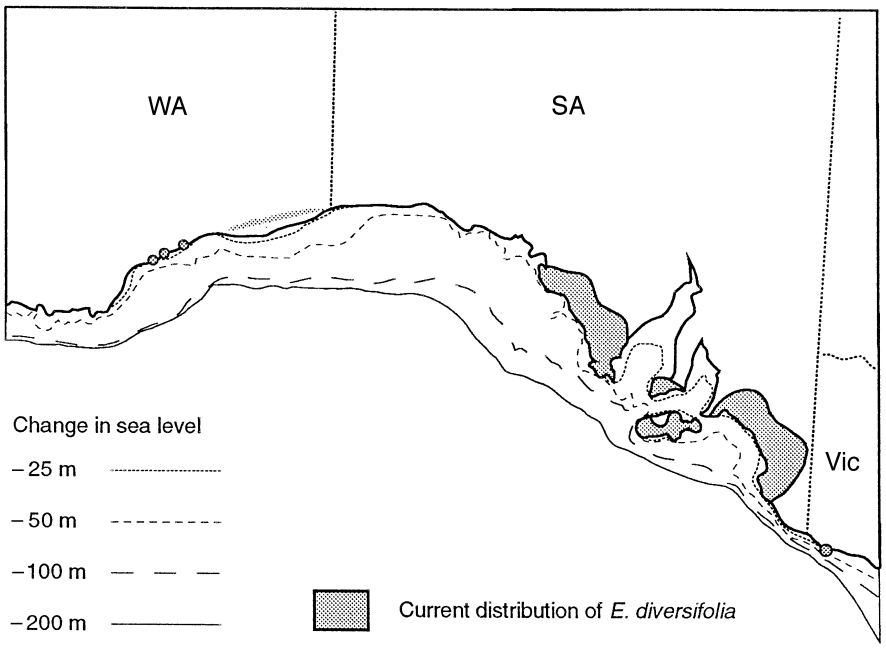


Fig. 11. Effect of Quaternary sea level changes on the southern coastline (after Nelson 1981). A drop in sea level of 50 m would be sufficient to create a narrow, but continous coastal land-bridge across the Nullarbor disjunction and join all eastern occurrences of *E. diversifolia* into a single land mass. By contrast, a sea-level change of only 25 m would not unite Kangaroo Island with the mainland, nor join the western region to those of the east by a coastal land-bridge.

Age of the Distribution Pattern in Eucalyptus diversifolia

With the exception of the Culver dune system, the calcareous sands on which *Eucalyptus diversifolia* occurs were deposited during the Pleistocene (Parkin 1969; Schwebel 1983; Parker *et al.* 1985), implying that its distribution reflects Quaternary events. However, microfossils contained within calcarenite from Kangaroo Island, Yorke and Eyre Peninsulas indicate that some of the parent material is of Miocene age, while on Kangaroo Island, additional material was laid down during marine incursions in the Pliocene (Milnes and Ludbrook 1986). Milnes and Ludbrook (1986) concluded that in these areas, while some deposits were formed during the Pleistocene, others were formed by reworking of Early Miocene sediments during the Late Miocene and Pliocene. Just as Pleistocene deposits provide suitable substrate for *E. diversifolia* today, older deposits may have provided a substrate for this taxon, or an ancestral taxon, prior to the Pleistocene. Marine sediments, exposed by the retreat of Eocene, Miocene, or Pliocene seas, could have acted as corridors for interchange of biota between formerly disjunct areas (e.g. Roberts and Maxson 1985). Thus, the age of the substrate on which *E. diversifolia* occurs cannot be used to infer the timing of the arrival of the taxon, or an ancestral taxon, to these areas. Interestingly, silicified fruit casts found near Woomera (Lange 1978; Ambrose *et al.* 1979), of possible Eocene or Miocene age, have a single opercular scar and broad discs, similar to those of eucalypts in series *Diversiformes*, and various stringybarks (series *Pachyphloiae*). This evidence is consistent with the presence of the *E. diversifolia* lineage in southern Australia in the Middle or Late Tertiary, implying that the age of the distribution pattern is older than has been previously suggested.

Conclusions

Patterns of geographic variation in morphological and chemical characters of *E. diversifolia* are congruent in distinguishing individuals, and their progeny, into three

groups: (1) those to the west of the Nullarbor disjunction; (2) South Australian populations to the east of this disjunction; and (3) those from Cape Nelson. Formal taxonomic recognition of the three forms at subspecific level is considered appropriate (Appendix 1). Examination of adult material used in this study, and additional herbarium material, indicates that intermediate individuals occur which, without viewing seedling material or performing chemical analyses, would be difficult to place in any one particular group. Rather than recognition at specific level, splitting of the taxon at subspecific rank would allow indeterminate material to be referred to a species name (Thiele 1994).

Patterns of geographic affinity between populations are consistent with a hypothesis of genetic exchange between disjunct, regional populations of *E. diversifolia*, via coastal land bridges exposed during periods of low sea level. Although this species is characteristically found on substrates of Pleistocene age, suitable substrate may have been available since the mid Tertiary.

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Appendix 1. Taxonomy

Eucalyptus diversifolia Bonpl., Description des Plantes Rares cultivées à Malmaison et à Navarre 35 (1814); Chippendale, *Flora of Australia* **19**: 129 (1988).

Type specimen grown from seed collected from Kangaroo Island, South Australia; that is, equivalent to South Australian form in this study (*E. diversifolia* subsp. *diversifolia*; distribution: coastal and sub-coastal areas from north-west Eyre Peninsula to Beachport).

Key to Subspecies in *E. diversifolia*

1. Fruit > 1.2 cm wide. Juvenile foliage deep green, broad-lanceolate to orbicular; intranode and petiole development occurring at nodes 13–14. Occurring at Cape Nelson, Victoria. . . . . *E. diversifolia* subsp. *megacarpa*

1. Fruit < 1.2 cm wide. Juvenile foliage glaucous to deep green, lanceolate to broad-lanceolate; intranode and petiole development occurring > node 13. . . . . .
   2. Mallee < 3 m in height with grey bark, fibrous towards stem bases. Juvenile foliage glaucous, lanceolate. Adult leaves narrow-lanceolate, lanceolate or falcate. Restricted to limestone scarp of the Hampton Tableland (extending from around Madura to just east of WA–SA border) and 'Culver System' of cliff-top dunes (Beard 1975), Western Australia. . . . . .
   . . . . . .*E. diversifolia* subsp. *hesperia*
   2. Mature trees to 6 m in height, may be single- or multi-stemmed. Bark commonly grey and smooth to stem base. Juvenile foliage glaucous to deep green, lanceolate to broad-lanceolate. Adult leaves lanceolate or falcate. Occurring in coastal and sub-coastal regions of South Australia from north-west Eyre Peninsula to Beachport. . . . . *E. diversifolia* subsp. *diversifolia*

Eucalyptus diversifolia* ssp. *hesperia Wright & Ladiges, subsp. nov.

A subspecie typica foliis adultis juvenilibusque generaliter angustioribus, foliis juvenilibus pallidovirentibus et fructibus minoribus differt; et a subsp. *megacarpa* eadem dimensionibus foliorum juvenilium fructuumque differt; et utrisque seminibus fuscatioribus differt.

It differs from the typical subspecies in the generally narrower adult and juvenile leaves, lighter green juvenile leaves and smaller fruit; from subsp. *megacarpa* it differs strongly in the same juvenile leaf and fruit dimensions; from both it differs in the darker seeds.

Type: Madura, Western Australia. Limestone scarp opposite Madura turn-off from Eyre Highway (31° 53′ 55″ S, 127° 01′ 19″ E), Jan. 1995, *IW 29* (*holotype*: MEL 2037230; *isotype*: PERTH 046 23444).

Mallee to 3 m with grey bark, mostly smooth but fibrous towards base. Prostrate individuals to 0.5m occur on exposed coastal cliffs. Adult leaves alternate, somewhat erect, moderately thick, dull, concolorous, grey-green to blue-green, narrow-lanceolate, lanceolate or falcate, 6.6–12 cm long, 1–2.8 cm wide, rarely acuminate with tip to 5 mm long. Petiole 0.8–1.8 cm long. Flowering time November to January, but also intermittently in some other months. Up to 13 buds per inflorescence. Operculum conical, same width as hypanthium. Stamens irregularly flexed, white. Fruit obconical to cup-shaped, 0.5–0.8 cm long and 0.8–1.2 cm wide; peduncle 0.6–1.6 cm long, sometimes flattened; pedicels 0.1–0.4 cm long. Disc broad, generally flat to slightly convex, sometimes strongly convex or descending; 4-loculate, but 3 not uncommon. Fertile seeds dark brown to red-brown, D-shaped. Sterile seeds (‘chaff’) red-brown. Cotyledons reniform to slightly bilobed, stalked, 1–2 cm long and 0.7–1.3 cm wide. Abaxial anthocyanin variously absent, restricted to veins, or abundant. Seedling leaves opposite, sessile, discolorous, elliptical, 3.8–7.6 cm long, 2.4–4.3 cm wide; grading into juvenile leaves by nodes 5 or 6. Juvenile leaves opposite, amplexicaul, discolorous, glaucous, lanceolate, 4.9–10.1 cm long, 2.4–4.9 cm wide. Intranode and petiole development occurs after node 17.

Etymology

From the Latinised Greek for west, *hesperius*, referring to the distribution of the taxon.

Eucalyptus diversifolia* ssp. *megacarpa Wright & Ladiges, subsp. nov.

A subspecie typica fructibus generaliter majoribus, cotyledonibus latioribus orbicularioribusque et foliis juvenilibus atrovirentibus differt; et a subsp. *hesperia* eadem characteribus fructuum cotyledonum et foliorum plantularum valde differt.

It differs from the typical subspecies in the generally larger fruit, wider cotyledons, wider and rounder seedling leaves and darker green juvenile leaves; from subsp. *hesperia* it differs strongly in the same fruit, cotyledon and seedling leaf characters.

Type: Cape Nelson, Victoria. Roadside, Portland–Cape Nelson road, 300 m before state park entrance, December 1994, *IW 2* (*holotype*: MEL 2037230).

Spreading mallee, although sometimes single-stemmed in wetter and more sheltered sites, ranging from 3 to 6 m. Bark smooth and grey, decorticating in ribbons to reveal cream to light brown new bark. Adult leaves alternate, somewhat erect, moderately thick, dull, concolorous, grey-green to blue-green, lanceolate to broad-lanceolate, sometimes falcate, 7.5–10.7 cm long, 1.3–2.5 cm wide, sometimes acuminate with tip to 8 mm long. Petiole 1.4–2.1 cm long. Flowering time November to January. Up to 13 buds per inflorescence. Operculum conical to domed, same width as hypanthium. Stamens irregularly flexed, white. Fruit cup-shaped, 0.9–1.2 cm long and 1.2–1.6 cm wide; peduncle 0.6–1.6 cm long, sometimes flattened; pedicels 0–0.5 cm long. Disc broad, generally flat to slightly convex; 4-loculate, but 3 not uncommon. Fertile seeds light tan, D-shaped. Sterile seeds (‘chaff’) tan, difficult to distinguish. Cotyledons reniform to slightly bilobed, stalked, 1.2–2.3 cm long and 1.3–1.8 cm wide. Abaxial anthocyanin faintly present, or restricted to veins. Seedling leaves opposite, sessile, discolorous, orbicular to elliptical, 4.3–5.4 cm long, 3.2–4.6 cm wide; grading into juvenile leaves by nodes 5 or 6. Juvenile leaves opposite, amplexicaul, discolorous, deep green, broad-lanceolate to orbicular, 4.8–8.9 cm long, 2.7–6.8 cm wide. Intranode and petiole development occurs at nodes 13 or 14.

Etymology

From the Greek *megas* (huge) and *karpos* (fruit).

Notes

Hybrids with *E. baxteri* occur on siliceous sands, distinguished by larger, broad leaves, fruit with warty protrusions; seedlings with deep green to bright green seedling and juvenile leaves, may be shiny; raised oil glands on leaf margins and stems.

Appendix 2. Collections used in the study

(All collections made in January 1995, except where indicated. IW: I. J. Wright; PYL: P. Y. Ladiges; JCM: J. C. Marginson)

Eucalyptus diversifolia* subsp. *hesperia

WESTERN AUSTRALIA: Madura, limestone scarp (Hampton Range) on west side of road opposite Madura turn-off from Eyre Highway (31° 53′ 55″ S, 127° 01′ 19″ E), *IW 25–29*, *IW 32–37*; Moodini Bluff, c. 24.2 km E of Madura on limestone scarp north of Eyre highway (31° 54′ 43″ S, 127° 16′ 20″ E), *IW 38, 39*; East Madura 1, 63.8 km E of Madura on limestone scarp (31° 53′ 32″ S, 127°41′ 01″ E), *IW 40*, *41*. East Madura 2, c. 110 km E of Madura on limestone scarp (31° 49′ 58″ S, 128° 07′ 32″ E), *IW 42, 43*; Eucla, 39.5 km W of Eucla, on rift in limestone scarp (31° 46′ 40″ S, 128º 29′ 30″ E), *IW 20–24, IW 44,45*; SA–WA border South Australia, coastal cliff near roadside parking area, Eyre Highway, C. 16.8 km E from border (31° 39′ 36″ S, 129° 10′ 05″ E), *IW 18,19*.

Other Specimens Examined

(selection only)

WESTERN AUSTRALIA: Eucla, *Baron F. von Mueller*, (no date) (MEL); Kuthala Pass, 1.0 km N of Mundrabilla roadhouse, *K. Hill 2164 & L. A. S. Johnson*, 3 Nov. 1986 (CBG, FRI, MEL, NSW, PERTH); Madura Pass, *M. I. H. Brooker 5623*, 2 Apr. 1977 (AD, MEL, NSW, PERTH); 15.2 km S of highway on track to Eyre telegraph station, *K. Hill 2175 & L. A. S. Johnson*, 3 Nov. 1986 (CBG, FRI, MEL, NSW, PERTH); near Moodini Rockhole, on south scarp of Hampton Tableland, c. 22 km E of Madura, *E. N. S. Jackson 3485*, 25 Sept. 1977 (AD, NSW).

Eucalyptus diversifolia* subsp. *diversifolia

SOUTH AUSTRALIA: Minnipa (1) roadside, c. 14 km N of Minnipa town centre on Minnipa–Yardea Road (32° 42′ 00″ S, 135° 10′ 30″ E), *IW 9–11*; Minnipa (2) roadside, 23.3 km W of Minnipa on Eyre Highway (32° 45′ 47″ S, 134° 57′ 05″ E), *IW 12–17*. Streaky Bay, c. 34 km SE of Streaky Bay along Flinders Highway roadside (32° 59′ 15″ S, 134° 29′ 11″ E), *IW 46*, *47*; Lock, roadside, 8.4 km N of Lock on Kyancutta–Port Lincoln Road (33° 30′ 54″ S., 135° 42′ 03″ E), *IW 48–52*; Proper Bay, 5.3 km SW of Port Lincoln racetrack on Proper Bay Road, c. 50 m from beach (34° 46′ 53″ S, 135° 48′ 37″ E), *IW 53–57*; East Eyre, 4.3 km west of Port Neill along Port Neill–Karkoo Road (34º 04′ 56″ S, 136º 17′ 33″ E), *IW 58–60*; Yorke Peninsula, c. 20.2 km from Stenhouse Bay on Stenhouse Bay–Warooka Road (35° 08′ 53″ S, 137° 05′ 12″ E), *IW 61–65*; West Bay, Kangaroo Island, 1.5 km SW along West Bay Road from West Bay camp ground, Flinders Chase National Park (35° 53′ 31″ S, 136° 33′ 52″ E), *IW 75–79*. Cygnet River (Kangaroo Island, SA): roadside, Playford Highway, 19.3 km E from Parndana (35° 44′ 8″ S, 137° 26′ 45″ E), *IW 73, 74*. American River, Kangaroo Island: track off main road out of American River (35º 48′ 41″ S, 137° 44′ 32″ E), *IW 68–72*; Parson's Beach, roadside, Parsons Beach Road, Newland Head Conservation Park (35° 37′ 37″ S, 138° 28′ 37″ E), *IW 80–84*; Keith, roadside, 0.4 km W along Mt Monster Road, from highway between Keith and Naracoorte (36° 12′ 04″ S, 140° 19′ 50″ E), *IW 85–89*; Lake George (SA), 4.3 km W along road to Lake George from main Robe–Beachport road (37º 21′ 44″ S, 139° 56′ 09″ E), *IW 90–95*.

Other Specimens Examined

(selection only)

SOUTH AUSTRALIA: Roadside of Mt Thisby, Kangaroo Island, *M. I. H. Brooker 8277*, 19 Aug. 1983 (FRI, NSW); 12 km S of Penneshaw, beside Wilson River, Kangaroo Island, *F. E. Davies 1458 & B. Hadlaw*, 30 Nov. 1989 (AD, CBG, MEL, NSW); coastal sand dunes, Sturt Bay, c. 14 km SE of Warooka, Yorke Peninsula, *A. E. Orchard 2833*, 15 July 1970 (AD, NSW); Thistle Island, Spencer Gulf, *J. H. Maiden*, Jan. 1907 (NSW); 6 miles SE of Keith, *D. E. Symon 3517*, 27 Aug. 1965 (AD, CANB, K, NSW); 17.2 km S of Nora Creina turnoff on road around Lake George, *M. I. H. Brooker 11546*, 7 Aug. 1993 (AD, CANB, MEL, NSW); 57 km SE of Streaky Bay, towards Port Kenny, *M. I. H. Brooker 7448*, 25 Apr. 1982 (FRI, NSW).

Eucalyptus diversifolia* subsp. *megacarpa

VICTORIA: Cape Nelson (1), roadside, Portland–Cape Nelson road, 300m before state park entrance, *IW 1–5*, Dec. 1994; Cape Nelson (2), roadside, unnamed road, heading east from Portland–Cape Nelson Road, near previous locality (38° 25′ 25″ S, 141° 32′ 40″ E), *IW 6*, Dec. 1994.

Other Specimens Examined

(selection only)

VICTORIA: Cape Nelson, *K. L. Wilson 1158 & L. A. S. Johnson*, 18 Feb. 1975 (NSW); Cape Nelson area, *P. C. Heyligers 80033*, 31 July 1980 (AD, FRI, NSW); Cape Nelson, *I. Clarke 2210*, 2 Dec. 1992 (MEL); Cape Nelson, *P. Carolin*, 24 Oct. 1985 (MEL); 6 miles SW of Portland, Cape Nelson, *J. H. Willis*, 15 June 1964 (MEL).

Eucalyptus* aff. *diversifolia

WESTERN AUSTRALIA: Jimberlana Hill, c. 5 km NE of Norseman (32° 08′ 53″ S, 121° 48′ 37″ E), *IW 30, 31*. Also *PYL 438, 441*, May 1983; *PYL 264*, Nov. 1981.

Eucalyptus baxteri

VICTORIA: Otways National Park (1), Lower catchment of Parker River (38° 49′ S, 143° 31′ 30″ E), *JCM 133*, Nov. 1978; (2) Parker River Road., near Blanket Bay intersection (38° 50′ S, 143° 34′ E), *JCM 134*, Nov. 1978.

***Eucalyptus diversifolia* × *E. baxteri* Hybrids**

VICTORIA: Cape Nelson roadside, unnamed road, heading east from Portland–Cape Nelson road (38º 25′ 25″ S, 141° 32′ 40″ E), *IW 7, 8*, Dec. 1994.

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