

Population differentiation within *Eucalyptus obliqua*: implications for regeneration success and genetic conservation in production forests

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Summary

Genetic differentiation at the site level was investigated within *Eucalyptus obliqua* L'Hérit. by comparing the progeny of ecologically distinct populations from two contrasting sites on topographic gradients at each of two localities, Lune and Forestier, in southern Tasmania. Progeny from all four sites of seed collection were planted together in trials at or near each collection site.

At the Lune locality there were significant differences in growth rates and susceptibility to leaf-spotting fungi between progeny grown from seed collected from mid-slope and plain populations respectively. At the Forestier locality, there were no differences between progeny from gully and ridge populations for growth rate and frost resistance. The gully population, however, had lower susceptibility to infection by leaf spotting fungi and browsing by native mammals than the ridge population.

The results indicated that differential selection forces may result in genetic differences between populations of *E. obliqua* over the scale of hundreds of metres, in addition to the tens or hundreds of kilometres normally associated with broad geographic variation at the ecotypic or provenance level.

Variation at the site level has important implications for the conservation of genetic diversity and for the probability of successful regeneration and long-term adaptation and growth within the typically heterogeneous native forest coupes in production forests.

Keywords: genetic variation; provenance; population structure; site types; artificial regeneration; seed collection; *Eucalyptus obliqua*

Introduction

Forest gene pools can be conserved in situ as reserves, or ex situ in plantings or seed stores (Pederick 1976). The reserve system generally relies upon the formal dedication of land areas such as national parks for specific conservation purposes. However, a useful complement to the formal system of in-situ conservation can be provided by adopting suitable management regimes in multiple-use forests. A general objective common to the forest practices codes of both Tasmania and Victoria is that the regeneration of native forests should maintain species

patterns and contribute to the conservation of local gene pools (CFL 1989; Forest Practices Board 2000). Forest management activities such as timber harvesting may be compatible with the objectives of gene conservation provided that appropriate regeneration systems are used (Shepherd 1974; Pederick 1976). Inappropriate regeneration systems, such as the use of foreign seed in artificial sowings and plantings, may cause changes in the genetic structure of local populations, often leading to a loss of genetic diversity (Gömöry 1992).

Special seed collection guidelines have been developed for clearfelled stands that are to be regenerated by artificial sowing within Tasmania and Victoria (Forestry Commission 1991; CNR 1994). These guidelines recommend that the seed to be used for regeneration purposes should be collected from the area to be regenerated, or from similar forest types in the same general locality. The rationale for the guidelines is that seed from a site that closely matches the area to be regenerated is most likely to contain genotypes best adapted to the site, thus minimising the risk of poor quality regeneration. In addition, the guidelines specify that collections should be made from an adequate number of trees in order to minimise the risk of narrowing the genetic base.

In practice, the proportion of on-site seed used to regenerate clearfelled areas has been very low. This is generally caused by small seed crops in areas scheduled for logging, a problem exacerbated by the temporal irregularity of good seed crops in most of the major commercial species (Cremer *et al.* 1978). As a result, forest managers collect large quantities of seed during good seed years in order to meet the expected needs for the next five-year regeneration program (Forestry Commission 1991). Subsequent sowing programs are then organised to conform as closely as possible to the guidelines by matching the seed source to the regeneration area on the basis of altitude, dryness, coldness, height of the mature forest and geographic proximity. The closeness of matching depends upon the availability and the economic cost of alternative sources of seed and a judgement about the level of similarity that is necessary in order to meet the general objective of using seed that is likely to be adapted to the sowing site. This judgement is based on current knowledge about the patterns of genetic diversity within individual species, and the extent to which this diversity can be captured and maintained by seed collection and sowing practices.

In a review of isozyme studies, Moran (1992) concluded that most genetic variation within eucalypt species occurs within, rather than between, populations. For species with a widespread distribution, Moran estimated that the mean proportion of total diversity due to differences between populations was less than 15%. Similarly, in a review of quantitative genetic variation expressed in common environment trials, Potts and Wiltshire (1997) reported relative levels of between population differentiation ranging from 1% to 87% of the total phenotypic variation, with the levels varying with species and trait. Tibbits and Reid (1987) attributed 75% of the variation in frost resistance of *E. nitens* to differences between provenances. In the case of *E. globulus*, variation between geographic races is high (15–22%) for leaf size, bark thickness, vegetative phase change and wood density, but low for growth and survival (Potts *et al.* 2004). Zobel and Talbert (1984) argue that in forest trees generally, 90% of all variation is due to differences between provenances and to differences between individual trees, and that the relative contribution of either will depend upon the particular trait under study. It is therefore apparent that seed collection programs for the regeneration of native forests should endeavour to capture genetic diversity at two levels. Initially, programs should aim to maintain a high level of genetic diversity in order to meet the general objectives of gene conservation. At a finer level of detail, the program should identify those traits that are most likely to be of substantial adaptive value and to ensure that the appropriate alleles are maintained within the collected gene pool.

This paper reports on the variation in survival, growth, frost tolerance, susceptibility to leaf spotting and to browsing between progeny grown from seed collected from adjacent but ecologically distinct populations of *E. obliqua* in Tasmanian native forests.

Materials and methods

Seed sources

The sources of *E. obliqua* seed used in these studies were selected from two geographic localities, Forestier and Lune, in southern Tasmania (Fig. 1). These localities are regarded as comparable seed zones on the basis of the close matching of the environmental attributes of altitude, dryness and coldness (Forestry Commission 1991). They are identified as separate geographic sources, however, because they are separated by a direct distance of 100 km (largely across a water surface) or an indirect distance of about 130 km via a continuous land surface. Both areas contain distinctive ecological gradients that are examples of those commonly found within the extensive range

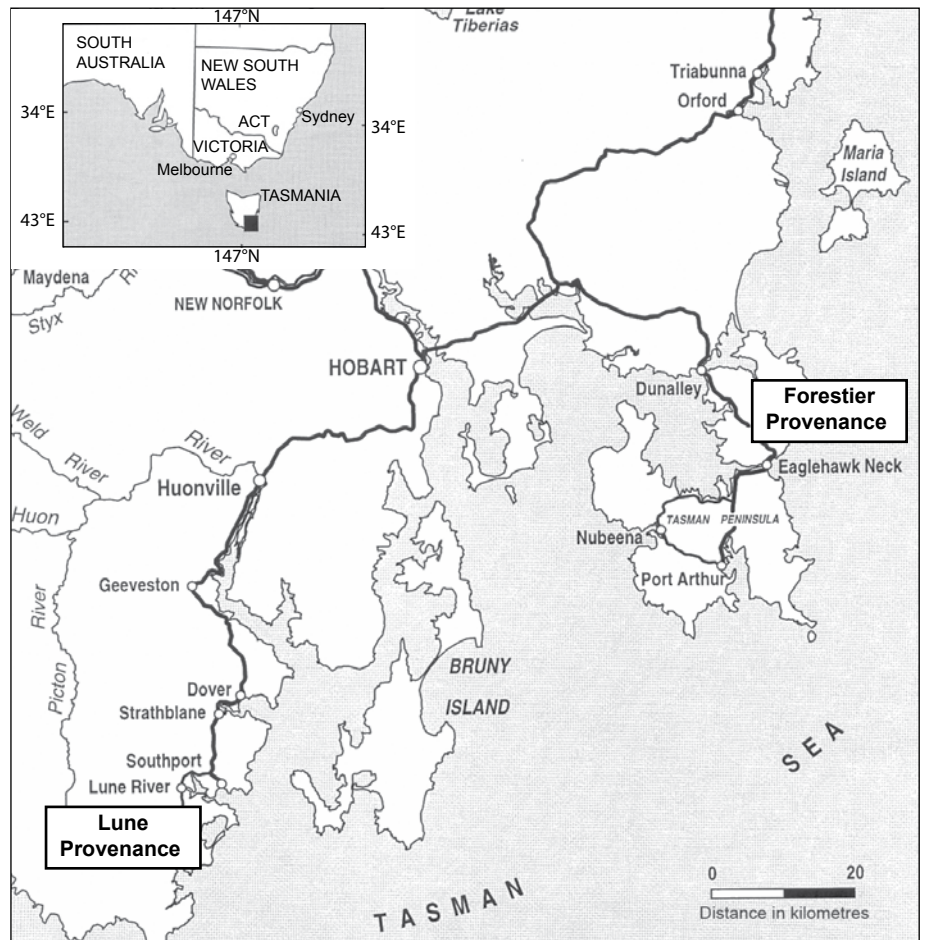


Figure 1. Location of the Forestier and Lune provenances

of *E. obliqua*. For the purposes of this study, the Forestier and Lune localities are regarded as provenances. Two forest types were selected from a topographic gradient (ridge to gully at Forestier and mid-slope to plain at Lune) within each provenance to study variation at the site level (Zobel and Talbert 1984). The four seed sources are referred to as populations in the current study. The gully and ridge populations occurred within 80–920 m of each other within the Forestier provenance and the mid-slope and plain populations were 180–620 m apart within the Lune provenance. Site descriptors for the four populations are shown in Table 1; further details are provided in Wilkinson (1995).

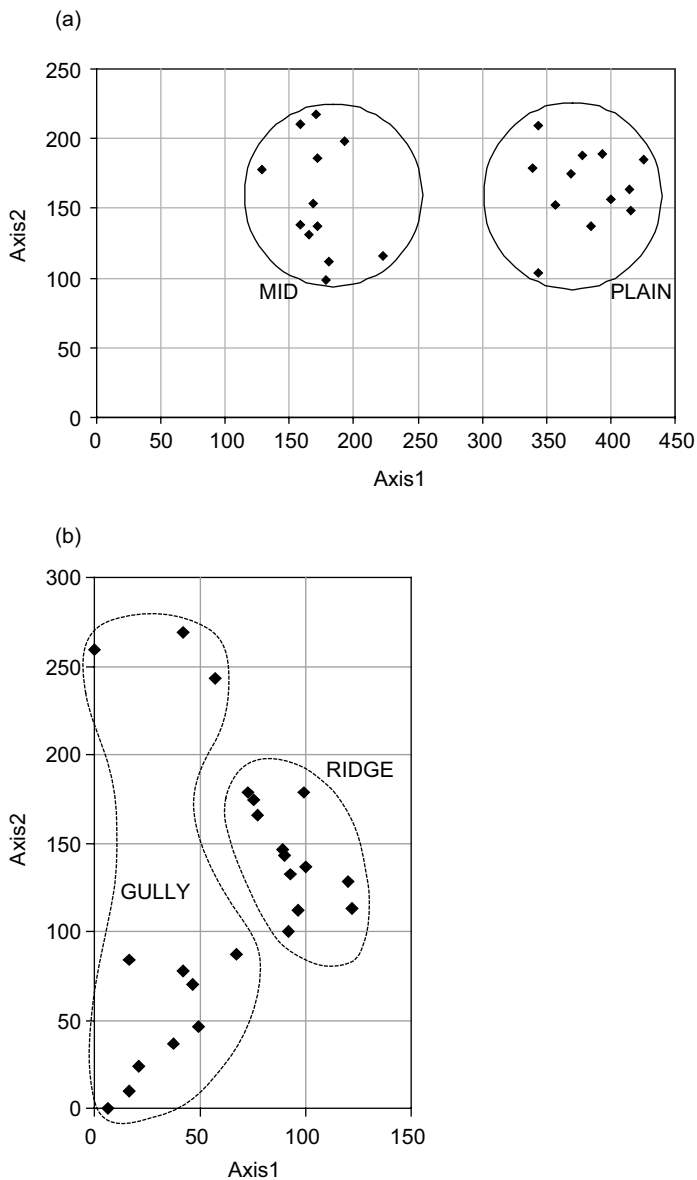
Seed was collected from 12 trees within each population. The criteria for selection of maternal trees were as follows:

- dominants or co-dominants within the main overstorey stratum
- spatially separated by a distance equivalent to no less than twice the tree height, to reduce the possibility of inbreeding effects
- sufficient seed available for experimental purposes (a minimum of 10 g).

Botanical surveys were undertaken at each selected tree. The tree, shrub and ground strata vegetation within a 10 m radius of each tree were sampled using TASFORHAB (Peters 1983) plots and species lists entered into the ECOPAK program (Minchin

Table 1. Site descriptors for the four populations

Population	Soil type	Altitude (m)	Rainfall (mm)	Forest type	Mean height (m)
Forestier Gully	Xanthozems	180–240	890	Wet sclerophyll forest within a moist gully	30.1
Forestier Ridge	Krasnozems	240–260	890	Open, dry sclerophyll forest along an exposed ridge	25.1
Lune Mid-slope	Xanthozems	70–90	1400	Wet sclerophyll forest on well-drained mid-slope	28.6
Lune Plain	Peaty podsols	30–50	1400	Open woodland on lower slopes adjoining poorly-drained plain	18.3

**Figure 2.** Ordination of vegetation plots on the basis of floristic similarity: (a) Lune Mid-slope and Lune Plain populations; (b) Forestier Gully and Forestier Ridge populations

1986) and subjected to an ordination analysis using the detrended correspondence analysis (DCA) technique of Hill and Gauch (1980). Results of this analysis indicated that the adjacent populations were clearly delineated on the basis of species composition (Fig. 2).

Mature capsules (containing seed) were collected from the selected maternal trees, mostly by the removal of one or more secondary branches from an open, sunny part of the upper canopy using climbing and hand sawing techniques. Two trees were felled in order to collect sufficient seed.

Survival and growth of planted seedlings

At each site, the initial selection of maternal trees was carried out within an area containing a recently clearfelled (<2 y) patch of forest so that a planting site could be located within the area occupied by each population or on a nearby site that had similar environmental attributes. Planting sites were located within the boundaries of the populations at Forestier Ridge, Forestier Gully and Lune Plain, whilst a nearby coupe was selected as the planting site to represent the Lune Mid-slope population. All sites except for Lune Plain were prepared for planting by burning the slash on the felled areas; Lune Plain had recently been burnt by a wildfire. Weed control was undertaken to remove existing vegetation and fences were constructed to reduce browsing by mammals.

Seedlings from the 48 families (12 families \times 4 populations) were raised in a glasshouse for 15 weeks using standard nursery techniques. The seedlings were removed from the glasshouse in July 1990 and then moved to an outside location for hardening-off until planting commenced, 4–5 weeks later. During this time the seedlings were subject to normal low winter temperatures (diurnal range 3–12°C) but were not subjected to frost. At the time of planting, the average height of the seedlings was 8.9 cm.

The trials were planted in a randomised complete block (RCB) design in August 1990. All 48 families were randomised separately within each block and within each planting site. Each family was represented by a plot of 6 trees within each block. There were 4 blocks at each site, giving a total of 1152 plants (12 families \times 4 populations \times 4 blocks \times 6 trees) for each trial.

Survival and tree height were assessed at 5, 9, 15, 21, 33 and 45 mo after planting, except at the Lune Plain site where survival was assessed at 5, 9, 15, 26, 31 and 41 mo and height growth measured from age 26 mo. Diameter was measured at breast height (1.3 m) over bark (dbhob) using vernier calipers, with the diameter recorded as the mean of two measurements taken at right angles around the stem. Diameter measurements were recorded when the mean tree height exceeded about 1.8 m, at ages 33 and 45 mo on the Lune Mid-slope and Forestier Gully sites, and at age 45 mo on the Forestier Ridge site. Total stem volume under bark was determined for each tree using the Opie equation (Opie 1976).

Measurements of survival and growth to age 45 mo were based on the total number of surviving trees within each plot. Survival was recorded as the percentage survival of seedlings within each plot. The data were subjected to the arcsine transformation prior to analysis to ensure that the residuals were normally distributed. Plot data for height and diameter growth were calculated as the mean value for surviving trees within the plot; volume data were expressed as the total volume of all trees within the plot.

Data analyses were initially undertaken separately for each planting site. Survival data were analysed by ANOVA, using the mean across the four blocks and treating the families as random terms, since the families within each population are not constant. Height and volume data at the Forestier Gully, Forestier Ridge and Lune Mid-slope planting sites were subjected to restricted maximum likelihood (REML) variance components analysis using the GenStat software package (Payne 2002). The limited height growth recorded at the Lune Plain site was analysed using ANOVA techniques as for survival (above). REML analysis of the plot mean data at the other three sites was undertaken using the following models given in GenStat model notation (Wilkinson and Rogers 1973):

Random model: population.family + block.population.family

Fixed model: constant + block + population
+ block.population

Hypothetical tests on fixed terms used Wald statistics, which can be compared to chi square critical values (Payne 2002). The estimate of the variance for the random term population.family was compared to its estimated standard error, which gives an approximate guide to the significance of the associated random variability. Differences between pairs of means of the populations were compared with the standard error of the difference using the *t*-test.

Mean and standard error were calculated for survival across all populations within each planting site. Analysis of height and volume data across the planting sites was undertaken using REML variance components analysis. The analysis was restricted to three sites (Forestier Gully, Forestier Ridge and Lune Mid-slope), with the Lune Plain site omitted because of very low survival and growth rate. Data from the age 45 mo measurement were used in the REML analysis. The following model was used to analyse the plot mean data across the sites:

Random model: site.block + population.family
+ site.population.family

Fixed model: constant + site + population + site.population

Sub-model: constant + site + population.

The analysis was used to determine the magnitude of the site × population interaction by calculating the deviance between the sub-model and full fixed model. The variance components for the random terms were estimated from a REML analysis carried out on the individual (tree) values using the following model:

Random model: site.block + population.family
+ site.population.family
+ site.block.population.family

Fixed model: constant + site + population + site.population.

Frost tolerance

Thermometers installed at the Lune Plain planting site indicated that the seedlings were subjected to frosts as low as -4.5°C during the first week after planting. Frost damage was evident as necrotic tissue on the leaf lamina four weeks after planting. All seedlings from the four populations planted at this site were visually assessed for the proportion of the total lamina area maintained as green tissue on each seedling. Seedlings were assessed and assigned to a percentage damage class (after Wilcox 1982; Tibbits and Reid 1987) as follows:

Score	Fraction of green leaf tissue (%)
0	<10
20	11–30
40	31–50
60	51–70
80	71–90
100	>90

Plot means were calculated and the data were subjected to REML variance components analysis, using the following models:

Random model: population.family

Fixed model: constant + population + blocks
+ population.blocks.

The Wald test was used to determine the significance of fixed effects. Differences between the means of the populations were compared with the standard error of the difference using the *t*-test ($P = 0.05$).

Fungal leaf spotting and browsing damage

Assessments of the leaf spotting disease *Mycosphaerella cryptica* were made by an ocular assessment of the proportion of necrotic leaf tissue on each seedling in the planting sites. Damage classes selected for the assessments were as follows:

Leaf spotting rating	Fraction of leaf covered by necrotic tissue (%)
1	0
2	1–10
3	11–20
4	21–30
5	31–75
6	>75

Assessments were carried out at the planting sites when the seedlings were in their third growing season. The ratings for leaf spotting were considered a multinomial response and were analysed by fitting an ordinal regression using composite link functions and REML analysis using GenStat. Initially, class probabilities were determined from a logistic distribution with the nominal class intervals dividing up the percentage scale to produce cut-points of 10, 20, 30 and 75. The nominal cut-points were then used as initial values to estimate cut-point parameters in a random effects, proportional-odds ordinal regression model (Candy 1997; Candy and Wilkinson 1997).

The effect of seedling height on susceptibility to leaf spotting was evaluated by covariate analysis, using the mean height of the seedlings at age 2 y (i.e., the approximate height of the seedlings during the period of initial leaf infection) as a covariate. Leaf spotting was analysed as the plot mean across the four blocks, using families as random effects. The relationship between the height and the infection severity of individual seedlings was determined by regression analysis, using the nominal cut-points for percentage damage of 0, 10, 20, 30, 75 and 100 for ratings 1 to 6 respectively.

Fences erected at the planting sites totally excluded browsing animals, except at the Forestier Gully site where seedlings were damaged by the brushtail possum (*Trichosurus vulpecula* (Kerr)). Browsing intensity was determined by recording the severity of leaf stripping on individual seedlings 15 mo after planting, using the following rating system:

Browsing rating	Intensity of defoliation
0	Nil
1	Loss of growing tips
2	<50% loss of foliage
3	>50% loss of foliage

The probability of browsing was determined by analysing the browsing data as a binomial distribution of unbrowsed (rating 0) and browsed (ratings 1–3) using the generalised linear mixed model (GLMM) (Breslow and Clayton 1993; Candy 1997) and REML analyses of GenStat. The GLMM analysis used the Logit link function with the following model:

$$\text{model} = [\text{families (random term)}] + [\text{constant} + \text{population} + \text{block} + \text{population.block (fixed terms)}].$$

The probability of a seedling being browsed (P_b) was determined as:

$$P_b = [(\exp(\text{model})) / [1 + \exp(\text{model})]]$$

Differences between the populations were compared with the standard error of the difference using the *t*-test.

Results

Survival of seedlings

Survival rates of >80% were maintained for all populations at the Forestier Gully, Forestier Ridge and Lune Mid-slope planting sites and there were no significant differences between the four populations or site \times population interactions over the first 45 mo (Table 2). In contrast, survival of all populations at the Lune Plain site was significantly lower than at the other sites, with high mortality occurring within the first 5 mo after planting. At age 41 mo, survival at the Lune Plain site was only 20%.

Height growth

The mean height of seedlings varied significantly on all four planting sites (Fig. 3). The tallest height growth was recorded at the Forestier Gully site and there was negligible growth at the Lune Plain site. At age 45 mo, there were significant differences between populations across the three sites at Forestier Gully,

Table 2. Site \times population interaction for the survival, height and volume of four populations at age 45 mo on three planting sites (Forestier Gully, Forestier Ridge and Lune Mid-slope)

Character	Deviance	d.f.	Significance
Survival	7.28	6	ns
Height	11.48	6	ns
Volume	17.95	6	0.01

ns = not significant

d.f. = degrees of freedom

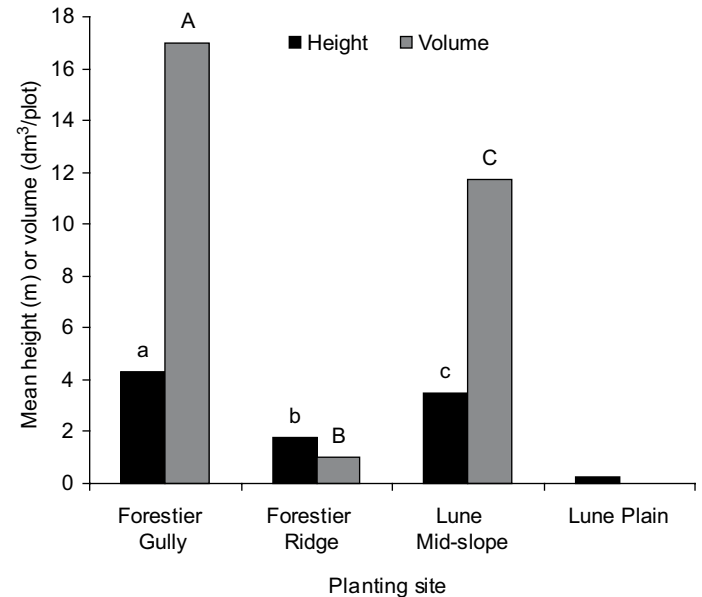


Figure 3. Mean height and total plot volume of all populations at age 45 mo on four planting sites (identical letters indicate non-significant subsets $P > 0.05$, with the Lune Plain site not included in the analysis)

Forestier Ridge and Lune Mid-slope (Fig. 4). The Lune Mid-slope population was significantly taller than all other populations, and the Lune Plain population significantly shorter than the Lune Mid-slope and Forestier Gully populations. The site \times population interaction was not significant (Table 2) and the population rankings were maintained across each of the three sites (Fig. 5).

Differences in the height growth of populations at the Forestier Gully site were expressed by age 9 mo (Table 3) when the Lune Mid-slope population was significantly taller than the other three populations. This trend continued to at least age 45 mo (Fig. 5). Differentiation of populations occurred slightly later (15 mo) at the Forestier Ridge site with the Lune Mid-slope population recording better height growth than the Lune Plain and Forestier Gully populations. By age 45 mo, the Lune Mid-slope, Forestier Gully and Forestier Ridge populations had similar heights and were all significantly different to the Lune Plain population (Fig. 5). Early differences between the Lune Mid-slope and Lune Plain populations were evident at the Lune Mid-slope site at age 9 mo. From age 21 mo, the Lune Mid-slope population was significantly taller than the other populations and the Lune Plain population was significantly shorter.

Analysis of the variance components for height growth across the planting trials indicated that most of the variation due to random terms occurred between trees (74%) and plots (16%) (Table 4). The Forestier Gully site was the only planting site to record significant variation between families within populations, with up to 26% of the total random variation attributed to this source (Table 3).

Height increment at the Lune Plain site averaged less than 4 cm y⁻¹ to age 45 mo (Fig. 3) and there were no significant differences

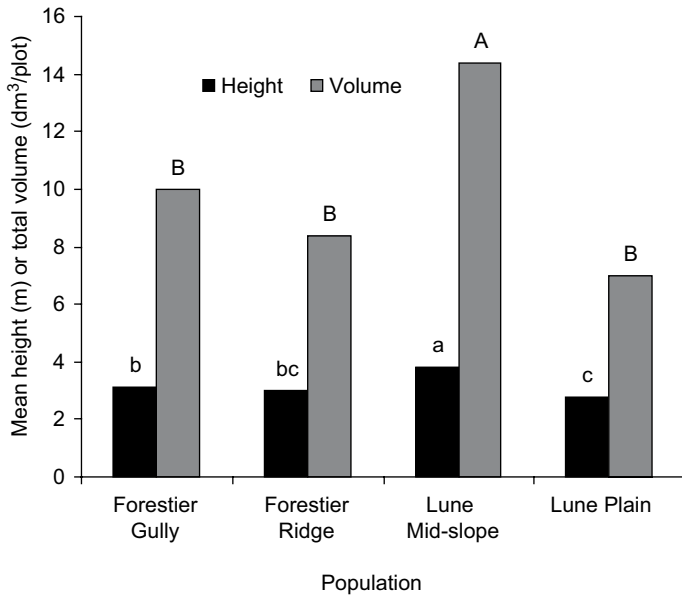


Figure 4. Mean height and total plot volume of each population at age 45 mo across three planting sites at Forestier Gully, Forestier Ridge and Lune Mid-slope (identical letters indicate non-significant subsets $P > 0.05$)

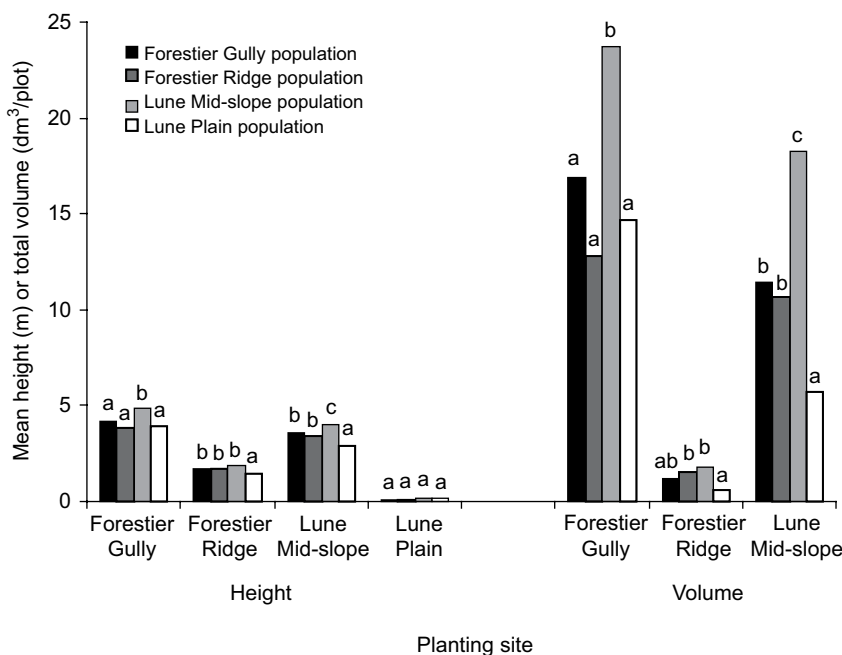


Figure 5. Mean height and total plot volume of four populations at age 45 mo on each of four planting sites (identical letters indicate non-significant subsets $P > 0.05$)

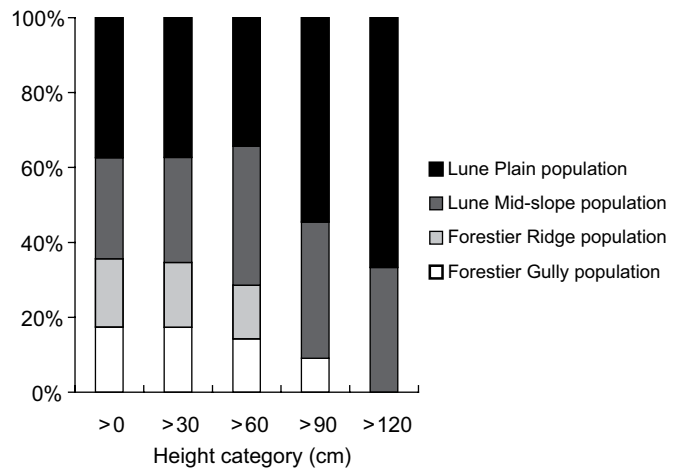


Figure 6. Proportion of seedlings from four populations within various height categories at age 60 mo in the Lune Plain planting trial

between populations. However, segregation of the surviving seedlings into height classes showed that the Lune Mid-slope and Lune Plain populations represented an increasingly larger proportion of the seedlings in the taller height classes (Fig. 6). The future survival of seedlings < 90 cm in height was assessed to be unlikely because of severe competition from the dense understorey vegetation. The Forestier Ridge and Forestier Gully populations were not represented in the >90 cm and >120 cm height classes respectively.

Volume

Total volume production varied significantly between planting sites, with the Forestier Gully site producing 14 times the volume of the nearby Forestier Ridge site at age 45 mo (Fig. 3). Growth at the Lune Plain site was insufficient to allow for the calculation of volume. Across the other planting sites, there were significant differences between populations, with the Lune Mid-slope population producing the greatest volume (Fig. 4). There was also a significant interaction between populations and sites (Table 2). This interaction mainly affected the magnitude of differences, rather than the ranking of populations at each site. In particular, the difference between the Lune Mid-slope and Lune Plain populations was much larger at the Lune Mid-slope site than at other planting sites (see below).

Differences in volume production were recorded at the Forestier Gully site at 33 and 45 mo after planting (Table 5, Fig. 5). At these ages, the Lune Mid-slope population had produced significantly more volume than the other populations.

Volumes produced by the Lune Mid-slope, Forestier Gully and Forestier Ridge populations were not significantly different at the Forestier Ridge site at age 45 mo (Table 5, Fig. 5). However, the Lune Plain population produced

Table 3. Results from REML variance components analysis of data for height of surviving trees at various ages on the Forestier Gully, Forestier Ridge and Mid-slope planting sites

Age (mo)	Planting site	Fixed term	d.f.	Wald statistic ^{#1}		Random term	Variance components \pm SE ^{#2 #1}			
5	Forestier Gully	block	3	55.6	**	pop.fam	9.62	\pm 3.59	(26%)	*
		pop	3	5.7	ns	block.pop.fam	26.95	\pm 3.32		
		block \times pop	9	4.3	ns					
	Forestier Ridge	block	3	27.8	**	pop.fam	6.65	\pm 4.10	(18%)	ns
		pop	3	7.6	ns	block.pop.fam	36.94	\pm 5.11		
		block \times pop	9	10.7	ns					
	Lune Mid-slope	block	3	3.4	ns	pop.fam	3.35	\pm 2.37	(11%)	ns
		pop	3	4.9	ns	block.pop.fam	27.97	\pm 3.44		
		block \times pop	9	4.3	ns					
9	Forestier Gully	block	3	25.8	**	pop.fam	67.6	\pm 36.1	(15%)	ns
		pop	3	12.9	**	block.pop.fam	371.3	\pm 45.1		
		block \times pop	9	9.7	ns					
	Forestier Ridge	block	3	47.3	**	pop.fam	5.27	\pm 3.28	(13%)	ns
		pop	3	7.4	ns	block.pop.fam	36.66	\pm 4.51		
		block \times pop	9	12.4	ns					
	Lune Mid-slope	block	3	8.0	*	pop.fam	2.2	\pm 13.7	(1%)	ns
		pop	3	11.2	*	block.pop.fam	216.4	\pm 26.6		
		block \times pop	9	6.6	ns					
15	Forestier Gully	block	3	31.0	**	pop.fam	184.3	\pm 79.9	(21%)	*
		pop	3	19.2	**	block.pop.fam	706.4	\pm 87.0		
		block \times pop	9	11.0	ns					
	Forestier Ridge	block	3	90.9	**	pop.fam	7.18	\pm 6.94	(7%)	ns
		pop	3	9.4	*	block.pop.fam	90.55	\pm 11.15		
		block \times pop	9	12.6	ns					
	Lune Mid-slope	block	3	10.5	*	pop.fam	12.7	\pm 36.6	(2%)	ns
		pop	3	20.6	**	block.pop.fam	556.0	\pm 68.4		
		block \times pop	9	8.0	ns					
21	Forestier Gully	block	3	38.5	**	pop.fam	581	\pm 271	(19%)	*
		pop	3	16.4	**	block.pop.fam	2548	\pm 314		
		block \times pop	9	10.8	ns					
	Forestier Ridge	block	3	74.0	**	pop.fam	19.8	\pm 20.8	(7%)	ns
		pop	3	12.2	**	block.pop.fam	276.8	\pm 34.1		
		block \times pop	9	11.1	ns					
	Lune Mid-slope	block	3	4.8	ns	pop.fam	27	\pm 106	(2%)	ns
		pop	3	29.4	**	block.pop.fam	1635	\pm 201		
		block \times pop	9	8.4	ns					
33	Forestier Gully	block	3	21.3	**	pop.fam	1692	\pm 928	(15%)	ns
		pop	3	12.4	**	block.pop.fam	9709	\pm 1195		
		block \times pop	9	10.2	ns					
	Forestier Ridge	block	3	78.0	**	pop.fam	47	\pm 91	(3%)	ns
		pop	3	12.6	**	block.pop.fam	1327	\pm 163		
		block \times pop	9	12.2	ns					
	Lune Mid-slope	block	3	3.7	ns	pop.fam	325	\pm 362	(6%)	ns
		pop	3	34.9	**	block.pop.fam	4884	\pm 601		
		block \times pop	9	11.2	ns					
45	Forestier Gully	block	3	31.8	**	pop.fam	3273	\pm 1348	(22%)	*
		pop	3	13.0	**	block.pop.fam	11338	\pm 1396		
		block \times pop	9	10.5	ns					
	Forestier Ridge	block	3	68.3	**	pop.fam	780	\pm 657	(9%)	ns
		pop	3	12.8	**	block.pop.fam	8255	\pm 1016		
		block \times pop	9	9.3	ns					
	Lune Mid-slope	block	3	4.3	ns	pop.fam	118	\pm 222	(4%)	ns
		pop	3	29.1	**	block.pop.fam	3256	\pm 401		
		block \times pop	9	9.4	ns					

^{#1} * = $P < 0.05$, ** = $P < 0.01$, ns = not significant ($P > 0.05$)^{#2} (% of total random variation)

Table 4. Estimated variance components for the height and volume of four populations at age 45 mo on three planting sites (Forestier Gully, Forestier Ridge and Lune Mid-slope)

Random term	Height			Volume		
	Component	S.E.	Fraction of variation (%)	Component	S.E.	Fraction of variation (%)
site.block	1288	683	5	0.403	0.222	4
population.family	724	365	3	0.096	0.097	1
site.population.family	684	416	3	0.109	0.148	1
site.block.population.family (plot)	4000	538	16	1.465	0.224	14
units (tree)	18668	539	74	8.696	0.251	81

Table 5. Results from REML variance components analysis of data for the total volume at the Forestier Gully, Forestier Ridge and Mid-slope planting sites

Age (mo)	Planting site	Fixed term	d.f.	Wald statistic ^{#1}	Random term	Variance components ± SE ^{#2 #1}			
33	Forestier Gully	block	3	38.4 **	pop.fam	2.22	±1.57	(12%)	ns
		pop	3	15.6 **	block.pop.fam	18.17	±2.26		
		block × pop	9	19.5 *					
	Lune Mid-slope	block	3	9.2 *	pop.fam	0.91	±1.26	(5%)	ns
		pop	3	18.2 **	block.pop.fam	17.40	±2.16		
		block × pop	9	12.0 ns		2.22	±1.57	(12%)	ns
45	Forestier Gully	block	3	38.5 **	pop.fam	32.2	±15.2	(18%)	*
		pop	3	11.9 **	block.pop.fam	143.6	±17.7		
		block × pop	9	21.8 **					
	Forestier Ridge	block	3	31.4 **	pop.fam	0.159	±0.213	(4%)	ns
		pop	3	10.1 *	block.pop.fam	3.933	±0.484		
		block × pop	9	11.6 ns					
Lune Mid-slope	block	3	5.9 ns	pop.fam	5.9	±8.3	(5%)	ns	
	pop	3	27.5 **	block.pop.fam	117.1	±14.4			
	block × pop	9	15.5 ns						

^{#1} = significant at $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant ($P > 0.05$)

^{#2} percentage of total random variation shown in brackets

significantly less volume than the Lune Mid-slope and Forestier Ridge populations.

Large differences in volume production were recorded at the Lune Mid-slope site (Table 5, Fig. 5). At age 33 mo, the greatest volume was produced by the local (Lune Mid-slope) population. Further differentiation occurred by age 45 mo, when the Lune Mid-slope population had produced significantly more volume and the Lune Plain population significantly less volume than the other populations. At this age, volume production by the Lune Plain population was only 50% of that produced by the Forestier Gully and Forestier Ridge populations, and only 31% of that produced by the Lune Mid-slope population (Fig. 5).

Analysis of the variance components for volume indicated that most variation occurred between trees (81%) and plots (14%) (Table 4). Within the individual planting trials, variation between families within populations was generally not significant and represented less than 18% of the total random variation (Table 5).

Frost tolerance

There were significant differences between populations for the frost resistance of seedlings planted at the Lune Plain site (Wald statistic = 9.9, $P < 0.05$). Seedlings from the Lune Plain population had the lowest levels of frost damage with necrotic leaf tissue representing $42.4 \pm 6.4\%$ of total leaf area (compared with 56.1%, 58.2% and 61.1% for the Lune Mid-slope, Forestier Gully and Forestier Ridge populations respectively). Significant differences were also attributed to blocks (Wald statistic = 8.7, $P < 0.05$) and there were no significant interactions between populations and blocks (Wald statistic = 13.9, $P > 0.05$).

Fungal leaf spotting and browsing damage

The severity of leaf spotting was highest at the Lune Mid-slope planting site where 64% of all seedlings had necrotic tissue covering $>20\%$ of the total leaf surface area. Across the three planting sites, there were significant differences in damage

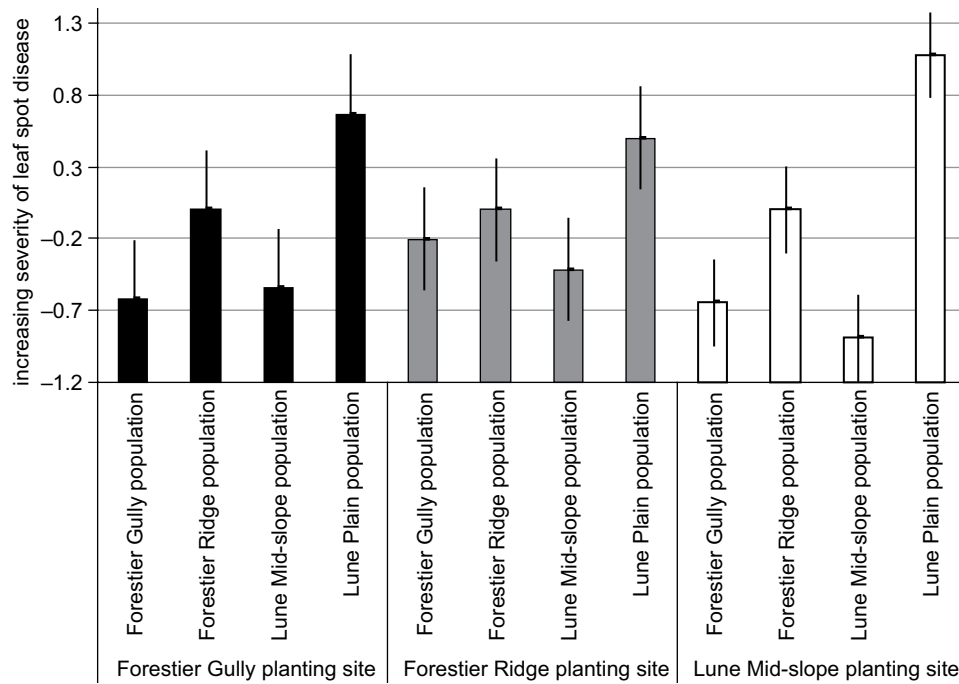


Figure 7. Calculated damage score and SE of differences for leaf spotting damage sustained by four populations at the Forestier Gully, Forestier Ridge and Lune Mid-slope planting sites

levels between populations (Fig. 7). Covariate analysis indicated that mean damage rating for leaf infection was not significantly correlated with the mean plot height (F -ratio = 0.01) but was highly significant for populations (F -ratio = 6.28, $P < 0.001$).

The Lune Plain population had significantly more damage than the Lune Mid-slope and Forestier Gully populations at all sites. Significant differences were also recorded between the Forestier Gully and Forestier Ridge, and between the Forestier Ridge and Lune Plain populations at the Lune Mid-slope site. Block \times population interactions were significant ($P < 0.01$) at the Forestier Gully site but not at the Forestier Ridge and Lune Mid-slope sites. The interaction at the Forestier Gully site was mainly due to differing severity between blocks, with lower levels of infection in one block.

The probability of a seedling being browsed at the Forestier Gully site varied significantly between populations (Fig. 8). The 'local' populations (Forestier Gully and Forestier Ridge) were more than twice as likely to be browsed as the two 'non-local' populations (Lune Mid-slope and Lune Plain). In addition, the Forestier Ridge population had a significantly higher probability of being browsed than the Forestier Gully population.

Discussion

Genetic differentiation between sites may arise as a result of geographic isolation or differential selection forces (Barber and Jackson 1957; Moran 1992; Potts and Wiltshire 1997; Steane *et al.* 2006). The natural distribution of *E. obliqua* within Australia can be regarded as widespread, with several disjunct populations. Within south-eastern Tasmania the distribution pattern is relatively continuous over extensive distances. Isozyme studies suggest that disjunct populations are likely to be associated with high levels

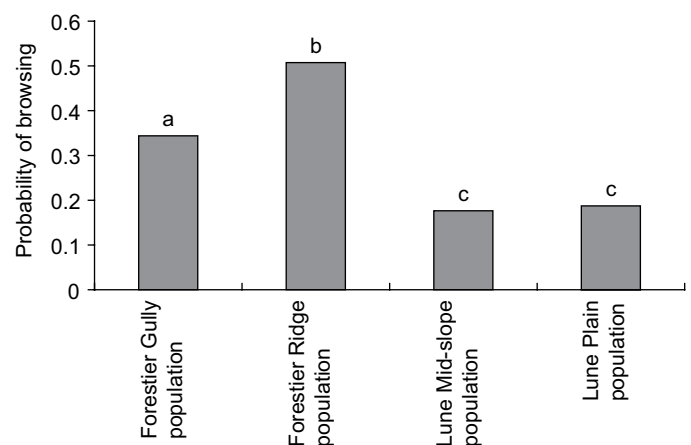


Figure 8. Probability of seedlings being browsed at the Forestier Gully planting site (identical letters indicate non-significant subsets $P > 0.05$)

of genetic variation between populations, whilst the continuous populations are likely to have high levels of both total genetic diversity and variation within populations, but low levels of variation between populations (Moran 1992). This pattern appeared to be confirmed by previous studies of *E. obliqua* which have demonstrated clear differences in the allelic frequencies of the major 'disjunct' populations in NSW, Victoria, Tasmania and South Australia (Brown *et al.* 1975), and little difference between populations within Tasmania (Brown *et al.* 1976).

The distribution pattern between the Forestier and Lune provenances in the current study is relatively continuous over a land distance of about 130 km, although there are some discontinuities on drier sites of up to 10 km where *E. obliqua* is displaced by

other species. Eucalypt seed has a dispersal distance restricted to about 60–90 m (Cremer 1966) and most gene flow is likely to result from pollen dispersal, which generally occurs over short distances of 50–300 m (Barber 1965; Potts and Reid 1983; Barbour *et al.* 2005) but may extend to over 1 km (Ashton 1981). Genetic divergence, however, is more likely to be related to evolutionary time since separation than to current geographic isolation between populations (Coates and Sokolowski 1989). The minor discontinuities over the geographic distance between these two provenances are therefore unlikely to have provided major barriers to gene flow. Within the study localities the two sets of adjoining populations formed a continuous distribution, with the distance between the nearest and most distant maternal trees from each population being 80–920 m at Forestier and 180–620 m at Lune. It could therefore be assumed that very active gene flow would occur within and between the populations on the adjacent sites at Forestier and Lune.

Population differentiation for growth rate was clearly evident between the Lune sites but not between the Forestier sites. Rapid early growth on wet forest sites allows seedlings to gain site occupancy and establish an advantage over the competing vegetation. On less favourable sites selection may be more related to tolerance of environmental stresses such as frost, drought and low soil fertility (Pederick 1976). There were significant differences in the growth of seedlings at the Forestier Gully and Forestier Ridge planting sites, with the mean height of seedlings at the Forestier Gully site 2.5 times greater than at the Forestier Ridge site and the total volume production 14 times greater at age 4 y. Nevertheless, the lack of differentiation in growth between the Forestier Gully and Forestier Ridge populations implies that similar selection forces for growth operate at these two sites, at least for seedlings up to the age of 5 y. In contrast, the highly significant difference between the Lune Mid-slope and Lune Plain populations implies differential selection for growth between these two sites. Rapid early growth would be of substantial adaptive value on the Mid-slope site, where prolific regeneration of the sedge *Gahnia grandis* provides intense competition to young eucalypt seedlings. In contrast, slow growth during the seedling stage on the Lune Plain site may have adaptive value in minimising the risk of damage to soft new growth by harsh environmental factors such as frost and summer drought. Slow growth would need to be balanced against the need to compete with the regeneration of sedges such as *Gymnoschoenus sphaerocephalus*. This understorey forms a very dense but relatively low stratum 0.5 to 2.0 m high (Marsden-Smedley 1993) and the eucalypts are likely to be suppressed unless their growing tips remain above the general height of the sward.

Leaf disease may be a primary cause of poor health and growth loss in eucalypts (Marks *et al.* 1982; Carnegie and Ades 2003) and is also regarded as a secondary symptom of other stress, such as that associated with plantings of species or provenances on unsuitable sites (Wardlaw 1990). Population variation in disease susceptibility has been reported in eucalypts at the provenance level (Harris *et al.* 1985; Purnell and Lundquist 1986; Carnegie and Ades 2005) and the current study has found that differentiation can occur at the site level, with significant differences between progeny from adjoining populations. Conditions conducive to high infection levels are most likely to occur within dense stands of regeneration on wet sites (such as the Lune Mid-slope site). On

such sites, the lower susceptibility of wet forest populations would be important for the maintenance of vigorous growth.

Differences in susceptibility to browsing by native mammals could result from direct selection, or may indicate parallel or unrelated selection for differences in leaf characteristics (Gill 1992; Haukioja *et al.* 1991). Browsing pressure is highly variable from one forest area to another (Wilkinson and Neilsen 1995). The current study provided evidence of substantial variability in browsing pressure over the distance of 500 m that separated the planting trials at the Forestier Gully and Forestier Ridge sites, with the heaviest browsing pressure at the Forestier Gully site. Severe browsing can result in very high mortality and growth losses, and these effects are exacerbated by vigorous competition from understorey vegetation on wet forest sites (Wilkinson and Neilsen 1995). Accordingly, the reduced susceptibility of the Forestier Gully population to browsing may indicate direct selection for this attribute. In contrast, the Forestier Ridge population may have an alternative strategy for tolerating browsing pressure, such as a higher capacity for recovery from lignotuberous shoots.

Variation between populations at the site level, as reported in this study, has important implications for the maintenance of genetic diversity within native forests. In forests being managed for wood production, the genetic diversity of artificially regenerated stands may be substantially lower than that of virgin stands, probably as a result of the restricted number of maternal trees that contribute to the sowing mix (Gömöry 1992). The maintenance of high levels of diversity is fundamental to the maintenance of potential for an adaptive response to environmental change. This may be particularly important for species that occupy a broad ecological range. Native forest sites often consist of a mosaic of heterogeneous sites that are associated with distinctive patterns of niche differentiation at the species level. Clearfelling followed by artificial sowing with seed from a mixture of species generally leads to vigorous natural selection and the maintenance of the original species patterns (Elliott *et al.* 1991). The omission of naturally-occurring species from the sowing mix can lead to a loss of species diversity and the occurrence of poorly adapted species on specific sites (Elliott *et al.* 1991).

The differentiation of populations in the current study indicates that similar selection forces may also operate at the intra-specific level. The potential narrowing of the genetic diversity within seed mixtures used for regeneration purposes could therefore result in long-term effects on adaptation and growth. For example, the use of seed from the Lune Plain population could result in substantially lower productivity on highly productive sites compared to seed from the Mid-slope population. In contrast, the Lune Plain population may have a higher probability of long-term survival and growth on harsh sites.

Variation at the site level therefore has important implications for the management of seed collection and sowing programs in native forest regeneration areas. Forest management units are mapped by photographic interpretation (PI) to a minimum area of about 3 ha (G. Dowl, Forestry Tasmania, *pers. comm.*). PI units are mapped on the basis of forest type characteristics, such as mean tree height, tree density and age structure. Harvesting coupes are generally about 30–70 ha in size, and can therefore encompass a mosaic of many different patches. Ecological gradients, such as

the topographic sequences used in the current study, commonly occur within coupes, particularly in steep country where there can be pronounced differences between the ridge-top forest and the gully forest. In such situations the collection of seed from the lower gully position is often very difficult, for two reasons. Firstly, the gully sites are generally occupied by wet forests, which tend to produce smaller and less frequent seed crops than the large open-crowned trees of the dry forests on the ridges (Edwards 1995). Secondly, the road system is generally constructed along ridge tops and physical access to the seed crops in the gully forest is often very difficult. In the past, phenotypic variation within a coupe has been largely attributed to environmental factors, and although seed collection guidelines prompted collection from as many trees as possible across the coupe (Forestry Commission 1991), there was no specific provision for variation at the site level.

Genetic diversity across heterogeneous sites in wood production forests could be maintained in a number of ways. Firstly, natural regeneration systems can be used by adopting partial logging systems in appropriate forest types. Such systems are currently applied to most dry forest types in Tasmania, and their extension into suitable wet forest types is continuing to be reviewed (Wilkinson 1992; Hickey *et al.* 2001). Natural regeneration systems have the advantage of using in-situ seed sources at the local site level. However, the level of retention and degree of relatedness of potential seed sources must be considered, since low retention levels can lead to a loss of genetic diversity (Gömöry 1992) and the creation of patches of related individuals that are more extensive than those found within natural forests (Richmond 1971).

In forest types not currently suitable for natural regeneration systems, genetic diversity could be maintained by the delineation and treatment of heterogeneous patches, or by sowing diverse seed mixtures across all sites. The first option would be appropriate where it is possible to clearly delineate major changes in forest type. Collections of seed could be undertaken from within these patches and then applied to the specific sites using ground sowing techniques (Forestry Commission 1991) or by using aerial application that allows areas as small as 0.25 ha to be individually sown. The second option for highly heterogeneous types would be to ensure that seed is collected from the full range of sites for mixing and application across all sites. This system relies on the processes of natural selection to maintain patterns of genetic diversity.

The delineation of specific sites for special seed collections will be determined by the likelihood of genetic variation, as indicated by the phenotypic variability of the stands and the environmental heterogeneity of the sites. The current results provide evidence of significant differentiation between adjoining populations for a range of attributes that may be associated with long-term adaptation and growth. Collection of seed across the range of sites within heterogeneous management units may therefore be important for the long-term maintenance of genetic diversity within native forests.

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