DETECTION AND PREDICTION OF HETEROSIS IN *EUCALYPTUS GLOBULUS*

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ABSTRACT

Significant specific combining ability effects (SCA) for two year growth were detected in a factorial of *Eucalyptus globulus* ssp. *globulus*. This factorial was established across 5 locations in Australia and involved crossing between and within two provenances (King Island and Taranna) in addition to crossing males from both provenances to a single female from south Flinders Island. We attempted to predict heterosis using genetic distance between parents calculated using RAPD markers. We isolated DNA from all 8 females and 19 out of 26 male parents. Data were collected from 99 different RAPD bands. Sixty-six out of the 99 RAPD bands scored were polymorphic. A UPGMA dendrogram based on Nei's distance resulted in parents within provenance being clustered together in all but one case. The degree to which genetic distance predicted heterosis was statistically significant but accounted for less than 5% of the variation in SCA, and mainly reflected the greater genetic differentiation between parents involved in interprovenance crossing. SCA effects within provenances were unpredictable.

Key words: *Eucalyptus globulus*, RAPD, prediction of heterosis, SCA, genetic distance, breeding values

INTRODUCTION

The exploitation of heterosis is a common objective in plant breeding (MAYO 1987). In forest tree breeding this usually takes the form of strategies to avoid inbreeding depression (ie. negative heterosis) or the exploitation of positive heterosis through deployment of high performing full-sibs in either family or clonal forestry. Heterosis is measured through crossing designs that allow the calculation of specific combining ability (SCA) of sets of parents (NIKLES 1992). Testing SCA with trees is an expensive endeavour. It would therefore be useful if heterosis could be predicted before mating, to reduce the number of parents to be tested.

Attempts to predict SCA have used morphological diversity (PRASAD & SINGH 1986; GHADERI *et al.* 1984; COWEN & FREY 1987a), kinship coefficient (LEFORT-BUSON *et al.* 1987b; COWEN & FREY 1987b), distance between geographic origin (LEFORT-BUSON *et al.* 1987a; MOLL *et al.* 1965) and genetic distance calculated from various genetic markers. Genetic markers used to assess the distance between parents include isozymes (FREI *et al.* 1986; LAMKEY *et al.* 1987), seed proteins (LEONARDI *et al.* 1991) and RFLPs (MELCHINGER *et al.* 1990; DUDLEY *et al.* 1991). In some annual plant species heterosis first increases with increasing genetic divergence then decreases at higher levels of divergence (MOLL *et al.* 1965; WASER & PRICE 1989). This response is believed to be due to a balance between inbreeding depression at one extreme of relatedness and outbreeding depression in widely divergent genotypes at the other (LYNCH 1991). The rationale for using genetic markers as a predictor is that up to a point, the genetic distance between two parents at neutral loci may be indicative of the closeness of their relationship and as such may be predictive of firstly, the number of deleterious alleles shared by the two parents and secondly the degree of expected heterozygosity in the progeny. No previous studies have been undertaken with tree species.

Random amplified polymorphic DNA (RAPD) (WELSH & MCCLELLAND 1990; WILLIAMS *et al.* 1990) markers are a type of genetic marker, based on the polymerase chain reaction, that seems particularly useful with trees. RAPDs require only small amounts of plant tissue, polymorphism for RAPDs is abundant, and most importantly the technique is easy to use and to automate (GRATTAPAGLIA *et al.* 1992). RAPDs have

also been found to be more time and cost efficient than RFLPs when the number of samples is small (RAGOT & HOISINGTON 1993).

Eucalyptus globulus ssp. *globulus* is an important pulpwood tree species (VOLKER & ORME 1988). It is widely planted throughout the temperate world especially in Australia, Chile, Portugal and Spain (ELDRIDGE *et al.* 1993). Domestication of spp. *globulus* has started, with most programs just commencing their first generation of breeding (ELDRIDGE *et al.* 1993). High levels of inbreeding depression occur in this taxon (HARDNER & POTTS 1995) and significant SCA effects occur for early growth (HODGE *et al.*, in press; POTTS *et al.* 1995). The objective of this study is to compare the magnitude of SCA effects in different populations of *Eucalyptus globulus* ssp. *globulus*.

MATERIALS AND METHODS

Crossing and trial designs

Twenty-six *E. globulus* males from Tasmanian native stands on King Island (K) and Taranna (T) were mated to three females from King Island, four from Taranna and one from South Flinders Island (SF) in a factorial manner as indicated in Table 1. Taranna and King Island are separated by c. 430 km. Trees from Taranna and King Island were both collected along c. 25 km of roads. This design resulted in a series of intra– and interprovenance crosses. The provenance locations were given in POTTS & JORDAN (1994). The progeny of most crosses was grown at up to five widely separated locations in Australia (Table 1 and 2). All sites were planted using an alpha lattice design (PATTERSON &

Table 1 The number of sites on which each cross in the incomplete factorial (8 females × 26 males) was planted (T = Taranna, K = King Island and SF = South Flinders Island)

Males	Females							
	K1	K2	K5	SF8	T1	T4	T5	T7
T139	5	5	1	1	1	5	5	5
T140	3	1	1	1	3	2	5	1
T141	5	5	–	–	3	5	2	4
T142	2	5	–	1	–	2	5	4
T143	5	2	–	1	–	5	5	–
T144	5	2	5	3	1	5	5	5
T145	5	5	5	2	2	5	5	4
T146	5	5	3	1	1	2	5	2
T147	5	5	1	1	5	5	5	–
T148	5	5	1	2	–	4	5	2
T149	5	3	1	4	3	4	5	5
T150	5	3	1	1	2	5	5	2
T151	5	5	1	5	–	2	5	4
T152	–	3	1	1	1	4	4	4
T153	5	5	2	1	1	1	5	–
T154	5	4	2	2	2	5	2	3
K155	4	5	1	2	–	1	5	–
K156	3	5	5	4	1	–	1	3
K157	5	–	3	5	–	–	1	5
K158	5	5	2	3	2	–	4	4
K159	5	2	–	2	–	–	1	5
K160	5	5	5	4	–	–	–	–
K161	5	2	4	3	–	–	2	–
K162	5	5	5	5	–	1	1	–
K163	5	5	3	3	–	–	1	2
K164	5	5	5	5	–	1	–	–

Table 2 Details of trial design and sites on which *E. globulus* factorial was grown

Trial	Bega (NSW)	Flynn (Vic)	Parkham (Tas)	West Ridgley (Tas)	Manjimup (WA)
Latitude	36°36'	38°18'	41°26'	41°09'	34°12'
Longitude	149°46'	146°41'	146°37'	145°46'	116°01'
Altitude (m)	120	170	205	185	240
Average annual rainfall (mm)	875	620	1,025	1,200	1,069
Establishment date	8/90	7/90	8/90	7/90	7/90
Measurement date	5/92	6/92	8/92	8/92	3/93
No. of replicates	4	4	4	4	7
No. incomplete blocks/replicate	10	11	13	15	11
Trees/plot	5	5	5	5	3
No. families	81	111	134	168	90

WILLIAMS 1976). There were generally four replicates of up to 25 incomplete blocks per trial with trees of each family arranged in line plots (Table 2). Diameter and height were measured at age two for 4 sites and age three for the Manjimup site (WA). Conic volume was calculated following POTTS & JORDAN (1994).

Parameter estimation

The conic volume of each tree was log transformed and then standardised by multiplying by the ratio of the pooled within plot variability across all sites over the pooled within plot variability of the corresponding site (eg. VISCHNER *et al.* 1991). Analysis of the *E. globulus* factorial was undertaken according to the model –

$$y = X_1 b + X_2 c + Z_1 a + Z_2 s + Z_3 p + e$$

where y is an n×1 vector of individual volume observations, b is a vector of fixed effects for incomplete blocks, c is a vector of fixed cross (T×T, K×K, K×T, T×K, SF×T and SF×K) effects, a is a vector of unobservable additive genetic effects (ie. breeding values of individuals and parents), s is a q×1 vector of random genetic effects common to each full–sib family (ie. specific combining ability), p is a p×1 vector of random effects common to each plot (ie. plot effect) and e is an n×1 vector of residuals which includes three quarters of the dominance variance and environmental error. X_1, X_2, Z_1, Z_2 and Z_3 are known incidence matrices relating observations y to effects in b, c, a, s and p, respectively. This analysis and the estimation of additive (σ^2_a), SCA (σ^2_s), plot (σ^2_p) and error (σ^2_e) variances were undertaken with a derivative-free restricted maximum likelihood method (DFREML) as implemented by MEYER (1991) following an individual tree model. Full details of this approach are given in VOLKER *et al.* (1994).

Estimates of σ^2_a, σ^2_s, σ^2_p and σ^2_e were used to calculate individual narrow–sense heritabilities (h^2) and the proportion of dominance variance (d^2) as:

$$h^2 = \sigma^2_a / (\sigma^2_a + \sigma^2_s + \sigma^2_p + \sigma^2_e)$$

$$d^2 = \sigma^2_d / (\sigma^2_a + \sigma^2_s + \sigma^2_p + \sigma^2_e)$$

respectively, where $\sigma^2_d = 4 * \sigma^2_s$ (FALCONER 1981). Tests of significance were obtained by comparing differences in likelihoods ($-2\log L$) from the fitted model to those obtained when either h^2 or d^2 were set to zero and then testing the deviance as a χ^2 with one degree of freedom (MCCULLOCH & NELDER 1989, pp. 476–478). Analyses were undertaken with and without the fixed cross effect using data from all 26 males and 8 females as well as for different subsets of the data (i.e. T×K and K×T; T×T; and K×K).

Once estimates of additive, SCA, plot and error variance were obtained, best linear unbiased predictions of the breeding values (BV) of the parents, the specific combining ability (SCA) of each full-sib family and estimates of the fixed effects were calculated with the mixed model program PEST (GRONEVELD 1990). This analysis was undertaken using the model excluding cross-type effects. Thus our BVs directly include population differences and our SCAs include between population interaction effects. The difference in breeding value between parents (DBV) was then calculated for each family.

RAPD markers

Total DNA was isolated from young leaf tissue using modifications of the CTAB method of DOYLE & DOYLE (1990). These modifications were as follows: twice as much extraction buffer was used per gram of leaves (8

Table 3 Estimates of genetic parameters for transformed volume in the full *E. globulus* factorial with fixed cross type effects in the model (Total), with fixed cross type effects removed (Pooled for interprovenances crosses (K×T; includes T×K), and intraprovenance crosses (K×K and T×T) amongst King Island (K) and Taranna (T) parents. The female from south Flinders Island (SF8 was included only in the Total and Polled estimates. The approximate standard error of the heritability estimate is in parenthesis and the significance of the likelihood ratio test for the difference of the heritability (h^2) and dominance variances (d^2) from zero are indicated

	Total	Pooled	K×T	K×K	T×T
No. parents	34	34	32	13	20
No. families	172	172	62	28	57
No. progeny	10,793	10,793	3,646	2,293	3,,702
Mean transformed volume	2.84	2.84	2.88	2.90	2.69
Additive variance (V_A×100)	4.2	3.4	4.5	1.4	6.7
Dominance variance (V_D×100)	7.5	6.1	4.6	0.7	3.2
Plot variance (V_P×100)	8.3	8.2	9.3	9.6	6.6
Error variance (V_E×100)	32.7	34.0	37.4	32.5	31.3
Phenotypic variance (V_P×100)	52.6	51.8	55.9	44.2	47.8
h^2	0.07	0.08	0.08	0.03	0.14
(se)	(0.02)	(0.02)	(0.05)	(0.03)	(0.06)
	***	***	***	*	***
d^2	0.14	0.12	0.08	0.01	0.07
	***	***	*	ns	*
V_A/V_D	0.56	0.56	0.98	2.2	2.1

ns = not significant, * P < 0.05; ** P < 0.01; *** P < 0.001

mL/g of leaves); 5% insoluble PVP (Sigma Co. Cat No. P–6755, St–Louis MO 63178 USA) was added to the extraction buffer. Following the first precipitation, the solubilized DNA was treated with RNAse and then re–precipitated in the presence of 2.0M NaCl (FANG *et al. 1*992).

We isolated DNA from all 8 female and 19 out of 26 male parents in the factorial. The DNA from each was assayed for Random Amplified Polymorphic DNA (RAPD) markers (WILLIAMS *et al.* 1990). Primers were obtained from Operon Technologies Inc (10000 Atlantic Ave., Alameda CA 94501 USA). Primers OPC–01, OPC–02, OPC–05, OPC–11, OPC–19, OPD–02, OPD–03, OPD–05, OPD–08, OPD–11, OPD–12, OPE–03, OPE–07, OPE–14, OPM–02, OPM–04, OPM–12, and OPM–16 were used. Amplification conditions were as in WILLIAMS *et al.* (1990) except that 150 mg/mL of BSA (Bovine Serum Albumen) was added to each reaction. Twenty mg of DNA per reaction was used. Consistency of interpretation was established by repeated blind scoring. Using presence/ absence of bands, the genetic distance (D) between parents was calculated using the formulas of Nei (NEI 1972), Euclidian distance (SAS 1991) and the Bray Curtis distance (BRAY & CURTIS 1957). Genetic distances were calculated according to procedures in SAS. Cluster analysis (UPGMA) was performed on the genetic distance matrix using a computer program written by MILLER (1991).

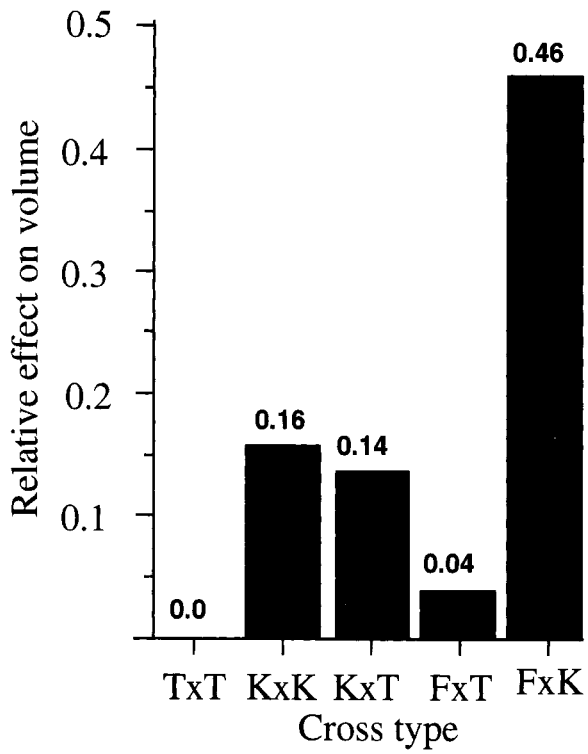

Figure 1 The effect of cross type on transformed conic volume. Population codes are T for Taranna, K for King Island and F for South Flinders Island. Values are expressed relative to T×T. The South Flinders Island population is represented by a single female (SF8)

Spearman Correlation Coefficients between the predicted SCAs, Nei's genetic distance (D) and the difference in breeding value (DBV) between parents were calculated with PROC CORR in SAS. The regressions of SCA and DBV on D were calculated using PROC REG in SAS. DNA could not be extracted from all parents used in the factorial, nor could SCA values be calculated for all combinations of parents from which DNA was extracted due to the failure of some matings. Therefore, these analysis were performed using only the data set in which both SCA and D could be calculated (n = 117).

RESULTS

Genetic parameters for conic volume

The proportion of genetic variation in early growth across the five trials is relatively small ($h^2 + d^2 < 22\%$; Table 3), yet when the whole factorial is considered, a significant proportion of this genetic variation can be attributed to dominance genetic variation (d^2 = 12–14%). The estimates of additive genetic variation for two year volume were low. However, the likelihood ratio test indicated that individual narrow–sense heritabilities were significantly different ($P < 0.001$) from zero in all but the King Island cross type (K×K) (Table 3). The T×T intraprovenance crosses had higher levels of both additive and non–additive genetic variation for growth than the K×K, with the T×T crosses, containing nearly 5 times more total genetic variation. Narrow–sense heritability was significantly ($P < 0.05$) higher in the T×T than in the K×K cross type, whereas the proportion of dominance variation in the two cross types was not significantly different.

Dominance genetic variation was only half that of the additive genetic variation (V_A/V_D) in intraprovenance crosses. Whereas in interprovenance crosses the ratio was close to one (Table 3). Dominance variation was more important in interprovenance crosses than in intraprovenance crosses. In contrast, the additive genetic effects in K×T were intermediate between that of T×T and K×K. When the female from south Flinders Island was included, dominance variation became highly significant and was nearly double that of the additive genetic variation in the factorial, even when

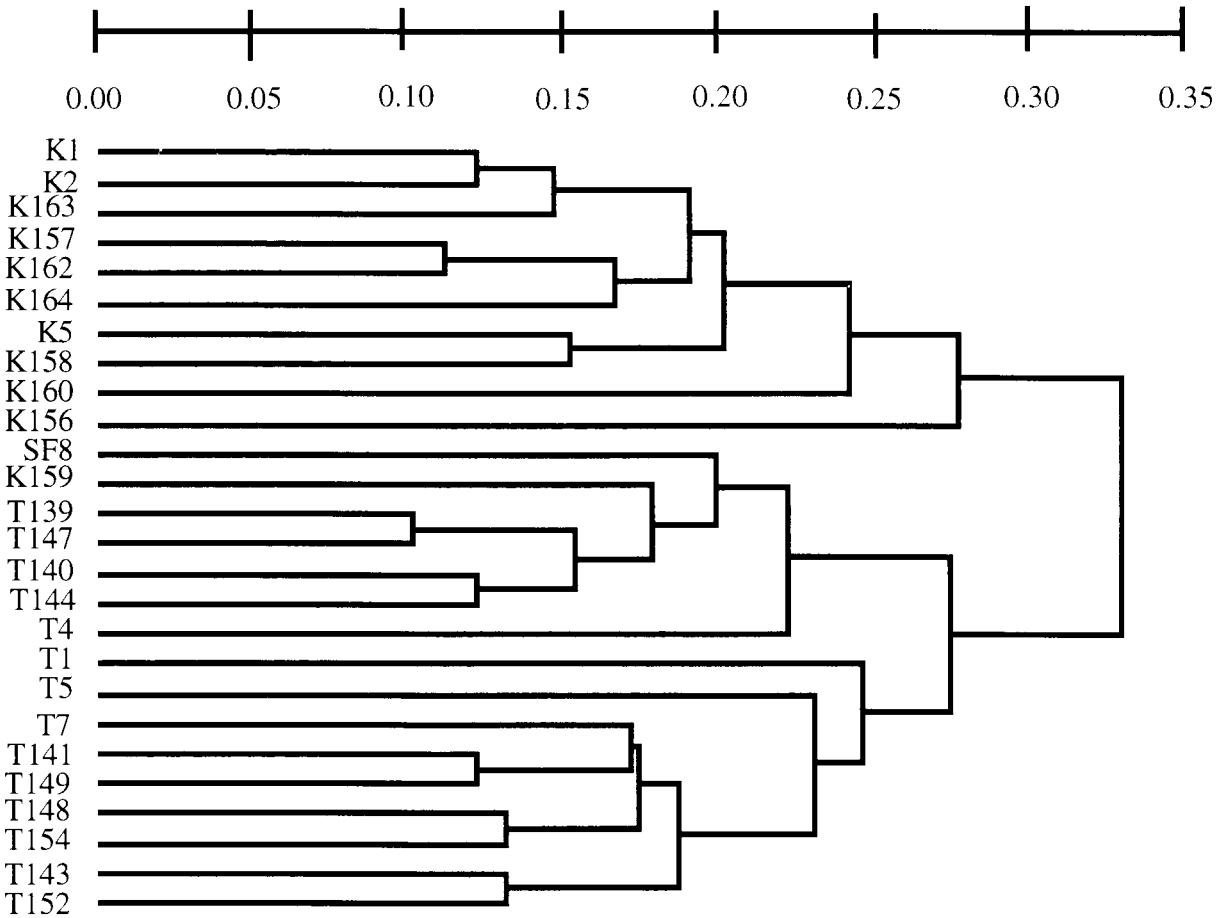

Figure 2 UPGMA dendrogram based on Nei's genetic distance between 26 *Eucalyptus globulus* trees of three provenances, King Island (K), South Flinders Island (SF8) and Taranna (T) using 99 RAPD markers

Table 4 Nei's genetic distance (*D*) specific combining ability (SCA) and difference in breeding value (DBV) for *E. globulus* ssp. *globulus* F_1's by cross type

Measure	Cross type[a]	N	Mean	SD	Min	Max
D	All	117	0.23	0.05	0.13	0.39
	K×T	41	0.25	0.06	0.13	0.39
	K×K	22	0.21	0.04	0.14	0.28
	T×T	37	0.22	0.04	0.15	0.30
SCA[b]	All	117	4.2	8.5	−26.6	28.2
(×100)	K×T	41	2.4	5.8	−8.6	13.4
	K×K	22	−3.3	8.1	−16.4	15.8
	T×T	37	−0.8	8.6	−26.6	15.5
DBV	All	117	0.23	0.13	0.01	0.54
	K×T	41	0.24	0.16	0.01	0.54
	K×K	22	0.17	0.07	0.05	0.28
	T×T	37	0.26	0.13	0.04	0.48

[a] Interprovenance cross type are coded as K×T, intraprovenance cross types are coded according to provenance origin: T = Taranna and K = King Island

[b] Both SCA and BVD effects were calculated using the complete data set of 172 families ignoring cross type and excluding the outlier family shown in Fig. 2

Table 5 Regression analysis of genetic distance (D) on specific combining ability (SCA), D on the difference in breeding value (DBV), and DBV on SCA

Variable[a]	N	F value	Prob > F	R^2 (%)
D on SCA[b]				
Ignoring provenance	117	5.0	0.03	4.2
Interprovenance K×T	41	3.0	0.09	7.2
Intraprovenance T×T	37	0.2	0.63	0.7
Intraprovenance K×K	22	0.3	0.59	1.5
D on DBV				
Ignoring provenance	117	3.1	0.09	2.6
Interprovenance K×T	41	0.9	0.36	2.2
Intraprovenance T×T	37	4.1	0.05	10.5
Intraprovenance K×K	22	0.7	0.43	3.1
DBV on SCA				
Ignoring provenance	117	5.2	0.02	4.3
Interprovenance K×T	41	0.3	0.62	0.6
Intraprovenance T×T	37	4.0	0.05	10.3
Intraprovenance K×K	22	1.5	0.24	7.0

[a] The independent variable is followed by the dependent variable,

[b] Both SCA and BVD effects were calculated using the complete data set of 172 families ignoring cross type and excluding the outlier family shown in Fig 2.

cross type effects (T×T, K×K, K×T, T×K, SF×K and SF×T) were removed (Table 3). The effect of cross type was significant ($\chi^2 = 6.7$; $P < 0.01$), due mainly to the better performance of the south Flinders Island female when crossed to King Island males (Fig. 1).

RAPD data

RAPD data were collected from 99 different RAPD bands, sixty-six of which were polymorphic. The three different distance measures (Nei's, Euclidian and Bray

Curtis) were all highly intercorrelated (Pearson Correlation Coefficients ranged from 0.97–1.0). Therefore, the data presented below used only one measure, Nei's genetic distance. A UPGMA dendrogram was produced using Nei's distance (Fig. 2). The two provenances of *E. globulus* were well separated in this dendrogram. All trees from Taranna (T) were clustered together. All trees from King Island, except for one (K159), clustered together. This grouping is consistent with the larger genetic distances observed between parents involved in interprovenance (T×K) compared to intraprovenance crosses (Table 4). Within provenance, no obvious correlation between clustering and spatial proximity (geographic distance between trees in native stands) was found.

Prediction of SCA

Genetic distance (D) between parents explained a low (R^2 = 4.2%), but statistically significant (P < 0.05) proportion of the variation in SCA using all data and ignoring provenance effects (Table 5). Likewise, the Spearman correlation coefficients between SCA and D (r = 0.20; P = 0.03) was marginally significant. This association mainly reflected the tendancy for interprovenance crosses to perform better than the average of the corresponding intraprovenance crosses, coupled with the better performance of the single female from Flinders Island when crossed to King Island males (Figure 1), from which it was genetically more divergent (Figure 2). Within provenances there was no significant association between D and SCA (Table 5). The regressions of D on DBV were not significant (Table 5). However, the T×T cross type, which had relatively high levels of additive genetic variation for growth, had a significant Spearman rank correlation (r = 0.32; P = 0.04). DBV explained a significant (P < 0.05), but low (4.3%), proportion of the variation in SCA when ignoring provenance (Table 5). Greater divergence in breeding values was associated with lower SCA when ignoring provenance (Spearman r = –0.21) and in the intraprovenance crosses (T×T Spearman r = –0.28, K×K Spearman r = –0.31). One cross (K159 × T7) had a very negative SCA (SCA = –0.46) as shown in Fig. 3. All analyses were performed with and without this outlier. This made little difference to the results. The outlier was therefore removed from all results shown in Tables 4 and 5.

DISCUSSION

The T×T cross type, which is in the main core of the distribution of *E. globulus* in Tasmania, was phenotypically and genetically more variable than the K×K cross type (Table 3). Genetic variance in the T×T cross type

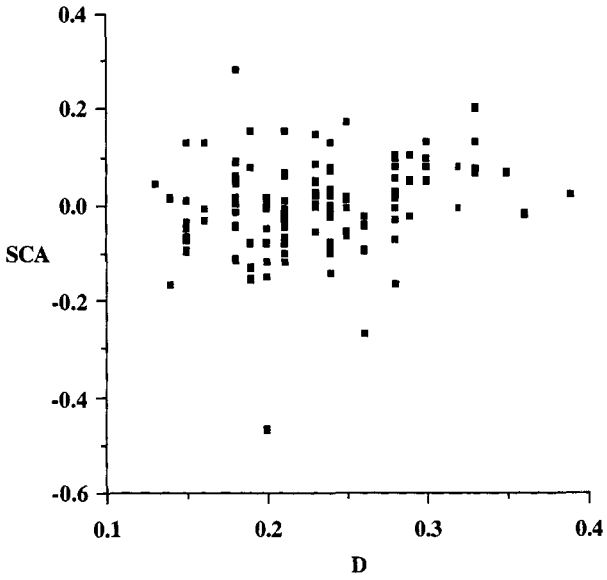

Figure 3 Scatter plot of Nei's genetic distance (D) versus specific combining ability (SCA) when using all data (n = 117)

was nearly five times greater than observed in the K×K cross type (Table 3). This difference in the quantitative genetic variability was of interest since the average Nei's genetic distance between trees within provenance differed little between the two provenances (T×T vs. K×K, Table 4). Non-additive gene effects are clearly a major component of the genetic variation in early growth of *E. globulus* ssp. *globulus*. The increased relative relative importance of SCA effects with inter provenance crosses (Table 3) should be taken into account where breeding populations comprise selections from a wide range of provenances.

In the present study, the total and T×T SCA effects were statistically significant. However, any prediction of these SCA effects across provenances, using genetic distance, accounted for only 4.2% of the variation in SCA (Table 5), and mainly reflected the greater genetic differentiation between parents involved in interprovenance crossing. SCA effects within provenances were unpredictable. There may be several reasons why the SCA of a cross could not be better predicted using genetic markers in *E. globulus*. This may simply be due to the fact that the dominance variation for early growth was just too small in this factorial. This may change with age (HARDNER & POTTS 1995). The measure of genetic distance appears to be appropriate. We chose RAPD markers because they could easily be utilised in a breeding program. However, RAPD markers have been criticized because they are a dominant type of molecular marker and are not as informative as codominant markers such as RFLPs or isozymes (STRAUSS *et al.* 1992). The fact that the cluster analysis clearly differentiated the Taranna and King Island provenances

demonstrates that RAPD markers are useful in measuring genetic distance in *E. globulus*, confirming the results of NESBITT *et al.* (in press), and could have been useful in predicting heterosis. We also examined whether similarity in breeding value (DBV) was related to genetic distance between parents and whether it could be used to predict heterosis. The relationship between D and DBV was poor. Interestingly, as parents diverged more in breeding values (larger DBV's), the SCA's became smaller. This trend was only significant ($P < 0.05$) in the full data set and remains unexplained. In no case in the present study did a predictor variable explain more than 11% of the variation in the dependent variable, suggesting little predictive value of the associations observed.

LEONARDI *et al.* (1991) theorized that the power of randomly selected genetic markers to predict heterosis would be low if heterosis was determined by relatively few quantitative trait loci (QTL) or QTL's with multiple alleles, that are randomly distributed among trees. This argument is supported by the fact that increasing the number of genetic markers made little difference to the correlation between genetic distance and heterosis (DUDLEY *et al.* 1991; MELCHINGER *et al.* 1990; SOUZA & SORRELLS 1991) . For example, in this study one interprovenance cross resulted in an F1 family with very poor performance. The very low SCA of this cross may be comparable to the effect of inbreeding in *E. globulus* and could be due to the chance presence of the same deleterious recessive allele(s) in these two unrelated trees. Such a rare event would be impossible to predict using genetic markers.

Another reason why genetic distance did not better predict SCA may be that it requires much closer degrees of relatedness between parents than exists in the present sample. Selfing *E. globulus* spp. *globulus* results in a 20% depression in height by two years of age (HARDNER & POTTS 1995). If the factorial had included some closely related parents, inbreeding depression resulting in large negative SCAs may have been detected. Our parents were randomly selected along c. 25 km of road in the natural populations and the parents were separated by at least 500 m. These geographic distances might have been too large to sample closely related trees within a stand and may have transgressed any neighborhood structure (ELDRIDGE *et al.* 1993) existing in these forests. Although in some studies significant levels of prediction were obtained, none of these predictors were good enough to be used in a breeding program. Using few genetic markers such as isozymes (FREI *et al.* 1986; LAMKEY *et al.* 1987) or a large number of markers such as RFLPs made little difference (DUDLEY *et al.* 1991; MELCHINGER *et al.* 1990; SOUZA & SORRELLS 1991).

In conclusion, genetic distances appear to have little value in predicting heterosis for early growth in *Eucalyptus globulus*. However, it remains to be tested whether inbreeding depression, from matings among closely related trees, can be predicted using RAPD markers.

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