EXPLORATION OF THE *EUCALYPTUS GLOBULUS* GENE POOL

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ABSTRACT

The first Europeans to discover *Eucalyptus globulus* were French explorers in 1792. Its seed was rapidly spread throughout the world in the 19th century and this was the species by which much of the world first knew the genus. However, it was in the industrial forests of the 20th century that this species, once considered the 'Prince of Eucalypts', achieved greatest prominence due to its fast growth and superior pulp qualities. Formal breeding first commenced in 1966 in Portugal and in the late 1980's large base population trials from open-pollinated seed collections from native stands were established in many countries. These trials have provided unprecedented insights into the quantitative genetic control of numerous traits of economic and ecological importance and how this variation is spatially distributed in the native range of the species. However with large, fully pedigreed breeding populations becoming available for quantitative analysis and the rapidly expanding knowledge of DNA sequence variation, we are now at the threshold of a new understanding of this important eucalypt gene pool. Indications of the significance of non-additive genetic effects are becoming available. The *E. globulus* chloroplast genome has now been sequenced and several genome maps have been published. Studies of the variation in nuclear microsatellites and the lignin biosynthesis gene *CCR* confirm the complex, spatially structured nature of the native gene pool. Strong spatial structuring of the chloroplast genome has provided a tool for tracking seed migration and the geographic origin of exotic landraces. Highly divergent lineages of chloroplast DNA have been discovered and studies of the hypervariable J_{LA+} region argue that some components of the *E. globulus* gene pool have been assimilated from other species following hybridisation.

INTRODUCTION

Eucalyptus globulus Labill. (Tasmanian blue gum) is one of the most important pulpwood plantation species in the world (Eldridge *et al.* 1993; Potts 2004). It is a forest tree with a native range on the island of Tasmania, the Bass Strait Islands and adjacent coastal regions of Victoria on continental Australia (Jordan *et al.* 1993; Dutkowski and Potts 1999; Fig. 1). It is one of four closely related taxa, which are variously given specific taxonomic status (*Eucalyptus globulus*, *E. bicostata*, *E. pseudoglobulus* and *E. maidenii* - Brooker 2000) or treated as subspecies of *E. globulus* (Kirkpatrick 1974; Pryor and Johnson 1971). These taxa are herein treated at the specific level (Brooker 2000). The cores of these four taxa are morphologically and geographically distinct, but linked by morphologically and geographically intermediate (intergrade) populations (Kirkpatrick 1974, 1975a; Jordan *et al.* 1993; Jones *et al.* 2002a; Fig. 1).

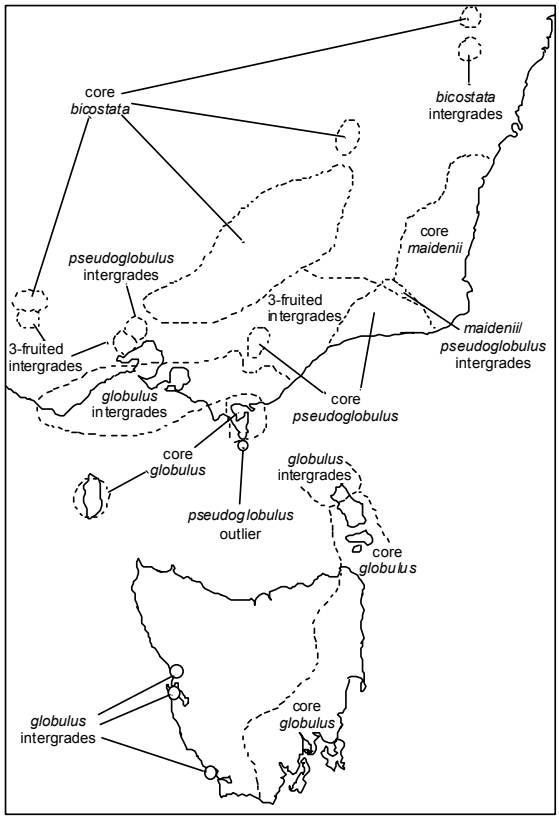


Figure 1. Distribution of core and intergrade populations of *E. globulus*, *E. bicostata*, *E. pseudoglobulus* and *E. maidenii*
Populations are classified based on morphological analysis of their capsule morphology (Jordan *et al.* 1993). Capsule size, shape and number per umbel are the main taxonomic features separating the four species. *Eucalyptus maidenii* has up to seven capsules per umbel and the smallest capsules. *E. globulus* has solitary fruit and the largest capsules. Both *E. bicostata* and *E. pseudoglobulus* are three fruited, but *E. pseudoglobulus* has smaller capsules and longer pedicels than *E. bicostata*. There is more or less continuous variation in capsule morphology between the cores. The populations sampled in the 1987/88 CSIRO collections were spread throughout the distribution of core *E. globulus* and *E. globulus* intergrades (modified from Jordan *et al.* 1993).

DISCOVERY
Eucalyptus globulus was discovered on the island of Tasmania (Recherche Bay; Fig. 2) in 1792 by French explorers and was one of the first eucalypt species to be formally described (Labillardière 1799). The primeval eucalypt forests of Tasmania were amongst the tallest forests in the world and *E. globulus* trees up to 101 m in height were recorded (Lewin 1906; Hickey *et al.* 2000). The proximity of native *E. globulus* to the coast ensured rapid use and knowledge of its timber by the early explorers and colonists (Potts and Reid 2003). The high-density timber was durable and resistant to the destructive Teredo sea-worm and it became prized for marine construction, including shipbuilding (Lawson 1949). By the late 1800's, trees 60-90 m high were regularly harvested from southeastern Tasmania and shipped throughout the world for wharf piles (100-120 feet in length and 20 inches square) (Lewin 1906). By 1905, four million feet of wharf piles had been supplied for British Admiralty contracts, acknowledged as '….*the longest and biggest and the most durable that had ever reached British shores*' (Lewin 1906). The timber was also in great demand for railway sleepers, street paving blocks and mine supports. However, while re-growth of this species was common, by 1925 the large mature timber trees of *E. globulus* were scarce (Irby 1925) and clearing for agricultural purposes in the late 19th Century had substantially reduced the natural distribution of *E. globulus* in Victoria (e.g. Gippsland) and on King Island.

DOMESTICATION
Following its discovery, *Eucalyptus globulus* seed was rapidly spread throughout the world. It was the first eucalypt to be extensively planted and known outside of Australia (Jacobs 1981; Doughty 2000). By the end of the 19th Century it had been introduced to France, Chile, South Africa, Portugal, Italy, India, Spain, USA, Uruguay, Algeria, Tunisia, Argentina, Peru, Ecuador, Zimbabwe, China, Ethiopia and Bolivia (Table 1). In most countries, it is likely that many independent seed introductions occurred throughout the 19th century, either directly from native Australian sources or from other exotic sources (Zacharin 1978). Much Australian seed was distributed by Ferdinand von Mueller, the director of the Royal Botanical Gardens in Melbourne, who considered *E. globulus* the "Prince of Eucalypts" and championed its introduction worldwide (Doughty 2000 p. 51; Hay 2002). However, France is believed to have been a key secondary distribution point (Zacharin 1978), and the species has been cultivated near major Mediterranean ports such as Toulon since 1813 (Doughty 2000), for example, seed for the 1863 planting of *E. globulus* near Santander in Spain was taken from the Iles de Hyeres, near Toulon (Orme 1977). The Frenchman, Prosper Ramel, played a major role in establishing *E. globulus* plantations in southern Europe and North Africa (Zacharin 1978).

Table 1. The spread of *E. globulus* as an exotic during the 19th Century
The date of first recorded introduction (1st record) into each country, the estimated area (ha) planted to *E. globulus* and the number of major breeding programs

1st record	Country	Comments	Area (ha)[date]	No. of major breeding programs
native	Australia	Mainly established since 1995	349,796[2004]	2
c. 1804	France	Probably from the 1800 Baudin expedition which visited Tasmania (Zacharin 1978). By 1810 trees of this species are recorded at Château Malmaison (Penfold and Willis 1961, see also Duyker 2003 p. 233)	NA	
1823	Chile	27 plants of unknown origin intended for Peru were planted between Valparaiso and Arauco, more genetic material came from France in 1838 (Andrade and Vecchi 1920) and 1865 (Zacharin 1978)	232,000[2003]	4
1828	South-Africa	9 plants from Australian seed raised in Mauritius were planted in the Governor's garden at Cape Town. Once the most ubiquitous exotic tree, pest and disease problems, particularly *Gonipterus scutellatus*, resulted in planting virtually ceasing by the 1940's. Most of the 1,016 ha estate at that time is believed to have descended from the original 9 trees (Poynton 1979)	70[2004]	
1829	Portugal	Villa Nova de Gaia near Oporto Zacharin 1978), main plantations probably derived from Australian introductions from mid to late 19th century	700,000[2002]	2
1829*	Italy	Possibly even earlier as plants growing in the Camalduli Gardens near Naples and described in 1829 as *E. gigantea* by Dehnhardt were later identified by von Mueller as *E. globulus* (Zacharin 1978)	70,000[1987]	
1843	India	Nilgiri Hills, followed by importation of large quantities of seed for many years (Zacharin 1978)	NA	1
1849*	New Zealand	Record of a single ornamental planting at Nelson Zacharin 1978, but probably earlier introductions. Plantings ceased by 1960's due to insect pests	140[2004]	
1860*	Spain	Possibly as early as 1847 (Zacharin 1978)	500,000[2002]	1
1853*	USA	Suisan Valley near San Fransisco, California (Helms 1988)	NA	
1853	Uruguay	Initial seed from Cape Town, South Africa (Brussa 1994; Cardozo *et al.* 2003)	200,000[2004]	3
1854	Algeria	Seed from France through Prosper Ramel	30,000[1977]	
1854	Tunisia	Seed from France through Prosper Ramel	30,000[1977]	
1857	Argentina	Plantings on the farms of Don Leonarde Pereyre Iraola (Zacharin 1978)	17,000[2004]	1
1860	Peru	In the Sierras	NA	
1865	Ecuador	Introduced for reforestation of elevate windswept plateaux	31,863[1994]	
1872	Indonesia	Experimental plantings on Mt Sindoro grew well, as did later plantings on Mt Perahu (1700-2300m) (Zacharin 1978)	NA	
1890	Zimbabwe		NA	
1890	China	Possibly even earlier as a large 'blue gum' was noted in the grounds of the French legation in Kunming in 1894 (Morrison 1895). 1894-96 introductions to South China from Italy probably included *E. globulus* (Zacharin 1978)	c. 48,000[2004]	
1895	Ethiopia	Near Addis Ababa, via France (Zacharin 1978), mainly for fuel wood and charcoal (Pohjonen and Pukkala 1990)	100,000[1990]	1
1900	Bolivia	Seed from Argentina	NA	

NA = not available * not from Doughty (2000)

The spread of *E. globulus* around the world was aided by the unpalatability of its juvenile foliage to cattle, sheep and goats (Jacobs 1981). *Eucalyptus globulus* was also believed to reduce the occurrence of diseases such as malaria by helping to dry out swampy soils and through its leaves producing beneficial volatile chemicals (Doughty 2000). It became known as the 'fever tree' and in the latter half of the 19th century millions of trees were planted to combat malaria

in many countries (e.g. Tre Fontaine Monastery near Rome, Italy) (Stanford 1970; Zacharin 1978). Simultaneously, cultivation of *E. globulus* for timber, fuel and oil production expanded rapidly in many parts of the world. Its spread into California, for example, occurred during the gold rush where it was widely promoted by Government agencies and private investors as a source of construction timber and fuel (Santos 1997; Doughty 2000). However, by the beginning of the 20th century warping and splitting problems in the exotic-grown timber greatly reduced interest in its use for construction. Nevertheless, younger trees were used for firewood, charcoal and mine props. The latter was one of the main reasons for the early plantings in Chile (near Lota in the VIII Region, Bío-Bío).

By the mid to late 1800's, *E. globulus* plantings were well established in southern Europe and Northern Africa, Chile, California and India (Andrade and Vecchi 1920; Penfold and Willis 1961; Jacobs 1981). The modern development of Ethiopia and creation of its capital Addis Ababa has been directly attributed to the successful introduction of *E. globulus* in 1894-95 (Penfold and Willis 1961; Pohjonen and Pukkala 1990). This is one of the great successes of rural afforestation and *E. globulus* is now integral to the life of most Ethiopians around Addis Ababa as a source of fuel wood, charcoal, poles for house construction and numerous other products. However, the industrial plantations of the late 20th century, established to feed voracious pulp and paper markets, brought *E. globulus* to its greatest prominence. No sector of world forestry has expanded as rapidly as this industrial use of eucalypts (Turnbull 1999) and the eucalypt kraft pulp industry is dominated by only two species. *Eucalyptus globulus* is the premier species (Cotterill *et al.* 1999; Grattapaglia 2003; Villena 2003) and favoured in temperate regions, whereas *E. grandis* and its hybrids are used in the subtropics and tropics. While exact figures are difficult to obtain, it is estimated that 800,000 hectares had been planted with *E. globulus* by 1973 (Poynton 1979), 1.7 million ha in 1995 (Tibbits *et al.* 1997), and planting rates in the last decade would suggest that it may now exceed 2.5 million ha. Countries where *E. globulus* is grown include Australia, Chile, China, Columbia, Ethiopia, India, Peru, Portugal, Spain, USA and Uruguay (Eldridge *et al.* 1993). The Iberian Peninsula has the main concentration of plantations. It's the main eucalypt species cultivated in Portugal, with the plantation estate estimated at c. 60,000 ha by 1961 (Penfold and Willis 1961), 430,000 ha by 1988 (Eldridge *et al.* 1993), 550,000 ha by 1997 (Tibbits *et al.* 1997) and about 700,000 in 2002 (Toval Hernandez 2002). Spain has a similar estate, estimated at 390,000 ha in 1988 (Eldridge *et al.* 1993) and 500,000 ha in 2002 (Toval Hernandez 2002). The *E. globulus* estate in Chile was estimated at 44,561 ha in 1960 (Penfold and Willis 1961) and 232,000 ha by 2003 (Raga 2001). Australia has an estimated 349,796 ha of *E. globulus* plantation, most of which has been established since 1995 (National Forest Inventory 2004).

Despite the early spread and utilization of *E. globulus* around the world, it took more than 150 years before formal domestication (provenance testing and breeding) started. By this stage, landraces adapted to their exotic environments had developed on most continents (Eldridge *et al.* 1993), no doubt under a combination of natural (e.g. adaptation to harsher frosts - Almeida *et al.* 1995 and drought - Toro *et al.* 1998) and artificial (e.g. improved form - Lopez *et al.* 2001a) selection. Some of these landraces are thought to have originated from a narrow genetic base (Eldridge *et al.* 1993), which for example may have contributed to the poor performance of *E. globulus* in South Africa (Table 1; Poynton 1979; Gardner *et al.* 2003). Through the 1960's and 70's, small experimental trials of Australian provenances of *E. globulus* were undertaken in many countries for species selection, increasing interest in the species for industrial pulpwood plantations (e.g. Orme 1977; Eldridge *et al.* 1993). Formal breeding first commenced in 1966 in Portugal using phenotypic selections from local, landrace plantations (Dillner *et al.* 1971; CELBI 1979a, 1979b). By 1973, an extensive genecological study of natural variation in the *Eucalyptus globulus* complex had been completed (Kirkpatrick 1975a; Kirkpatrick 1975b), followed in 1975-76 by the first major native stand seed collection for provenance testing for forestry ('Orme collection' - Orme 1977). The Orme collection encompassed provenances from all four species in the complex, and was planted in more than 20 trials in Australia, Colombia, Portugal, Spain, Uruguay and USA between 1977 and 1985 (Orme 1988; Eldridge *et al.* 1993; Almeida *et al.* 1995; Barbour and Butcher 1996). By the late 1980's, growth and wood property results from bulk provenance and family trials established from this collection in Australia and overseas had clearly focused temperate zone breeders on *E. globulus* and its intergrade populations (results are reviewed in Eldridge *et al.* 1993). Local landrace provenances of *E. globulus* included in these trials did not necessarily grow better than at least some of the native stand provenances, and there was no clear evidence of major differences in growth rate between the native Australia provenances.

With increasing worldwide interest in breeding *E. globulus* for pulpwood plantations, the largest ever native-stand seed collection of *E. globulus* and intergrade populations (in the Otways and Strzelecki Ranges) was undertaken in 1987 and 1988 by the Australian Tree Seed Centre (ATSC) of CSIRO in collaboration with Australian and overseas forestry companies (616 parent trees from 49 collecting localities - Gardiner and Crawford 1987, 1988; Jordan *et al.* 1993; Dutkowski and Potts 1999). While studies of the Orme collection continued through the 1990's (Volker *et al.* 1990; Almeida *et al.* 1995; Kube *et al.* 1995), particularly for wood properties (Miranda and Almeida 2001, Miranda *et al.* 2001b, a, Miranda and Pereira 2001,

Miranda and Pereira 2002), the main focus of breeding programs rapidly turned to exploiting variation in this new collection. This collection was planted as family lots in over 50 progeny trials worldwide, including Australia (Jarvis *et al.* 1995), Chile (Sanhueza and Griffin 2001; Infante and Prado 1989, 1991, Ipinza *et al.* 1994), China (Zang *et al.* 1995), Ethiopia (Gizachew 2002), Portugal (Araújo *et al.* 1996) and Spain (Vega Alonso *et al.* 1994; Soria and Borralho 1998; Toro *et al.* 1998) and formed the base (e.g. Jarvis *et al.* 1995) or a key fusion population (e.g. Griffin 2001) for many breeding programs. Several other native stand seed collections have been undertaken since. However, these have been smaller and targeted specific areas. There are now active breeding programs for this species in at least 7 countries (Table 1), many of which are 2 or more generations from the native or landrace population. While most countries with well developed landraces have based their original breeding population on local landrace selections (e.g. India, Venkatesan *et al.* 1984; Portugal, Dillner *et al.* 1971), many are infusing into it new material from more recent Australian collections (e.g. Griffin 2001).

THE GENE POOL

THE QUANTITATIVE GENETICS VIEW

The base population trials established from native stand seed collections have provided invaluable insights into the genetic control of numerous traits of economic and adaptive value and how the genetic variation affecting these quantitative traits is structured in nature. The large CSIRO seed collections of 1987 and 1988 provide the best perspective on the geographic patterns of quantitative genetic variation in *E. globulus*. Many trials only contain seedlots from one of these collections. However, our studies have focused one five trials established by APPM Forest Products (now Gunns Ltd) in northern Tasmania that combine both collections. These trials were established in 1989 across a range of planting environments, using randomized incomplete block designs (5 replicates, 21-28 incomplete blocks per replicate, 2-tree plots). They contain between 450 and 596 open-pollinated families (Jordan *et al.* 1994; Dutkowski and Potts 1999).

Spatial structure of the gene pool: Our studies of these five trials show highly significant, often independent and spatially structured, quantitative genetic differentiation between the 49 sampling localities in virtually all traits examined (Table 2). Maps of the broad-scale distribution of genetic diversity in key economic and biological traits are now available (see references in Table 2). The overall pattern of genetic variation amongst localities, in what could be viewed as mainly adaptive traits, has been summarized by classification of the *E. globulus* gene pool into a hierarchy of 13 races and 20 subraces (Fig. 2; Dutkowski and Potts 1999). This classification includes morphologically intermediate populations (i.e. the *E. globulus* intergrades - Fig. 1) and accounts for a significant component of the genetic variation in these trials (Fig. 3). While the genetic variation in growth and survival exhibited weak spatial structuring, there were clear regional patterns in bark thickness, wood density, flowering precocity and leaf morphology (Table 2; Dutkowski and Potts 1999). There were few simple correlations with climatic variables, although multivariate analyses indicated a major latitudinal cline differentiating King Island and intergrade populations on continental Australia and western Tasmania from core *E. globulus* populations in eastern Tasmania. This cline is paralleled by a genetically based trend for flowering to occur earlier in the growing season in eastern Tasmania and on the Furneaux Group of islands (Gore and Potts 1995; Apiolaza *et al.* 2001). However, there was marked continuous variation within these major groups. There is an east-west cline in bark thickness and drought tolerance in the Otway Ranges coincident with a decline in rainfall. A major north-south cline within the continuous populations of core *E. globulus* on the east coast of Tasmania suggests adaptation to some environmental change, but without a simple association with macro-climatic variables. Bark thickness increases in northern populations (Dutkowski and Potts 1999), as does drought tolerance (Dutkowski 1995a; Toro *et al.* 1998). There is also an increase in susceptibility to marsupial browsers (O'Reilly-Wapstra *et al.* 2001) in northern populations, associated with a decrease in defensive chemistry (O'Reilly-Wapstra *et al.* this volume).

An early discovery from these trials was the deviant nature of the shrub-like population of *E. globulus* sampled from exposed coastal cliffs at Wilsons Promontory, Victoria. This population exhibits rapid transition to adult foliage type, precocious flowering and maintains the dwarf habit in field trials (Jordan *et al.* 1999; Jordan *et al.* 2000). It became clear that previous grouping of seedlots from the Bass Strait island races, King Island together with Furneaux (Orme 1988) was inappropriate as these races were clearly genetically different in many adaptive (e.g. drought tolerance Dutkowski 1995b; Toro *et al.* 1998, susceptibility to herbivores Jordan *et al.* 2002) and economic traits (e.g. wood density - Dutkowski and Potts 1999). There is also nearly a 100 day difference in peak flowering time between these two races (Gore and Potts 1995; Apiolaza *et al.* 2001), resulting in virtual reproductive isolation when they are grown together. The core *E. globulus* populations on the Furneaux islands were clearly genetically differentiated from adjacent populations in northeastern Tasmania, having shorter juvenile leaves, thinner bark and more precocious flowering and vegetative phase change (Dutkowski and Potts 1999).

Table 2. Published studies of quantitative genetic variation in the five Tasmanian trials of a range-wide collection of OP seedlots of *Eucalyptus globulus* by CSIRO in 1987 and 1988, and average (and range) of single site estimates of heritability of within race variation (h^2_{op}) for each trait type. The h^2_{op} values are averaged across different ages, sites, or different ways of measuring the same trait (e.g. growth includes dbh, height and volume measures across ages 1 to 8 years) and the number of such variables averaged for each trait is indicated (N). Except where indicated[1], h^2_{op} values have been recalculated and values may differ slightly from those published. The mixed linear model used to estimate variance components treated race and replicate as fixed effects and family within race, incomplete block, and plot (where appropriate) as random terms. The heritability calculation follows Jordan *et al.* (1999), and assumes a coefficient of relatedness for open-pollinated families of 0.4. It should be noted that our parameter estimates also refer to the heritability of within-race or within subrace differences, and the phenotypic variance does not include the incomplete block term

Trait	N	h^2_{op} (range)	Publications
Cellulose content	1	0.84±0.27	Apiolaza *et al.* unpubl.
Flowering time	4	0.68 (0.60-0.81) [1]	Gore and Potts 1995, Apiolaza *et al.* 2001
Vegetative phase change	2	0.54 (0.46 - 0.63)	Dutkowski and Potts 1999, Jordan *et al.* 1999, Jordan *et al.* 2000
Frost resistance	1	0.52 (0.017)[1]	Tibbits *et al.* unpubl.[1] (seedling T50)
Wood density (Pilodyn)	5 2	0.46 (0.21 - 0.59) 0.56 (0.44-0.67)	McDonald *et al.* 1997, Dutkowski and Potts 1999 Muneri and Raymond 2001; Apiolaza *et al.* unpubl.
Predicted pulp yield	2	0.46 (0.42-0.49) [1]	Muneri and Raymond 2001; Apiolaza *et al.* unpubl.
Flowering precocity [2]	3	0.44 (0.37 - 0.50)	Chambers *et al.* 1997, Dutkowski and Potts 1999, Jordan *et al.* 1999
Bark thickness	5	0.42 (0.22 – 0.71)	Dutkowski and Potts 1999
Lignotuber development	2	0.35 (0.27-0.43)	Whittock *et al.* 2003 [1]
Juvenile leaf morphology	5	0.32 (0.13-0.46)	Potts and Jordan 1994a, Dutkowski and Potts 1999
Growth (2-8yr)	22	0.28 (0.17 - 0.39)	Potts and Jordan 1994b, Borralho *et al.* 1995, Borralho and Potts 1996, McDonald *et al.* 1997, Dutkowski and Potts 1999
Survival (8yr) [2]	5	0.27 (0.14 - 0.41)	Chambers *et al.* 1996, Dutkowski and Potts 1999
Microfibril angle	1	0.27±0.24	Apiolaza *et al.* unpubl.
Herbivory	4	0.23 (0.00-0.46)	Jones and Potts 2000 [1], Jordan *et al.* 2002, O'Reilly-Wapstra *et al.* 2001 [1]
Fibre length	1	0.16±0.17	Apiolaza *et al.* unpubl.
Coppicing	1	0.07	Whittock *et al.* 2003 [1]

[1] Not re-analysed, used published estimates. [2] Binomial model

Indirect assessment of wood density at age 5 based on Pilodyn penetration added to the knowledge of economically important traits (McDonald *et al.* 1997; Dutkowski and Potts 1999). Rapid growth was the main aim of selection during the 1980's and, while no major regional variation was detected, the King Island race had performed consistently well resulting in interest for seed collection (Volker and Orme 1988; Orme 1988; Eldridge *et al.* 1993). However, with the clear definition of a pulpwood-breeding objective in the early 1990's (Borralho *et al.* 1993), wood density and pulp yield also became important traits for selection. With the significant regional differences in wood density evident, seed collections during the 1990's for breeding and deployment were focused on specific components of the gene pool, particularly the fast growing, high density intergrade races such as the Strzelecki race. The previously favoured King Island race had the lowest wood density (Dutkowski and Potts 1999) and poorest drought tolerance (Toro *et al.* 1998) of all races sampled. The economic value of the various components of the diverse *E. globulus* gene pool may change again with more information on other wood properties relevant to present and future breeding objectives (fiber dimensions - Miranda *et al.* 2001b; extractives, cellulose content and pulp yield - Miranda and Pereira 2002, Muneri and Raymond 2000; microfibril angle Muneri and Raymond 2000; tension wood Washusen *et al.* 2001; Washusen and Ilic 2001; sawing properties Greaves *et al.* this volume).

While all traits we have examined show significant genetic variation among localities, the largest component of the genetic diversity in quantitative traits within the *E. globulus* gene pool lies within sampling localities, much of which is between families (Fig. 3). Localities were defined to ensure all the trees sampled occurred within 10km of each other (Potts and Jordan 1994b), but trees were at least 100m apart (Gardiner and Crawford 1987). The variation between these open-pollinated families could represent true additive genetic differences between the trees sampled or be due to other

factors, including variation in the parental outcrossing rates and hence variation in the expression of inbreeding depression (Hardner and Potts 1995; Hardner *et al.* 1996). In the former case, our studies have clearly indicated that fine-scale spatial structuring of genetic variation is superimposed on the broad-scale racial patterns of genetic variation discussed previously. This structure has no doubt arisen through a combination of limited seed dispersal (family groups <50m; Hardner *et al.* 1998; Skabo *et al.* 1998) and/or adaptive clines in response to environmental gradients (Jordan *et al.* 2000).

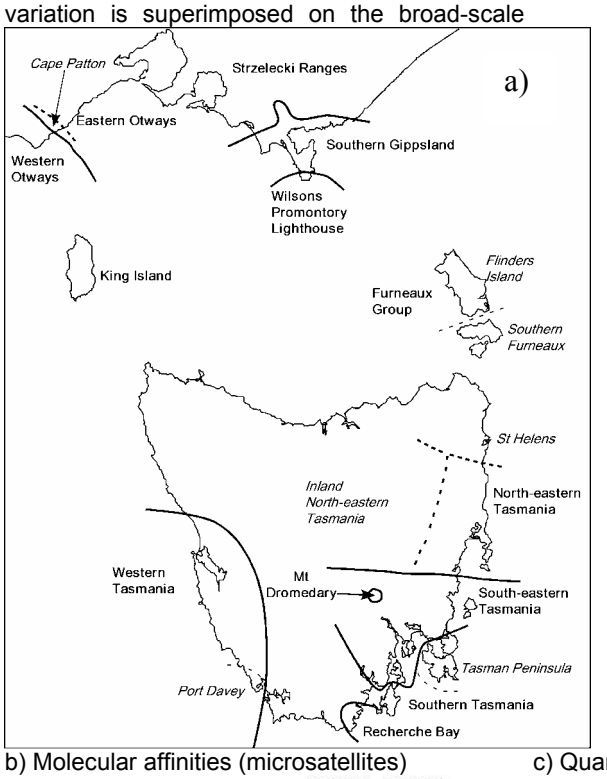
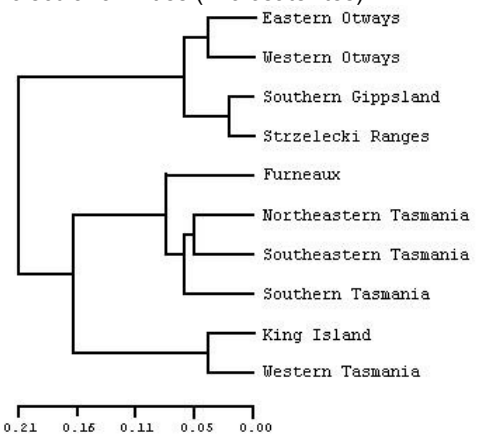
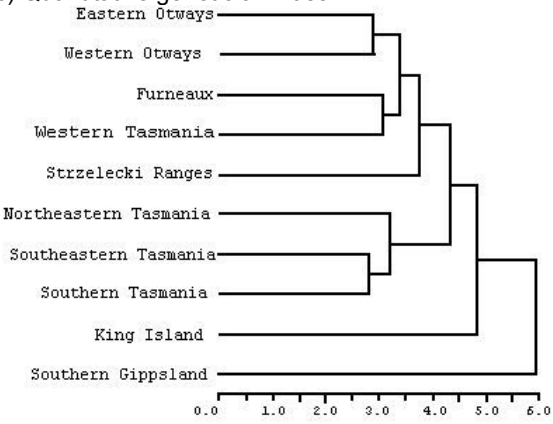


Figure 2. a) Racial classification of *Eucalyptus globulus* based on genetic differences in quantitative traits; and b) molecular and c) quantitative genetic affinities of the races
Races are separated by solid lines and labelled in roman type and subraces are separated by broken lines and labelled in italic type. The stippled area is the natural distribution of *E. globulus* and intergrade populations (see Fig. 1). The figure (left) has been modified from Dutkowski and Potts (1999) based on new information from Lopez *et al.* 2001b which changed the boundary between the Southern and South-eastern races. The classification and boundaries are being refined as more information becomes available and the latest version is available at http://members.forestry.crc.org.au/globulus/index.html. The UPGMA dendograms are based on b) Nei's genetic distances derived from an analysis of 8 microsatellite loci using nearly 400 native trees and c) Mahalanobis' distances based on 38 quantitative traits measured on families in the 5 Tasmanian base population trials (see Dutkowski and Potts 1999).

b) Molecular affinities (microsatellites)



c) Quantitative genetic affinities



OP genetic parameters: Most estimates of narrow-sense heritabilities and genetic correlations required to understand the response of *E. globulus* populations to selection are from open-pollinated (OP) progeny trials (e.g. Volker *et al.* 1990; Woolaston *et al.* 1991). *Eucalyptus globulus* has a mixed mating system (Hardner *et al.* 1996) and hence we have standardized our calculations of heritabilities (e.g. Lopez *et al.* 2002) and breeding values (Dutkowski *et al.* 2001) from open-pollinated progenies by assuming a coefficient of relatedness of 0.4 (termed h^2_{op}) rather than 0.25 used for complete half-sibs. It should be noted that our heritabilities and genetic correlations refer to the additive genetic (co-)variation within genetic groups (i.e. races, sub-races or localities), although between-race (co)variances may be accounted for in statistical models used for genetic parameter estimation. The within-race heritabilities derived from the five Tasmanian base population trials are summarised in Table 2. Other estimates for growth and wood properties are reviewed in Lopez *et al.* (2002) and Raymond (2002). Survival, growth and herbivore damage were the least heritable traits whereas flowering time, vegetative phase change and wood properties such as cellulose content and wood density were the most highly heritable traits in these trials (Table 2). Our average h^2_{op} of 0.25 for diameter across ages 4 and 8 in the five trials is consistent with the average of 0.21 reported from numerous trials by Lopez *et al.* (2002), although our average

heritability for Pilodyn penetration (0.46) is slightly higher than normally reported (Lopez *et al.* 2002; Costa e Silva *et al.* 2004a).

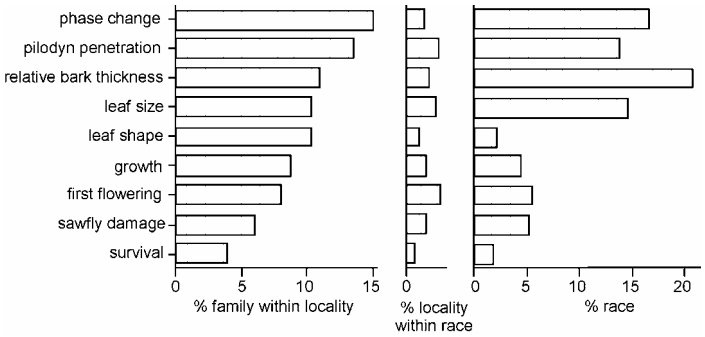


Figure 3. Hierarchical partition of quantitative genetic variation in *E. globulus*. Average % total phenotypic variation within replicates due to variation between families within localities, between localities within races, and between races for different types of traits (see Table 2). Sawfly damage represents two of the herbivory traits in Table 2. The additive genetic variation within localities is expected to be 2.5 times the family variance component (Potts and Jordan 1994b)

The expression of quantitative genetic variation amongst these open-pollinated families is surprisingly stable. Age-to-age correlations for growth were high at both the family within race (r_g, genetic correlation) and race (r_{race}, correlation between subrace least squares means) levels. Between dbh at ages 4 and 8 years, the average r_g was 0.93 (n=5) and the average r_{race} was 0.80 (n=5). Two-year old growth measurements were taken at only one trial and were significantly correlated with 8-year measurements at the family within race (r_g=0.71), but not the race level (r_{race}=0.57). There was also a strong correlation between the different measures of growth at a given age (e.g. dbh4 vs. height4: averages r_g=0.89 and r_{race}=0.80, n=5). For a given trait, the expression of genetic variation on the five sites was also highly stable, at least within races. Within races, the across-site correlations for the various growth traits (averages r_g=0.71 and r_{race}= 0.48; n=40), survival (averages r_g=0.85 and r_{race}=0.41, n=10), flowering precocity (averages r_g=0.68 and r_{race}=0.84, n=4), Pilodyn penetration (averages r_g =0.91 and r_{race}=0.67, n=10), and bark thickness (averages r_g=0.88 and r_{race}=0.89, n=10) were all positive but only 89% of the across-site correlations at the race level were positive. For growth and survival, r_{race} was clearly less in magnitude than r_g, suggesting that genotype x environment interaction is stronger at the racial level. This trend is clearly evident in the magnitude of the genotype x environment variance components in the partition of the variation in 4-year dbh and Pilodyn penetration shown in Fig. 4. Virtually all main effects and interaction terms were statistically significant. However, with the exception of the race x site interaction components for diameter, the interaction variance components were small compared to the site stable components of genetic variance (i.e. race and family within race terms).

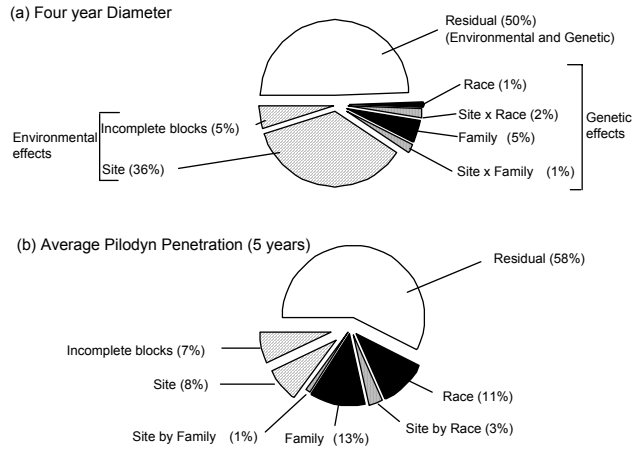


Figure 4. Percentages of variation across all five Tasmanian base population trials due to genetic, environmental and other causes in (a) diameter, and (b) Pilodyn penetration. The traits were standardised so that the residual variance for each site was 1. Pilodyn penetration was the average of two shots per tree. The terms indicated were fitted as random effects in the model (replicate was treated as fixed) using ASREML and their significance tested using a one-tailed likelihood ratio test. All interaction variance components were highly significantly (P<0.001) greater than zero except for the site by family interaction for Pilodyn (P<0.05)

Even beyond Tasmania and Australia, recent analyses (João Costa e Silva *et al.*, in preparation) suggest that the expression of growth differences between these open-pollinated families is still relatively stable (Table 3). In this case, across-site genetic correlations were estimated for DBH between families within subraces (r_g), and between subraces ($r_{subrace}$) rather than using the broader race as the genetic group. As expected, the across-site correlations decreased slightly as growth was compared between sites within regions in Australia (weighted averages r_g=0.85 and $r_{subrace}$=0.85, not shown), different regions within Australia (weighted averages r_g=0.76 and $r_{subrace}$=0.73, not shown) and from different countries (weighted averages r_g=0.70 and $r_{subrace}$=0.51, Table 3).

Subrace performance was less stable across countries than the performance of families from the same subrace, suggesting that genetic differences at the subrace level with impact on performance are more responsive to environmental variation.

Table 3. Average values for estimated across-site correlations in dbh of *E. globulus* from the 87/88 CSIRO collections. Ages of dbh measurements ranged from 4 to 6 years in Australia (15 sites), 3 to 4 years in Chile (3 sites), 3 to 5 years in Portugal (3 sites) and 4 to 5 years in Spain (3 sites). Across-site correlations were estimated at the subrace ($r_{subrace}$) and family within subrace (r_g) levels, and the numbers of site pairs used in the calculations are given in parenthesis. Although all sites had connections for subraces, there were pairs of sites with no connections for families, owing to the use of different family samples within subraces. The statistical analyses used bivariate mixed linear models which fitted unstructured correlation matrices for subrace and family within subrace effects (note that, in the absence of family connections, only $r_{subrace}$ was estimated and a diagonal matrix replaced the unstructured correlation form for family effects, which is analogous to assuming zero across-site covariances), as well as accounting for heterogeneous variances for these terms, design features (such as incomplete blocks, plots, etc) and residual effects (João Costa e Silva *et al*., in preparation)

	Family within subrace - r_g				Subrace - $r_{subrace}$			
	Australia	Chile	Portugal	Spain	Australia	Chile	Portugal	Spain
Australia	0.78 (93)	0.74 (27)	0.71 (31)	0.63 (27)	0.76 (105)	0.49 (45)	0.56 (45)	0.50 (45)
Chile		0.87 (3)	0.85 (6)	0.69 (9)		0.76 (3)	0.41 (9)	0.47 (9)
Portugal			0.73 (1)	0.63 (6)			0.60 (3)	0.53 (9)
Spain				0.66 (3)				0.39 (3)

Note: Using the numbers of site pairs as weighting factors: weighted average r_g within countries = 0.78; weighted average r_g across countries = 0.70; weighted average $r_{subrace}$ within countries = 0.75; weighted average $r_{subrace}$ across countries =0.51.

Reliability of OP genetic parameters: The genetic interpretation of the patterns and levels of quantitative genetic variation amongst the native stand OP families is a key issue in understanding the genetic architecture of *E. globulus.* Adjustment of the coefficient of relatedness to better estimate levels of additive genetic variance and breeding values from OP progeny, as discussed above, does not account for inbreeding depression, non-random mating and the variable levels of outcrossing which occur amongst OP families of *E. globulus* from native stands in Australia (Hardner *et al.* 1996; Jones *et al.* 2002b). This means that additive and non-additive genetic effects may be confounded in genetic parameter estimates based on OP progeny.

We have been studying an experiment established in 1990 specifically aimed at comparing genetic parameters and parental breeding values estimated from families derived from OP's with those obtained using pollen of the same 26 base parents in a factorial mating design (CP). Results at age 2 years (Potts *et al.* 1995; Hodge *et al.* 1996), now supported by those at 6 years (Volker 2002.), indicate that for growth traits: (i) variability within OP families is high compared to CP families, (ii) OP heritabilities are inflated even after adjustment (also Hardner and Potts 1995); (iii) breeding values estimated from OP and CP progeny are poorly correlated (e.g. DHB age 6yrs: a r_g between the CP and OP cross-types of -0.1, P>0.05; Volker 2002) and (iv) estimated levels of genotype x site interaction are less for OP progeny then CP, suggesting that OP estimates of additive x environment interactions are downwardly biased. Growth is one of the key traits affected by inbreeding depression, and the later finding is consistent with the suggestion that the high within race/subrace genetic correlations for growth across sites may well reflect stable differences in inbreeding depression amongst OP families rather than consistent expression of additive genetic effects. In contrast to growth traits, good correlations between OP and CP breeding values have been found for frost resistance (Volker *et al.* 1994), *Mycosphaerella* leaf damage (Dungey *et al.* 1997), leaf size and shape (Dungey 1991), Pilodyn penetration (Volker 2002) and the timing of transition to adult foliage and first flowering (Jordan *et al.* 1999). For these traits, variation amongst OP progeny should reliably estimate variation in additive genetic effects, but clearly the same cannot be said for growth traits.

The importance of non-additive genetic effects: Understanding the relative importance and nature of non-additive (dominance, epistasis and maternal) genetic effects in *E. globulus* is now a key research issue. This information is required, for example, to help decide whether to shift from OP seed orchards to more expensive clonal and full-sib family deployment strategies for the species. Additive and non-additive effects can only be separated with full pedigree control (CP), and dominance and epistatic effects can partly be partitioned where individuals within full-sib families have been cloned (Costa e Silva *et al.* 2004a). There are few CP studies of the relative importance of additive and non-additive genetics effects in *E. globulus* (Table 4). Evidence for significant non-additive genetic effects on growth in *E. globulus* comes from i) the existence of severe inbreeding depression (Hardner and Potts 1995; Hardner *et al.* 1998), ii) existence of positive mid-parent heterosis in inter-provenance/inter-race crosses (Vaillancourt *et al.* 1995; Hodge *et al.* 1996; Volker 2002; Lopez *et al.* 2003; see also Hardner *et al.* 1998) and (iii) significant non-additive interactions between parents from the same population (Table 4). In the latter case, results have been variable, but it appears that the non-additive genetic control of

growth (e.g. dominance ratio - d^2) can in cases be comparable to the additive one (CP heritability - h^2). Indeed, the additive genetic control of growth is clearly low and CP heritabilities for DBH (Table 4) tend to be approximately half the average of the many values reported from open-pollinated progeny trials of this species (Lopez *et al.*, 2002). However, in the study of Volker (2002) the non-additive effects on 6 year DBH were less stable across sites than the additive genetic effects. Costa e Silva *et al.* (2004a) is the only study to separate dominance and epistatic components of variation. This study used RAIZ breeding trials based on crosses amongst selections from the Portuguese landrace. Both non-additive components were small and insignificant for DBH and Pilodyn penetration (Table 4). Similar results were also obtained for height (Costa e Silva *et al.* 2004b). However, the possibility cannot be dismissed that epistasis may be more important in other populations.

Table 4. Estimates of the percentage of the phenotypic variation within *E. globulus* populations, which is due to additive, dominance and epistatic effects. Estimates are either (i) averaged over individual sites or (ii) calculated across sites and include gxe terms in the estimate of the phenotypic variance

Population	Trait	Additive (h^2)	Dominance (d^2)	Epistasis (i^2)	Reference
Taranna x King Island, 8x26 F_1 factorial (5 sites)	Across sites- Volume 2yr	0.08[1] 0.08 ± 0.01[2] 0.02 ± 0.01[3]	0.12 0.05 ± 0.03 0.15 ± 0.06		Vaillancourt *et al.* 1995 Hodge *et al.* 1996
	5 single sites -DBH 6yr - Pilodyn Across sites - DBH 6yr - Pilodyn	0.12 0.30 0.08 ± 0.03 0.25 ± 0.07	0.14 0.06 0.02±0.02 0.05±0.02		Volker 2002
Portuguese landrace breeding population (RAIZ)	DBH 4yr (5 sites) Pilodyn 4yr (2 sites)	0.10±0.04 0.17±0.07	0.04±0.05 0.00	0.004±0.04 0.05±0.06	Costa e Silva *et al.* 2004a
Australian breeding population (STBA)	DBH 1-3yr (5 sites) Density (3 sites)	0.11[4] 0.44[4]	0.11 0.14		Li *et al.* unpubl

[1] pooled within crosstypes [2]intra-provenance crosses [3]interprovenance crosses [4] combines between and within subrace dominance estimates whereas as other estimates refer to within subrace/provenance dominance alone

Lopez *et al.* (2003) is the only study to investigate the significance of maternal effects in *E. globulus*. The maternal effects on seed weight and germination rate were shown to only have a transitory effect on growth and were insignificant after 1 year of field growth. Additive genetic effects on growth became expressed by age 40 months, suggesting that maternal seed or other maternal environmental effects are unlikely to inflate variation between OP families. Interestingly, the fact that differences between reciprocal inter-race F_1 crosses were significant by one year after field planting raises the intriguing possibility that there may be some maternal genetic effects on growth (e.g. through maternally inherited plasmid genes or cytonuclear interactions).

THE MOLECULAR GENETICS VIEW

Our key molecular information on the structure of the *E. globulus* gene pool is from studies involving three different marker systems. Two studies have used nuclear microsatellites. These loci can effectively be considered neutral to selection and affinities between populations are more likely to be influenced by time since isolation, bottlenecks and recent pollen and seed mediated gene flow. One study examined the affinities of the commercially important Strzelecki race (Jeeralang population) to core *E. globulus*, *E. pseudoglobulus* and *E. bicostata* (Jones *et al.* 2002a). This Victorian *E. globulus* intergrade race is located in a geographically intermediate position between the three species (Fig. 1). Its capsule morphology is variable and ranges from large single capsules resembling *E. globulus* to smaller, three-fruited forms resembling *E. bicostata* or *E. pseudoglobulus*. The population is intermediate in microsatellite frequencies between core Tasmanian *E. globulus* and the other species, but has very close affinities to core *E. globulus* on the coastal plain in south Gippsland (Southern Gippsland race) (Fig. 5). The second microsatellite study involved 8 nuclear microsatellite loci assayed for nearly 400 native trees from 11 races of *E. globulus* collected from many of the same areas as sampled in the 1987/1988 CSIRO collections (Fig. 2, Steane *et al.* unpubl.). This study showed two independent clines in gene frequency associated with latitude and longitude. The latitudinal cline was the most differentiated. It appeared to be an extension of the cline between species (Fig. 5) and separated the Victorian races from those in eastern Tasmania, with the Western Tasmania and King Island races intermediate. The longitudinal cline separated western and eastern populations in Victoria, Bass Strait and Tasmania. The *E. globulus* races, as defined on genetic differences in morphology and putative adaptive quantitative traits, fall into three main molecular groups (Fig. 2b). The first comprises the races in western (Otway Ranges) and eastern (Strzelecki and South Gippsland) Victoria. The South Gippsland race within this group comprises many remnant farmland populations. It is an outlier in the quantitative genetic classification

(Fig. 2b), most likely due to high inbreeding depression in growth traits (Borralho and Potts 1996). It also classifies taxonomically as core *E. globulus* (Fig. 1), which does not match its underlying molecular genetic affinities (Figs. 2b). The second group comprises the continuous core *E. globulus* races in eastern Tasmania and the disjunct Furneaux race. This molecular affinity would suggest that the large quantitative genetic differences which occur between these races are adaptive and have evolved relatively recently or in the face of substantial gene flow. The third group comprises the intermediate King Island and Western Tasmania races, which has slightly closer affinities to the second group. In both the west and the east, the Bass Strait island races had closest molecular affinities to their nearest Tasmanian races, suggesting that a more recent connection or gene flow has occurred between the Bass Strait islands and Tasmanian races than with those in Victoria.

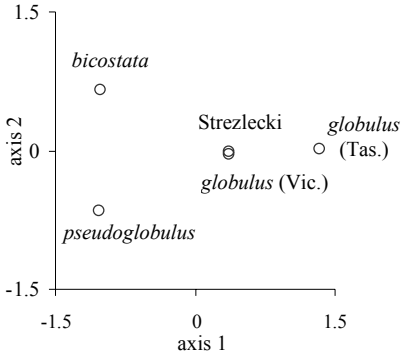


Figure 5. Ordination of Nei's (1972) genetic distances between populations of *E. globulus* and closely related species, based on 8 microsatellite loci. The figure shows that the species cores are clearly differentiated. The *E. globulus* intergrade population in the Strezlecki Ranges (Jerralang) and the populations morphologically resembling core *E. globulus* on the coastal plain in Gippsland, Victoria are intermediate and overlaping in the figure (Jones *et al.* 2002a).

The second type of molecular marker we have examined has provided a completely novel perspective on the *E. globulus* gene pool. We sequenced the hypervariable J_{LA+} region of the chloroplast DNA (cpDNA) from 264 samples from 157 different populations (Freeman *et al.* 2001; McKinnon *et al.* 2004a; McKinnon *et al.* 2004b). CpDNA is non-recombining and maternally inherited (McKinnon *et al.* 2001b) and therefore reflects patterns of variation driven by seed dispersal alone. The cpDNA of *E. globulus* is highly variable (we have identified more than 100 J_{LA+} haplotypes) and the gene pool contains several highly divergent lineages, the main ones are the Central (C), Southern (S), and Eastern Tasmanian (ET) lineages (Fig. 6). The variation in cpDNA is highly spatially structured geographically, at both the lineage and haplotype level, providing a ready tool for determining the geographic origin of unpedigreed germplasm such as the various *E. globulus* landraces (Fig. 6). The C lineage is widespread and found in many other species in the section *Maidenaria*. A sub-lineage, Cc, is widespread in Tasmania, occurs in the Otway Ranges, but has not been found in populations in eastern Victoria. The sub-lineage, Cg, is the dominant cpDNA in eastern Victoria but does not occur in Tasmania. Both the Cc and Cg lineages occur on the Furneaux Group of islands, but these Cc haloptypes are different to those in northern Tasmania. The lack of an eastern continuity in haplotypes strongly argues for an eastern barrier to seed migration into and out of eastern Tasmania (Freeman *et al.* 2001). In contrast, the sharing of the same Cc haplotypes between the Otways, King Island and Western Tasmania supports a more recent migration route. While the Western Tasmania, King Island and Otways races are separated by large disjunctions, this connection was most likely associated with the formation of a land bridge between Tasmania and continental Australia during the Last Glacial (McKinnon *et al.* 2004a).

The S and ET cpDNA lineages are less widespread and also have been found in other species of the section *Maidenaria* growing in southeastern and eastern Tasmania respectively (McKinnon *et al.* 2001a). Patterns of cpDNA variation within *Maidenaria* are more related to geography than species boundaries, suggesting extensive introgression between species (McKinnon *et al.* 2001a). In the case of *E. globulus*, the distribution of the S lineage is thought to coincide with a glacial refuge in which hybridisation and 'chloroplast capture' occurred. We now have evidence of sharing of specific haplotypes on a very localized scale between *E. globulus* and the rare Tasmanian endemic *E. cordata,* which is believed to be due to the widespread *E. globulus* assimilating the cpDNA of this rare species through hybridization (McKinnon *et al.* 2004b). Shared haplotypes are highest in *E. globulus* sampled within 2 km of known *E. cordata* populations and drops to zero at a distance of 25 km from the nearest known *E. cordata* population. This cpDNA evidence leads us to believe that *E. globulus* may be a 'compilospecies' that, through evolutionary time, has assimilated genes of other species into its gene pool through hybridisation.

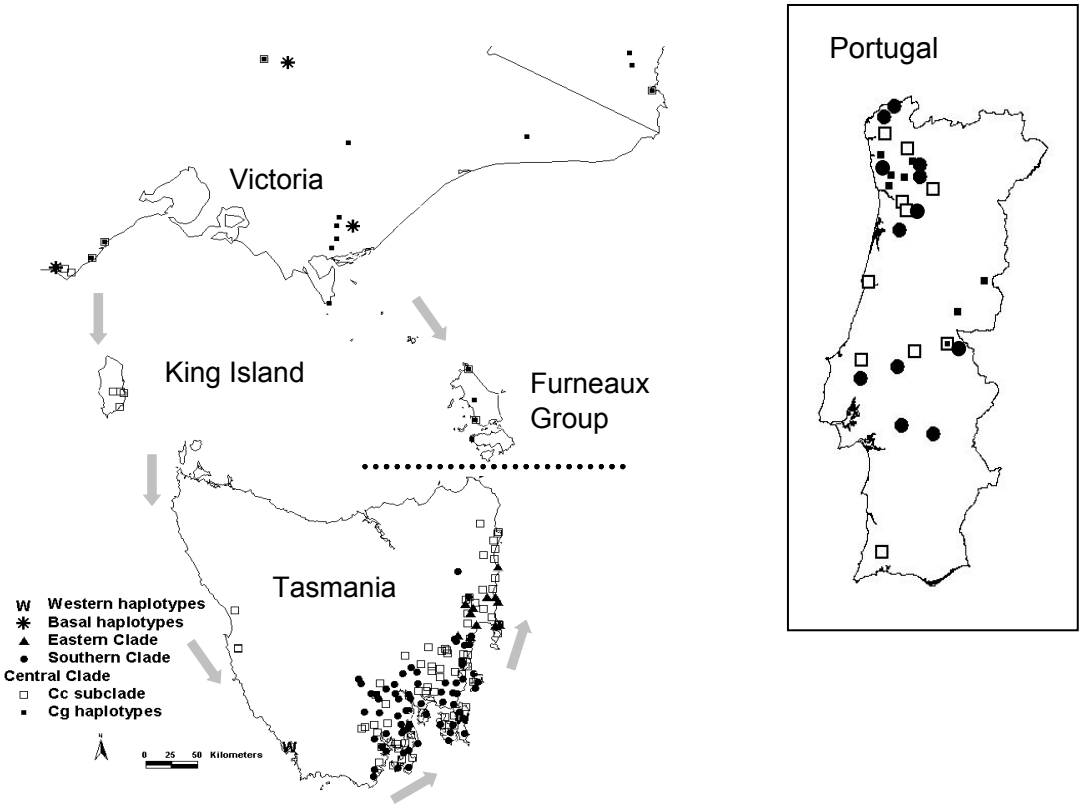


Figure 6. The marked spatial structure in variation in chloroplast DNA in *Eucalyptus globulus*.
(a) The distribution of major chloroplast DNA lineages in the native population (Freeman *et al.* 2001). Arrows show inferred seed-dispersal routes between continental Australia and Tasmania based on shared haplotypes; the dotted line shows an apparent block to seed dispersal between the Furneaux Group and Tasmania based on the distribution of specific haplotypes. (b) The distribution of the same chloroplast DNA lineages in plantations in Portugal established from the landrace. DNA was obtained from 26 samples from diverse Portuguese localities by Victor Carocha (RAIZ) and their cpDNA sequence compared to a large database of over 269 samples of *E. globulus* trees from known native localities in Australia. The Portuguese landrace contained 10 different chloroplast haplotypes; six have only been found in southeastern Tasmania, and one has only been found in eastern Victoria and Flinders Island. The remaining three haplotypes have as yet only been found in the Portuguese samples. These results suggest that the Portuguese landrace is likely to have different geographic origins and be genetically diverse.

While there is no obvious evidence of such hybridisation in the morphology of the *E. globulus* trees we sampled, it is logical that such hybridisation may have also contributed significantly to nuclear gene diversity in the *E. globulus* races in eastern Tasmania. This hypothesis is consistent with the finding that Eastern Tasmania not only has the highest cpDNA diversity, but also the highest nuclear microsatellite diversity (Steane *et al.* unpubl.). We used a third marker system to study whether we could detect evidence for hybridisation affecting genetic diversity in functional nuclear genes. We studied a lignin biosynthesis gene cinnamoyl coA reductase (*CCR*; McKinnon *et al.* in prep), which mapping studies have shown co-locates with QTL affecting cellulose and pulp yield production in *E. globulus* (Thamarus *et al.* 2004). It is a single-copy gene and exhibits considerable diversity in the species (Poke *et al.* 2003). PCR-RFLP analysis of *CCR* from 208 *E. globulus* samples combined with sequencing of specific haplotypes showed two major lineages of CCR are present in *E. globulus*. One lineage dominates southern and western Tasmanian *E. globulus* and is shared with other *Maidenaria* species. The other lineage is prominent in Victorian and northern Tasmanian *E. globulus*, and surprisingly shows homology to *CCR* from *Eucalyptus saligna* (section *Latoangulatae*). Consistent with the other marker systems, *CCR* shows a major latitudinal cline in the frequency of key haplotypes and greatest diversity in Tasmania. The relative frequencies of different *CCR* haplotypes divide the range of *E. globulus* into four geographic regions: (1) Victoria, (2) the Furneaux Group and northeastern Tasmania, (3) King Island and western Tasmania and (4) southeastern Tasmania. The eastern and western Victorian races are not differentiated

and there is a significant north-south cline in haplotype frequencies in the continuous populations of core *E. globulus* in eastern Tasmania. Drift in glacial refugia, selection and/or historical hybridisation may have been instrumental in generating this cline. A potential role for hybridisation is suggested by the finding that southeastern *E. globulus* with the same cpDNA lineage as the endemic *E. cordata* (S lineage) also have significantly higher levels of a *CCR* haplotype that is closely related to the dominant *CCR* haplotype in *E. cordata* than those *E. globulus* with C haplotypes.

CONCLUSION

A more detailed knowledge of the *E. globulus* gene pool is emerging from integrated studies of quantitative genetic variation in traits of potential adaptive significance with studies of DNA variation in the nuclear and chloroplast genomes, which are probably mostly neutral but may affect the phenotype in some cases. Each perspective reveals similarities and differences in the variation pattern as would be expected from combining genetic information affected by time since isolation, population size and gene flow with that driven by selection and resulting in both divergence and convergence in phenotype. There is increasing evidence that the *E. globulus* gene pool is not closed and that *E. globulus* has, through its evolutionary history, assimilated genes from co-occurring species into its gene pool through hybridisation. Differential rates of gene introgression during the hybridization process (e.g. *E. grandis* x *globulus* backcrossing - Myburg *et al.* 2004) could provide a mechanism for rapid recovery of parental phenotypes during backcrossing which would explain the relative constancy of taxonomic traits. Great genetic diversity at both the broad geographic and local scales is available for selection in virtually all traits examined, although their degree of additive genetic control varies markedly. There are now unique opportunities to use the molecular genetic framework being developed to better understand the quantitative genetic behaviour of breeding populations. Identification of the key genes responsible for the large quantitative genetic differences observed at all spatial levels is now a key research issue. Similarly we are just starting to understand the importance of non-additive genetic effects such as epistasis. If differential co-adaptation has occurred between the highly differentiated races, the effects of epistasis on growth may become greater as breeding programs proceed to advanced generations using inter-race hybrids. The highly diverse cpDNA and its strong spatial structuring provides unprecedented opportunities for tracing the geographic origin of maternal lineages and the recent sequencing of the *E. globulus* chloroplast genome (Steane *et al.* unpubl) opens opportunities to improve the resolution of this marker system. Whether the diverse cpDNA lineages within the gene pool are adaptive is unclear. However, there is clearly the possibility of maternal effects through these haplotypes affecting plant growth directly or through cytonuclear interactions (Lopez *et al.* 2003).

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