

# Genetic Variation Amongst and Within The Native Provenances of *Pinus radiata* D. Don in South-eastern Australia.

## 1. Growth and form to age 26 years

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### Abstract

Four hundred and sixty individual families of *Pinus radiata*, representing all provenances and populations in a 1978 seed collection, plus a local seed orchard control, were planted together in 1980 in a large trial in southern New South Wales, Australia. Provenance means and genetic parameters for growth measured at ages 3, 8 and 26 years plus stem straightness, branch angle and nodality at age 26 years are reported. Large provenance differences were apparent for all traits. The two island provenances, Cedros and Guadalupe, were significantly inferior to the mainland provenances and, due to competition effects, very few trees survived to age 26. Within the mainland provenances, the performance of Año Nuevo and Monterey was almost identical, with Cambria being less vigorous. The best performing seedlot for all traits was the local control.

Differences between populations within the mainland provenances were apparent for diameter at age 26 within Año Nuevo and Cambria but not Monterey. Año Nuevo also showed population differences for stem straightness. Heritabilities for early growth were similar within Año Nuevo and Monterey but by age 26, the heritability for diameter was higher in Monterey. Within Cambria, heritabilities for growth and tree form at age 26 were close to zero. Genetic correlations between traits showed similar patterns for each of the mainland provenances, with the exception of correlations with stem straightness within Año Nuevo. Results are discussed in light of recent molecular studies of genetic architecture, levels of inbreeding in the native stands and possible effects of this inbreeding.

**Key words:** *Pinus radiata*, provenances, Californian collection, heritability

### Introduction

*Pinus radiata* (D. Don), the predominant plantation softwood species in the southern hemisphere, is native to the California coast and two offshore islands. The three Californian mainland provenances, Año Nuevo, Monterey and Cambria, occur as disjunct populations along the coast south of San Francisco, between 35°N and 37°N (Figure 1). The two remaining provenances,

Guadalupe and Cedros, are further south and located on islands off the Mexican coast. Based on morphology, these two island provenances are considered to be separate sub-species (LIBBY, 1997). All provenances are located in a Mediterranean winter rainfall climate but receive additional summer moisture from ocean fog (Libby 1997). Provenances differ in soil type, elevation, temperature and total rainfall (Table 1) (LIBBY, 1997; MORAN *et al.*, 1988).

Monterey provenance is the most extensive, followed by Cambria, Año Nuevo, Cedros and Guadalupe (Table 1). The Guadalupe population is almost extinct, with only 220 trees distributed along the 8 km long ridge top (ROGERS, 2002).

Radiata pine was introduced into Australia, via England, in the mid 1800's (WU *et al.*, 2007; JOHNSON *et al.*, 2008). Initially the species was used for ornamental purposes in botanic gardens. During the 1860's FERDINAND VON MUELLER, then director of the Melbourne botanic gardens, distributed thousands of seedlings throughout Victoria and South Australia for ornamental plantings and windbreaks on farms (WU *et al.*, 2007; JOHNSON *et*



Figure 1. – Location of each provenance of *Pinus radiata* (from KARHU *et al.*, 2006).

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*Table 1.* – Descriptive characteristics of native provenances of *Pinus radiata* (from ELDRIDGE, 1978 and MORAN *et al.*, 1988).

Provenance	Latitude °N	Altitude (m)	Rainfall (mm)	Area (ha)	Soil
Año Nuevo	37	15-330	800	450	Sand, shaly clay loam
Monterey	36.5	5-580	400	3800	Varied fertility & base
Cambria	35.5	30-180	500	900	Sandy loam, localised poor drainage
Guadalupe	29	490-1220	300?	*	Rocky loam
Cedros	28	450-610	200?	150	Skeletal

\* 220 trees along ridge top.

*al.*, 2008). Further seed was imported directly from California to New Zealand in the 1870's with the largest consignment of 11 kg in 1876 probably originating from a large number of trees (WU *et al.*, 2007). Subsequently some of this seed, or seed collected from trees grown from these seedlots, was probably imported into Australia. By 1880 the early ornamental and windbreak plantings produced sufficient seed to meet the market and no further Californian material was introduced.

From the early 1900's seed from these early plantings was used to establish increasing areas of *radiata* plantation in southern Australia and New Zealand (MORAN and BELL, 1987; JOHNSON *et al.*, 2008). However, the exact origin and breadth of the original genetic base was unknown. In the 1950's structured breeding programs for *radiata* pine commenced and eventually the issue of the genetic base was raised. Using isozyme variation, MORAN and BELL (1987) determined that Australian *radiata* originated from the Monterey and Año Nuevo provenances and that the first generation of existing breeding programs had captured a substantial proportion of the genetic diversity (in terms of allelic representation) in these provenances. However, the three southern provenances did not appear to be represented in breeding programs.

As breeding programs became more sophisticated, thought was given to expanding the genetic base by re-collecting in the original stands in California. During the 1960's and 70's three major collections were done:

1) 1962 – cuttings were collected from juvenile trees in the three mainland provenances and planted in California (HOOD and LIBBY, 1980; GUNION *et al.*, 1982)

2) 1964 – seed was collected in both the mainland and island populations by FORDE (1964). Each of the mainland provenances was divided into 5 subpopulations and 20 trees sampled per location. This collection was subsequently planted as a provenance/progeny trial on 2 sites in New Zealand and was the subject of a major study published in 1992 (BURDON *et al.*, 1992 a to e)

3) 1978 – a more extensive seed collection of both the mainland and island populations was jointly funded by Australian and New Zealand Governments (ELDRIDGE, 1978; JOHNSON *et al.*, 1997; MORAN *et al.*, 1988). This collection aimed to provide ex-situ gene conservation stands and to expand the genetic base of *Pinus radiata* available for future breeding. The more widespread mainland provenances were divided, on a geographic basis, into a number of populations (see *Table 2*) and individual tree seed collections conducted on varying numbers of parents per population. Parent trees were

*Table 2.* – Details of provenances, populations and families of *Pinus radiata* collected in 1978 and numbers of families from each population established in the 1980 progeny trial.

Provenance	Population	Location	Families collected	Families in 1980 trial
Año Nuevo	01-1	Coastal strip	70	67
	01-2	Inland central	40	33
	01-3	Inland, south Swanston	40	35
	01-4	Inland northern	29	24
Monterey	02-1	Coastal, northern dunes	54	43
	02-2	Monterey Bay town	38	31
	02-3	Inland, Huckleberry Hill	36	33
	02-4	Inland, Jacks Peak	59	53
	02-5	Point Lobos - Yankee Point	22	20
	02-6	Carmel highlands	31	24
Cambria	03-1	Pico Creek	25	18
	03-2	Town area	50	47
	03-3	Scott Rock	25	19
Guadalupe	14		39	9
Cedros	15		26	4
Control		Tallaganda seed orchard		37 plots per rep

*Table 3.* – Published results from trials of native radiata provenances. The collection (Coll) used in each study is specified, along with the number of planting sites and the oldest age at which trials were assessed.

Location	Coll	Sites	Age	Source
California	1962	1	3 9	Hood & Libby, (1980) Guinon <i>et al.</i> , (1982)
New Zealand	1964	2	12	Burdon <i>et al.</i> , (1992a to e)
Australia, New South Wales	1978	8	8	Johnson <i>et al.</i> , (1997)
Chile	1978	3	6	Jayawickrama & Balocchi (1993)
Greece	1978	2	12	Matziris (1995)
New Zealand	1978	24	11	Burdon <i>et al.</i> , (1997, 1998)
South Africa	1978	6	8	Falkenhagen (1991)
Turkey	1978	1	6	Toplu <i>et al.</i> , (1987)

separated by at least 100 m and were selected as being large and with good form where possible (JOHNSON *et al.*, 1997).

The 1978 collection is the most extensively studied with provenance trials established between 1979 and 1982 at many locations in Australia, New Zealand and other countries (*Table 3*). Significant provenance differences for growth (to age 12 years) and many other traits have been found in most studies. However, little difference was found amongst populations within the mainland provenances. In addition, significant provenance by environment interaction has also been found in many studies. Of the mainland provenances, Monterey is generally considered to be the best overall for growth and Cambria the worst. Año Nuevo is the most interactive, growing well on high growth sites but very poorly on low growth sites in NSW (JOHNSON *et al.*, 1997) and New Zealand (BURDON *et al.*, 1997, 1998). In contrast, Cambria has been found to grow better than Año Nuevo on lower quality sites (BURDON *et al.*, 1998).

Of the mainland provenances, Año Nuevo has the best cold and snow tolerance (HOOD and LIBBY, 1980; MATZIRIS, 1995; BURDON *et al.*, 1998). Results for branching habit are mixed with Año Nuevo producing the lowest number of whorls in New Zealand (BURDON *et al.*, 1998) and greatest number in Greece MATZIRIS (1995). Año Nuevo had the greatest resistance to aphid attack in South Africa (FALKENHAGEN, 1991). Cambria is most susceptible to *Dothistroma* needle cast (BURDON *et al.*, 1998).

Cedros provenance appears to be the worst provenance for survival, growth and tree form at every site where it was tested. In contrast, Guadalupe provenance, whilst slightly slower growing than the mainland provenances, appears to have good stem straightness (JAYAWICKRAMA and BALOCCHI, 1993; MATZIRIS, 1995; BURDON *et al.*, 1997) and good frost resistance (MATZIRIS, 1995).

Despite the high number of native provenance trials established, only two studies established progeny trials where individual family identity was maintained – the New Zealand study of the 1964 collection (BURDON *et al.*, 1992 a to e) and the current study which reports on results from a progeny trial established with the 1978 collection. These progeny trials are important as they

allow genetic parameters to be determined within and between provenances and a fuller study of the genetic architecture of the species. The New Zealand progeny trials were abandoned at age 12 due to increasing competition effects from the more vigorous local seedlots which were then felled. The Australian progeny trial, which is the subject of this paper, was established in 1980 and included 460 parents originating from the mainland and island provenances. This trial has been assessed for growth on 3 occasions (1983, 1988 and 2006) and for stem form in 2006, at age 26 years. In 2006/7, the trial was also sampled for wood density and standing tree wood stiffness was assessed using acoustics. The growth and form assessments of this trial form the basis of this paper with the wood quality data to be published in subsequent papers.

The aims of this paper are to determine the genetic architecture of the native provenances of radiata pine by examining:

- Provenance differences in height and diameter growth at different ages and whether earlier provenance rankings were maintained to age 26 years
- Provenance differences for stem straightness, branch angle and nodality at age 26 years and their relationship to diameter growth
- Genetic control of height and diameter growth, stem straightness, branching angle and nodality in different provenances
- Relative performance of native provenances compared to locally selected seed orchard material
- Whether the genetic architecture observed for growth and tree form is compatible with that observed for molecular data from the same native provenance collection.

## Materials and Methods

### *Genetic material*

Individual family seedlots were collected from the three mainland plus the two island provenances as part of the 1978 native population collection (ELDRIDGE, 1978). Within each provenance a number of different populations were sampled (see *Table 2*) with different numbers of families included in each population.

### Field site

The provenance/progeny trial was established in 1980 as a collaborative project between CSIRO Division of Forest Research and the Forestry Commission of NSW on land owned by the Forestry Commission. The trial is located in compartment 777 of Green Hills forest, south west of Tumut (latitude 35°17', longitude 148°13') on the south western slopes of New South Wales. The trial site is at approximately 830 m altitude, has an average annual temperature at the site of 10.9°C, average summer temperature of 26°C, annual precipitation of approximately 1270 mm with a distinct winter-spring maximum and may receive light snow falls during winter. The soil is derived from granodiorite.

Seedlings were raised in the CSIRO nursery in Canberra. All populations and families were planted using an incomplete block design with 7 replicates each containing 23 incomplete blocks of 22 four-tree row plots with each plot being a single family (total of 14,168 trees). Planting spacing was 2.5 x 3 m. The trial was designed to allow for a thinning operation to occur without disrupting the trial integrity. Every third row throughout the trial was planted with routine seed orchard stock and the entire trial was surrounded by rows of routine buffer trees. These 3<sup>rd</sup> out-row trees were removed in a thinning operation at age 14 years.

All trees were measured for total height (metres) in 1983 and 1988, diameter over bark at 1.3 m above ground (DBH in cm) in 1988 and 2006 and in 2006 they were also scored for straightness (1 = crooked to 6 = straight), branch angle (1= steeper than 45° to 3 = less than 45°, tending to flat) and nodality (1 = uninodal, 2 = low or uneven nodality, 3 = moderately nodal and 4 = highly multinodal).

### Data analysis

The initial analysis aimed to determine whether significant differences existed between the provenances and/or populations within provenances by fitting the following model:

$$Y = \text{mean} + \text{Replicate} + \text{Incomplete block within Replicate} + \text{Provenance} + \text{Population within provenance} + \text{Female parent} + \text{Plot} + \text{error}$$

with Replicate, Provenance and Population treated as fixed effects and Incomplete block, Female and Plot as random. Two forms of this analysis were run: (1) including data from all provenances and (2) for the mainland provenances only.

Analyses were then run for each of the provenances separately, fitting the above model but with the provenance term excluded. Genetic parameters (and their standard errors) for each of the mainland provenances were calculated using ASReml (GILMOUR *et al.*, 2002) fitting a univariate model. Equivalent bivariate analyses involving pairs of traits were conducted, fitting covariances to all random terms in the model. Narrow-sense heritabilities (assuming random mating) and genetic correlations at the individual tree level were obtained from equations 1 and 2 respectively.

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} \quad \text{Equation 1}$$

where  $h^2$  is the heritability,  $\sigma_a^2$  is the additive genetic variance and  $\sigma_e^2$  is the residual variance. A coefficient of relationship for half-sib families of  $1/4$  was assumed (giving  $\sigma_a^2$  equal to 4 \* female component of variance) as data from molecular genetics studies of the 1978 native provenance collection indicated very low levels of selfing

Table 4. – Percentage survival for each provenance at ages 3, 8 and 26 years and total number of surviving trees.

Provenance	Age 3	Age 8	Age 26
Año Nuevo	95	95	57
Monterey	95	96	53
Cambria	95	95	37
Guadalupe	88	91	12
Cedros	55	59	4
Control	96	96	77
No. surviving trees	13,399	13,444	7,361

Table 5. – Provenance means and significance of differences between provenances and populations for Height (Ht in metres) at ages 3 and 8 years, diameter (DBH in cm) at ages 8 and 26 years. Results are presented for all provenances and for only the mainland provenances (Año Nuevo, Monterey and Cambria) due to lower survival of Guadalupe and Cedros. The same letter in the Sig. column indicates no significant difference between the means.

Provenance	Ht 3	Sig	Ht 8	Sig	DBH 8	Sig	DBH 26	Sig
Año Nuevo	8.70		15.46		12.04	a	30.11	a
Monterey	8.35		16.32		12.10	a	30.18	a
Cambria	7.99		16.07		11.59	a	22.87	
Guadalupe	6.55		12.97		7.51		18.87	b
Cedros	4.38		9.00		5.42		17.50	b
Control	9.22		16.97		13.67		37.27	
All Provs	**		**		**		**	
Population	ns		ns		ns		*	
Mainland Provs	**		**		**		**	
Population	ns		ns		ns		*	

\* P < 0.05, \*\* P < 0.01, ns = not significant.

Table 6. – Provenance means and significance of differences between provenances and populations for straightness (STR), branch angle (BR) and nodality (NOD) at age 26 years. Results are presented for all provenances and for only the mainland provenances (Año Nuevo, Monterey and Cambria) due to lower survival of Guadalupe and Cedros. The same letter in the Sig. column indicates no significant difference between the means.

Provenance	STR	Sig	BR	Sig	NOD	Sig
Año Nuevo	3.47	a	2.19	a	2.58	a
Monterey	3.65	a	2.03	b	2.66	a
Cambria	3.12	b	1.84	b	2.25	b
Guadalupe	3.51	a	1.83	b	2.35	b
Cedros	3.09	b	2.07	ab	2.22	b
Control	4.61		2.42		3.25	
All Provs	**		**		**	
Population	**		ns		ns	
Mainland Provs	**		**		**	
Population	**		ns		ns	

\* P < 0.05, \*\* P < 0.01, ns = not significant.

or inbreeding (MORAN *et al.*, 1988; VOGL *et al.*, 2002). Heritability estimates from a single site may be biased upwards as no family by site interaction term was available for inclusion in the denominator.

$$r_{Axy} = \frac{\sigma_{axy}^2}{\sqrt{\sigma_{ax}^2 \times \sigma_{ay}^2}} \quad \text{Equation 2}$$

where  $r_{Axy}$  is the genetic correlation between traits,  $\sigma_{ax}^2$  is the additive genetic variance of the first trait,  $\sigma_{ay}^2$  is the additive genetic variance of the second trait and  $\sigma_{axy}^2$  is the additive genetic covariance between the first and second traits.

## Results

Provenances differed significantly for survival (Table 4) and all growth and form traits (Tables 5 and 6) but little difference was found between populations within each provenance (Tables 5 to 8). Survival of Guadalupe, and particularly Cedros, was very low by age 26 due to trees of these provenances being out-competed. As only 4 trees from Cedros provenance (out of 112 planted) were still alive by 2006, the results for this provenance are of little value. Analyses of variance for testing provenance differences were rerun using data from only the mainland provenances but the significant provenance differences were still evident (Tables 5 and 6).

The control seedlot (Tallaganda seed orchard) had the highest survival and significantly out-performed all of the native provenances for every growth and form trait. Of the native provenances, Año Nuevo ranked the highest for height growth at age 3 but was overtaken for height at age 8 by both Monterey and Cambria. For diameter growth Monterey was slightly superior to Año Nuevo at both ages but the two provenances were not significantly different to each other. Monterey was also significantly better than Año Nuevo for straightness and nodality but poorer for branching. The third mainland provenance, Cambria, was significantly smaller than Monterey and Año Nuevo for all growth and form traits except height at age 8. Guadalupe was significantly poorer than the mainland provenances for all growth traits but ranked better than Año Nuevo and Cambria for straightness. Cedros ranked lowest for all traits.

Significant differences between populations within provenances were found only for diameter at age 26 and straightness (Tables 5 to 8). These population differences were only apparent in Año Nuevo and Cambria, with no significant population differences found in Monterey. Within Año Nuevo provenance, population 01–1,

Table 7. – Population means and ranks (R) for Height (Ht in metres) at ages 3 and 8 years and diameter (DBH in cm) at ages 8 and 26 years and survival at age 26 (Surv).

Provenance	Pop.	Ht 3	R	Ht 8	R	DBH 8	R	DBH 26	R	Surv
Año Nuevo	01-1	8.34	3	13.39	12	11.36	11	29.95	7	57
	01-2	8.44	2	13.35	13	11.36	10	30.31	5	59
	01-3	8.21	6	13.28	14	11.37	9	30.71	3	53
	01-4	8.30	4	13.70	11	11.72	4	31.96	2	58
Significance		ns		ns		ns		**		
Monterey	02-1	7.99	11	14.26	6	11.42	8	30.02	6	50
	02-2	8.14	8	14.27	5	11.60	6	29.39	11	53
	02-3	8.16	7	14.33	4	11.78	2	30.32	4	56
	02-4	8.26	5	14.64	2	11.73	3	29.94	8	55
	02-5	8.09	9	14.34	3	11.63	5	29.89	9	54
	02-6	8.09	10	14.14	7	11.47	7	29.51	10	48
Significance		ns		ns		ns		ns		
Cambria	03-1	7.63	14	13.99	8	10.91	14	22.73	14	24
	03-2	7.88	12	13.84	9	11.32	12	26.09	12	40
	03-3	7.83	13	13.76	10	11.15	13	24.93	13	40
Significance		ns		ns		ns		**		
Guadalupe	14	6.19	15	10.90	15	6.83	15	18.77	15	
Cedros	15	4.03	16	6.96	16	4.75	16	17.37	16	
Control		8.86	1	14.82	1	12.98	1	37.13	1	

\*\* P < 0.01, ns = not significant.

**Table 8.** – Population means and ranks (R) for straightness (STR), branch angle (BR) and nodality (NOD) at age 26 years.

Provenance	Pop.	STR	R	BR	R	NOD	R
Año Nuevo	01-1	3.58	9	2.16	3	2.58	5
	01-2	3.71	6	2.09	5	2.65	3
	01-3	3.77	3	2.13	4	2.53	6
	01-4	3.81	2	2.18	2	2.53	7
Significance		**		ns		ns	
Monterey	02-1	3.77	5	2.00	10	2.66	2
	02-2	3.57	10	1.99	11	2.48	12
	02-3	3.77	4	2.04	9	2.59	4
	02-4	3.62	7	2.05	8	2.53	8
	02-5	3.54	11	1.94	12	2.52	9
	02-6	3.53	12	2.05	7	2.50	10
Significance		ns		ns		ns	
Cambria	03-1	3.24	16	1.82	15	2.25	15
	03-2	3.51	13	1.87	14	2.46	13
	03-3	3.44	14	1.92	13	2.50	11
Significance		ns		ns		**	
Guadalupe	14	3.61	8	1.81	16	2.35	14
Cedros	15	3.28	15	2.07	6	2.22	16
Control		4.73	1	2.39	1	3.24	1

\*\* P &lt; 0.01, ns = not significant.

collected from the coastal strip, had the smallest diameter trees at age 26 and significantly lower straightness. For Cambria, at age 26 years the largest trees came from Cambria town (03–2) whilst the smallest diameter and least straight trees came from population 03–1, Pico Creek. Survival was also lower for 03–1.

Heritabilities for each trait (*Table 9*) indicate that the heritability for both height and diameter decreased with increasing age. Within-provenance heritabilities for Año Nuevo and Monterey were very similar for growth to age 8 but at age 26 the heritability for diameter is greater in Monterey. Interestingly, within Año Nuevo this drop in heritability for diameter at age 26 years corresponds with the appearance of significant population within provenance effects. For Cambria, the heritability was much lower for all growth traits, dropping to zero for diameter at age 26, again when significant population within provenance effects were evident.

Heritabilities for stem form follow a different pattern. Heritability for stem straightness was highest in Monterey but for branching and nodality heritability was

**Table 9.** – Heritabilities for combined data set and for each mainland provenance.

Provenance	Ht 3	se	Ht 8	se	DBH 8	se	DBH 26	se
Combined	0.31	0.03	0.22	0.03	0.27	0.03	0.18	0.03
Año Nuevo	0.39	0.06	0.21	0.05	0.27	0.05	0.14	0.04
Monterey	0.32	0.05	0.20	0.04	0.28	0.04	0.20	0.04
Cambria	0.11	0.02	0.16	0.03	0.06	0.02	0.00	0.00

Provenance	STR	se	BR	se	NOD	se
Combined	0.19	0.03	0.19	0.03	0.27	0.04
Año Nuevo	0.13	0.05	0.27	0.06	0.32	0.06
Monterey	0.21	0.05	0.13	0.04	0.29	0.05
Cambria	0.08	0.05	0.05	0.05	0.05	0.05

**Table 10.** – Genetic correlations (and standard error) for each mainland provenance.

Año Nuevo	HT3	se	HT8	se	DBH8	se	DBH26	se	STR	se	BR	se
HT8	0.79	0.04										
DBH8	0.83	0.03	0.77	0.05								
DBH26	0.63	0.08	0.41	0.13	0.81	0.05						
STR	-0.10	0.14	-0.33	0.15	0.03	0.15	0.16	0.19				
BR	0.15	0.12	0.00	0.13	-0.09	0.13	0.06	0.17	-0.05	0.16		
NOD	0.14	0.12	-0.11	0.13	0.12	0.12	0.06	0.17	0.35	0.14	0.40	0.12
Monterey	HT3	se	HT8	se	DBH8	se	DBH26	se	STR	se	BR	se
HT8	0.75	0.05										
DBH8	0.86	0.03	0.65	0.06								
DBH26	0.63	0.07	0.22	0.12	0.79	0.05						
STR	0.28	0.11	0.12	0.13	0.36	0.11	0.44	0.12				
BR	0.11	0.13	0.14	0.14	0.01	0.14	0.17	0.17	-0.05	0.16		
NOD	0.94	1.10	0.48	1.17	0.27	0.11	0.29	0.13	0.46	0.11	0.49	0.13
Cambria	HT3	se	HT8	se	DBH8	se	DBH26	se	STR	se	BR	se
HT8	0.71	0.08										
DBH8	0.81	0.06	0.57	0.11								
DBH26	0.72	0.10	0.38	0.18	0.82	0.08						
STR	0.44	0.17	-0.05	0.20	0.40	0.19	0.57	0.20				
BR	0.35	0.19	0.32	0.19	0.26	0.21	0.11	0.27	0.00	0.36		
NOD	0.34	0.21	-0.13	0.23	0.19	0.24	0.22	0.31	0.73	0.19	#	

# convergence failed due to negative sum of squares.

highest in Año Nuevo provenance. Within Cambria, heritability was uniformly low for stem form.

Genetic correlations between traits (*Table 10*) indicated that all height and diameter measures were strongly correlated. Stem straightness and branch angle were not related (all correlations close to zero) but nodality was strongly related to straightness and branch angle, particularly for Monterey and Cambria. When correlations were compared across provenances, the size and direction of the correlations were very similar with one exception – the correlations between height and straightness in Año Nuevo. These correlations were negative whereas for the other provenances they were positive. In addition, within Año Nuevo the correlation between diameter at age 26 and straightness was very low (0.16) whilst this correlation was much stronger for Monterey (0.44) and Cambria (0.57).

## Discussion

Detailed provenance studies, in many countries, of the three difference collections from the native provenances have yielded very similar results in terms of the genetic architecture of the species. Large provenance differences have been identified, especially between the island and the mainland provenances. Within each study arbitrary boundaries were allocated to define stands or populations within provenances but these boundaries differed between collections, with little overlap between the 1964 and 1978 collections. However, each study found limited variability between populations within the mainland provenances. In nearly every study, the local control seedlot (where included) outperformed all of the native collection seedlots.

The island provenances, Cedros and Guadalupe, were universally the worst performers in all studies. Real genetic differences between the mainland and island populations may exist, especially given that these island populations have historically been classified as different subspecies, based on morphology (LIBBY, 1997). However, these provenances are also geographically limited with the smallest numbers of surviving trees (*Table 1*). Inbreeding is a real possibility within such a limited population size and may be a cause of the observed poor performance.

Pines are considered to be relatively self infertile with enforced self pollination resulting in low seed set and very low viability of offspring with most failing to survive the nursery stage (ROGERS, 2002; VOGL *et al.*, 2002). Isozyme data from seeds of the 1978 collection (MORAN *et al.*, 1988) and microsatellite data from 20 year old trees in a provenance trials from the same collection (KARHU *et al.*, 2006) indicate that Guadalupe provenance had the lowest allelic richness and expected heterozygosity, followed by Cedros. Further, VOGL *et al.* (2002) found some selfs from the two island provenances surviving in the 20 year old trial and estimated the average inbreeding co-efficient for these provenances to be 0.1. These results appear to provide some support for the theory that inbreeding is an issue within these provenances. However, VOGL *et al.* (2002) argue that the continued existence of these provenances indicates inbreed-

ing depression must have been purged as, given the limited population size, mating between relatives would be frequent.

For the mainland provenances, Monterey is generally the best performer, Cambria the worst with results for Año Nuevo mixed. The early study of GUNION *et al.* (1982) in California found the average height of the mainland provenances was almost identical at age 3. By age 8 Año Nuevo and Monterey were still the same height with Cambria being significantly shorter. However, the proportion of variation attributed to differences amongst provenances and stands within provenances at each assessment was less than 2 percent, with the majority of the variation being within clones.

Similar results were reported for the 1964 collection from 2 sites in Kaingaroa forest on the north island of New Zealand (BURDON *et al.*, 1992 a to e). No significant differences between the mainland provenances were apparent for height or diameter growth up to age 8 (*Table 11*). By age 12 Cambria had dropped behind in diameter but Monterey and Año Nuevo were still very similar. The experiment was abandoned at age 12 due to increasing competition effects from the more vigorous local seedlots which were then felled. The native population trees were left standing for future use as a gene resource but only very limited further assessment of the trial was done.

The many provenance studies based on the 1978 collection (*Table 3*) also found that Monterey was generally the fastest growing provenance up to age 12, with the performance of Año Nuevo often being similar. The later age data from the current study supports these earlier findings, with significant differences apparent between the mainland provenances, for every trait measured (*Table 5*). Provenance differences were also apparent for survival (*Table 4*). At age 8 years all provenances, except Cedros, had over 90% survival. However, with increasing age and increasing between tree competition, the island populations became over-topped and subsequently started to die out. By age 26 years the highest survival was for the local control seedlot (77%), followed by Año Nuevo (57%) and Monterey (53%) with survival being very low for the island populations (12% for Guadalupe and only 4% for Cedros). These competition effects would have exaggerated the provenance differences for growth and also reduce the validity of the age 26 data for the island populations. Trees from Cambria appeared to be able to compete up until age 8 years with their height and diameter growth being similar to the other mainland provenances. However by age 26 Cambria was out-competed resulting in lower survival (37%) and reduced diameter.

Within the mainland provenances, the only differences found between the populations was at age 26 years, where populations differed for diameter within Año Nuevo and Cambria (*Table 7*), for straightness within Año Nuevo and for nodality within Cambria (*Table 8*). The lack of population differences in early growth contrasts with the results of BURDON *et al.* (1992b) for the 1964 collection where significant differences were found between populations within Año Nuevo for height and

diameter at age 8, height within Cambria and branch quality within Monterey (Table 11). The definition of the population boundaries differs between the two collections so a direct comparison of population differences is not possible. But, the Año Nuevo population with the largest diameter at age 26 (01–4) appears to overlap with the population with largest diameter (I) in the 1964 collection. However, for both studies the magnitude of the population within provenance differences is relatively small compared to the differences between provenances.

Within provenance heritability estimates from this study (Table 9) and the BURDON *et al.* (1992c) study indicate that heritability for height at age 8 was greater in New Zealand (Table 11) whilst heritability for diameter (age 8) was greater in Australia. For the New Zealand study heritability for height was greatest in Año Nuevo (0.32) provenance which also showed significant population differences. Heritability for height was similar within Monterey and Cambria whilst heritability for diameter was similar in all three provenances. In contrast, for the Australian data the heritability for height (age 8) was similar for the 3 provenances but for diameter (age 8) the heritability for Cambria was significantly lower than the other two provenances. One possible reason for these observed differences is that the two studies examined different collections from the native prove-

nances. Alternatively, site differences may be responsible for the different patterns of heritability observed.

At age 26 Año Nuevo and Monterey show different patterns of genetic variability for diameter. Significant population differences were apparent within Año Nuevo but not within Monterey and the heritability for diameter (0.14) was lower in Año Nuevo than Monterey (0.20). For Cambria, the heritability of diameter was zero but significant differences were apparent between the populations, indicating that all of the genetic variability was at the population level. However, this heritability estimate may be affected by competition effects from other more vigorous seedlots suppressing the Cambria trees and reducing their ability to express their true genetic merit.

For branching traits the New Zealand study scored branch quality (a 9 point score combining branch angle, size and nodality, BURDON *et al.*, 1992a) at age 8 whilst the Australian study scored branch angle and nodality at age 26. Heritability of branch quality was similar for Año Nuevo and Monterey (~0.20) in the New Zealand study (Table 11) and slightly higher for Cambria (0.26). These results are very different to those from the Australian study (Table 9) where, at age 26, heritability within Cambria was close to zero for both branch angle and nodality but a significant between population effect was present for nodality. Average branch angle was sim-

Table 11. – Published results for age 8 height (Ht in metres) and diameter (DBH in cm) data for each provenance and population from the New Zealand study of the 1964 collection (BURDON *et al.*, 1992 a to e). BRN is branch quality scored on a 1 (rough, steep angle) to 9 (light, flat) scale. Data for Guadalupe and Cedros were calculated from percentage performance data.

Provenance	Pop.	Location	Ht 8	DBH 8	BRN
Año Nuevo	I	Near northern limit	9.91	15.9	4.5
	II	Interior mid north	9.48	15.2	4.4
	III	Mid range	9.63	15.0	4.4
	IV	Coastal southern	9.83	15.5	4.4
	V	Inland southern	9.37	14.5	4.5
Significance			*	*	ns
Monterey	I	Seaward, NW edge	9.79	16.1	4.6
	II	South of Monterey	9.83	16.1	4.8
	III	Dry north facing slopes	9.60	16.2	4.5
	IV	Inland	9.78	16.1	4.9
	V	Near southern limit	9.44	15.3	5.3
Significance			ns	ns	*
Cambria	I	Near northern limit	9.57	16.0	4.4
	II	Coastal edge, W Cambria town	9.81	15.8	4.8
	III	Above Cambria town	9.39	15.5	4.6
	IV	Coastal southern	9.34	15.5	4.4
	V	Inland southern	9.74	16.0	5.1
Significance			*	ns	ns
Guadalupe			8.8	12.0	5.4
Cedros			7.0	8.8	5.0
Heritability					
Año Nuevo			0.32	0.14	0.18
Monterey			0.22	0.14	0.20
Cambria			0.23	0.16	0.26
Guadalupe			0.40	0.34	0.22

\* P < 0.05, ns = not significant.

ilar for Año Nuevo and Monterey (Table 6) but the heritability within Año Nuevo (0.27) was double that of Monterey (Table 9). However, these two provenances were very similar for both the mean and heritability of nodality.

Genetic correlations between traits (Table 10) indicate that the pattern of correlations was similar across the mainland provenances with the exception of correlations with straightness in Año Nuevo where the correlation with height at age 8 was significantly negative. Height at ages 3 and 8 were significantly correlated with diameter at all ages. The correlation for height and diameter at age 8 was very similar to the 0.76 reported by BURDON *et al.* (1992d) for his combined data set. Correlations between diameter at age 8 and straightness in Monterey and Cambria were almost identical to the 0.36 reported by BURDON *et al.* (1992d) but the correlations between height and straightness were much lower than the 0.33 of Burdon, possibly due to the lower heritability for height in the current study. Results for branch cluster frequency score in BURDON *et al.* (1992d) indicate a strong genetic correlation with both height and diameter at age 8 (0.50 and 0.52 respectively) which is very different to the low correlations for Año Nuevo and Cambria in the current study. Only Monterey had a similar correlation between height at age 8 and nodality (0.48).

The origin of the Australian and New Zealand populations of radiata is believed to be Monterey and Año Nuevo (MORAN *et al.*, 1987; BURDON, 1992e). However, the seedlot with the highest survival and fastest growth in the current study was the local control collected from a seed orchard established using trees selected in plantations in Australia (JOHNSON *et al.*, 1997). The superiority of this seed orchard material suggests that selection in the tree breeding program had been successful in producing locally adapted, higher performing genetic material.

In the BURDON *et al.* (1992a to e) study of the 1964 collection the best growing seedlots were the 2 local controls, which had been collected from stands which had passed through 2 generations in their local area but had not been part of an organised breeding program. The superiority of the local seedlot over the Californian material seems to suggest that a degree of natural selection to the local environment had occurred within New Zealand. BURDON *et al.* (1992b) provided two other suggestions: (1) that the local populations may be superior due to the lack of "neighbourhood inbreeding" effects present in the native stands and (2) possibly heterosis from crosses between the Monterey and Año Nuevo progenitors of the New Zealand (and Australian) plantation estates. However, little evidence was provided to judge the merits of these alternative explanations.

The large size of the mainland populations, and the fact that radiata is wind pollinated, makes it difficult to accept that neighbourhood inbreeding, or its effects, may be an issue. The isozyme data for adult trees from MORAN *et al.* (1988) indicated that the provenances were in Hardy-Weinberg equilibrium, suggesting that selfs have been eliminated. Subsequently, VOGL *et al.* (2002)

reported a limited amount of inbreeding in the mainland provenances and a very low average inbreeding coefficient of 0.04. Further the inbreeding study of WU *et al.* (1998, 2004) indicated that low levels of inbreeding have very limited effect on survival, growth or fecundity and that individual trees differed greatly in the actual effect of inbreeding. WU *et al.* (1998) conclude that the level of inbreeding depression within radiata pine is very low compared with other conifers and may indicate that many deleterious alleles have already been purged.

The alternative hypothesis for superiority of the local seedlots being due to heterosis in crosses between Año Nuevo and Monterey may still have some merit if there are real differences between these provenances. The genetic architecture of the species can be examined using both molecular markers and the quantitative data. The quantitative data from the current study indicates very little difference between these two provenances for the mean value of any trait, for heritability of most traits or for genetic correlations amongst the traits. Results from the many other provenance studies (see Table 3) also indicate similarity in growth at most ages of Monterey and Año Nuevo and the lack of differences between populations within the mainland provenances. These results are supported by the molecular data (MORAN *et al.*, 1988) whose cluster analyses indicated the two most closely related provenances were Monterey and Año Nuevo, with Cambria being distinctly different. Interestingly, their data also indicated that population 03-1, Pico Creek, was different to the other 2 Cambria populations, a result that is supported by the growth data. However, within Monterey, the lack of differences amongst populations for the growth data differs from the isozyme analysis which suggested that the coastal population (02-1) was different to the remaining Monterey populations. JAYAWICKRAMA and BALOCCHI (1993) also noted the similarity between provenance growth results in Chile and studies on variation in gene frequency found in the MORAN *et al.* (1988) and PLESSAS and STRAUSS (1986) studies.

Interestingly, the microsatellite marker study of KARHU *et al.* (2006) gave slightly different results, indicating that Monterey and Cambria were the closest pair of the mainland populations. Results for the two island populations were similar to those of MORAN *et al.* (1988) with the islands being the most divergent from the mainland and also from each other. Unfortunately the KARHU *et al.* (2006) study did not sample across the populations within provenances so does not provide further information.

As both the quantitative and isozyme data indicate the same genetic architecture, in terms of provenance and population differences, the arbitrary division of the provenances into populations may not be meaningful. MORAN *et al.* (1988) argued that their isozyme data indicate that gene flow between populations within mainland provenances has not been restricted in the recent evolutionary past with the opposite applying to gene flow between provenances. They found no association between environmental variables used to define the boundaries of each population and isozyme variation,

which suggests that the isozymes are neutral to the factors used to classify the stands. A fuller examination of the evolutionary history of radiata and genetic structures suggested by quantitative and molecular data are the subject of the third paper in this series.

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### References

- BURDON, R. D., M. H. BANNISTER, H. A. I. MADGWICK and C. A. LOW (1992a): Genetic survey of *Pinus radiata*. 1. Introduction, description of experiment and basic methodology. *New Zealand Journal of Forestry Science* **22**: 119–37.
- BURDON, R. D., M. H. BANNISTER and C. A. LOW (1992b): Genetic survey of *Pinus radiata*. 2. Population comparisons for growth rate, disease resistance and morphology. *New Zealand Journal of Forestry Science* **22**: 138–59.
- BURDON, R. D., M. H. BANNISTER and C. A. LOW (1992c): Genetic survey of *Pinus radiata*. 3. Variance structures and narrow-sense heritabilities for growth variables and morphological traits in seedlings. *New Zealand Journal of Forestry Science* **22**: 160–86.
- BURDON, R. D., M. H. BANNISTER and C. A. LOW (1992d): Genetic survey of *Pinus radiata*. 5. Between-trait and age-age correlations for growth rate, morphology and disease resistance. *New Zealand Journal of Forestry Science* **22**: 211–27.
- BURDON, R. D. (1992e): Genetic survey of *Pinus radiata*. 9. General discussion and implications for genetic management. *New Zealand Journal of Forestry Science* **22**: 274–98.
- BURDON, R. D., A. FIRTH, C. B. LOW and M. A. MILLER (1997): Native provenances of *Pinus radiata* in New Zealand: performance and potential. *New Zealand Forestry* **41**(4): 32–36.
- BURDON, R. D., A. FIRTH, C. B. LOW and M. A. MILLER (1998): Multi-site provenance trials of *Pinus radiata* in New Zealand. *Forest Genetic Resources* **26**: 3–8.
- ELDRIDGE, K. G. (1978): Refreshing the genetic resources of radiata pine plantations. CSIRO Division of Forest Research, Genetics Section Report, No. 7, 120 pp.
- FALKENHAGEN, E. R. (1991): Provenance variation in *Pinus radiata* at six sites in South Africa. *Silvae Genetica* **40**: 41–50.
- FALCONER, D. S. and T. F. C. MACKAY (1996): Introduction to Quantitative Genetics, 4<sup>th</sup> Edition. Prentice Hall, Pearson Education, Harlow, UK. 464 p.
- FORDE, M. B. (1964): Variation in natural populations of *Pinus radiata* in California. I. Sampling methods and branch characteristics. *New Zealand Journal of Botany* **2**: 213–236.
- GILMOUR, A. R., B. J. GOGEL, B. R. CULLIS, S. J. WELHAM and R. THOMPSON (2002): ASReml user guide release 1. VSN, Hemel Hempstead, UK.
- GUINON, M., J. V. HOOD and W. J. LIBBY (1982): A clonal study of intra specific variability in Radiata Pine. II. Growth and form. *Australian Forest Research* **12**: 199–201.
- HOOD, J. V. and W. J. LIBBY (1980): A clonal study of intraspecific variability in Radiata Pine. I. Cold and animal damage. *Australian Forest Research* **10**: 9–20.
- JAYAWICKRAMA, K. J. S. and C. BALOCCHI (1993): Growth and form of provenances of *Pinus radiata* in Chile. *Australian Forestry* **56**: 172–178.
- JOHNSON, I. G., P. K. ADES and K. G. ELDRIDGE (1997): Growth of natural Californian provenances of *Pinus radiata* in New South Wales, Australia. *New Zealand Journal of Forestry Science* **27**: 23–38.
- JOHNSON, I. G., I. M. COTTERILL, C. A. RAYMOND and M. HENSON (2008): Half a century of radiata tree improvement in NSW. *New Zealand Journal of Forestry* **52**(4): 7–13.
- KARHU, A., C. VOGL, G. F. MORAN, J. C. BELL and O. SAVOLAINEN (2006): Analysis of microsatellite variation in *Pinus radiata* reveals effects of genetic drift but no recent bottlenecks. *Journal of Evolutionary Biology* **19**: 167–175.
- LIBBY, W. J. (1997): Native origins of domesticated radiata pine. In: BURDON, R. D. and MOORE, J. M. IUFRO '97 Genetics of Radiata Pine. FRI Bulletin No. 203. Rotorua New Zealand. pp. 9–25.
- MATZIRIS, D. I. (1995): Provenance variation of *Pinus radiata* grown in Greece. *Silvae Genetica* **44**: 88–96.
- MORAN, G. F. and J. C. BELL (1987): The origin and genetic diversity of *Pinus radiata* in Australia. *Theoretical and Applied Genetics* **43**: 616–622.
- MORAN, G. F., J. C. BELL and K. G. ELDRIDGE (1988): The genetic structure and the conservation of the five natural populations of *Pinus radiata*. *Canadian Journal of Forest Research* **18**: 506–514.
- PLESSAS, M. E. and S. H. STRAUSS (1986): Allozyme differentiation among populations, stand and cohorts in Monterey pine. *Canadian Journal of Forest Research* **16**: 1155–1164.
- ROGERS, D. L. (2002): In situ Genetic Conservation of Monterey pine (*Pinus radiata* D. Don). Information and Recommendations. University of California, Division of Agriculture and Natural Resources, Genetic Conservation Program Report No. 26. Davis, California, USA.
- TOPLU, F., K. TUNÇTANER and M. TULUKCU (1987): Investigation on growth and resistance to European shoot moth (*Evertria buoliana* Schiff.) of radiata pine (*Pinus radiata* D. Don) origins in Kocaeli Peninsula. Poplar and Fast-growing Exotic Forest Trees Research Institute, Turkey, Annual Bulletin No. **23**: 13–27.
- VOGL, C., A. KARHU, G. F. MORAN and O. SAVOLAINEN (2002): High resolution analysis of mating systems: inbreeding in natural populations of *Pinus radiata*. *Journal of Evolutionary Biology* **15**: 433–439.

- WU, H. X., A. C. MATHESON and D. SPENCER (1998): Inbreeding in *Pinus radiata*. I. The effect of inbreeding on growth, survival and variance. *Theoretical and Applied Genetics* **97**: 1256–1268.
- WU, H. X., J. V. OWEN, A. ABARQUEZ and A. C. MATHESON (2004): Inbreeding in *Pinus radiata*. V. The effects of inbreeding on fecundity. *Silvae Genetica* **53**: 80–87.
- WU, H. X., K. G. ELDRIDGE, A. C. MATHESON, M. B. POWELL, T. A. MCRAE, T. B. BUTCHER and I. G. JOHNSON (2007): Achievements in forest tree improvement in Australia and New Zealand. 8. Successful introduction and breeding of radiata pine in Australia. *Australian Forestry* **70**: 215–225.

## The Effects of Drying Temperature and Method of Assessment on the Expression of Genetic Variation in Gross Shrinkage of *Eucalyptus globulus* Wood Samples

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### Abstract

Genetic variation in wood-sample gross shrinkage and basic density was examined in a *Eucalyptus globulus* base population trial growing in Tasmania, Australia. Gross shrinkage, which includes all components of shrinkage including collapse, was assessed in four ways (calliper- and visually-assessed tangential shrinkage, volumetric shrinkage and radial shrinkage) on samples dried at three temperatures (22°C, 60°C and 105°C). Significant differences between subraces were observed using all measures of gross shrinkage for two or more of the three drying treatments. Furthermore, significant additive genetic variation within subraces was observed in calliper- and visually-assessed gross shrinkage under two or more of the drying treatments, with narrow-sense heritabilities greater than or equal to 0.35. There was no obvious trend in heritabilities or coefficients of additive genetic variation with drying temperature. Under the 105°C drying treatment, subrace correlations among calliper-, visually- and volume-assessed gross shrinkage were positive and very strong ( $\geq 0.97$ ), while

these measures were less strongly correlated with radial gross shrinkage at the subrace level ( $\leq 0.77$ ). Within-subrace genetic correlations among the first three measures were also strongly positive ( $\geq 0.95$ ). These high genetic correlations suggest that different drying regimes and the calliper, visual and volume methods of assessment could be used interchangeably to select for reduced tangential gross shrinkage. Estimated subrace and genetic correlations between basic density and measures of gross shrinkage were universally negative (i.e. favourable), although not all were significantly different to zero.

*Key words:* Heritability, genetic correlation, genotype-by-drying-regime interaction, drying defect, shrinkage, collapse, basic density.

### Introduction

There are large areas of *E. globulus* plantation in Australia (c. 454000 ha in 2005; PARSONS *et al.*, 2006), Chile (c. 232,000 ha in 2003), Portugal (c. 700,000 ha in 2002) and Spain (c. 500,000 ha in 2002) (POTTS *et al.*, 2004). Although pulpwood production is the principal focus of most *E. globulus* growers, there is increasing interest in producing plantation-grown solid-wood products, such as appearance-grade sawn timber (RAYMOND, 2000; GREAVES *et al.*, 2004a; NOLAN *et al.*, 2005). Breeding, along with improved silviculture and the adoption of appropriate processing practices, is a possible means of improving the profitability of such plantations.

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