1 Introduction

1.1 General introduction

Anthropogenic exploitation of natural resources has resulted in unprecedented habitat restriction, destruction and modification (Young et al. 1996; Mimura et. al. 2009). Urgent information is required to ascertain the impact on species, and what it means for their long-term viability. In Australia, 48 flora species are listed in the *Environment Protection and Biodiversity Conservation Act* (1999) as having become extinct since 1788, 113 are listed as critically endangered and a further 1180 as endangered or vulnerable. Although species extinction is a natural process, the rate of extinction is presently thought to be 100 to 1000 times greater than estimated from the fossil record (Davidson 2000).

In Australia, land clearing is identified as a key threatening process (EPBC Act 1999). In many parts of the country, land clearing has reduced large continuous tree-dominated woodland communities to small isolated remnants, often within hostile urban and agricultural landscapes. While at present no trees are listed as extinct (Sjöström & Gross 2006) many are among those on the endangered and vulnerable listings. The absence of trees from the extinction list is, probably, in part due to longevity (Sjostrom & Gross 2006). Long generation times are a characteristic of trees (Petit & Hampe 2006) and in Australia many populations have been fragmented for only a small number of generations (i.e. European settlement only occurred 223 years ago). Because habitat fragmentation and landscape modification typically impact upon reproduction and regeneration (Hobbs & Yates 2003a), visible degeneracy of tree-dominated communities may not occur for a long period of time. What we have now, therefore, may not be an accurate reflection of what we are likely to have in the future.

Eucalyptus is the dominant genus of trees in Australia (Brooker & Kleinig 2006) and probably one of the most widely affected by fragmentation. The majority of vegetative communities on the continent are dominated by one or more species of eucalypt. While the body of research addressing eucalypt persistence in fragmented landscapes is growing (e.g. Prober & Brown 1994; Butcher et al. 2005; Byrne et al.

2008), the wide distribution of eucalypts and the large number of species affected indicates many more species specific enquiries are needed to understand how remnant communities will persist in the longer-term, and how we might mitigate their demise.

The long-term sustainability of remnant populations depends upon the capacity of the remaining individuals to maintain evolutionary processes. For evolution to proceed, a genetically variable population is required, (Frankham et al. 2002; Aguilar et al. 2008). Since genetic diversity is undoubtedly reduced in fragmented communities (Aguilar et al. 2008), maintaining genetic variability within fragmented forest remnants is critical for longer-term conservation. Further, since relatively low levels of genetic variability are widely documented to negatively impact upon plant reproduction and population fitness (Young et al. 1996; Reed & Frankham 2003; Leimu et al. 2006), maintaining high genetic variability may assist in enhancing immediate population persistence.

The contribution of genetic variation to population viability more broadly, however, has been heavily debated. Within the context of extreme degradation, several researchers have suggested remnants in a modified habitat are more likely to be threatened by non-genetic factors before being affected by genetic factors (Epperson & Alvarez-Buylla 1997; Lande 1998; Ouborg et al. 2006). This implies that a preoccupation with genetic factors may ignore more immediate threats. Further, while some species are able to persist over a wide geographical range (indicating significant ecological amplitude) with very little genetic diversity (e.g. low diversity in aquatic *Eichhornia crassipes,* Li et al. 2006); individuals of other species with overall relatively high levels of genetic diversity can exhibit fitness declines due to the relatively minor reductions of genetic variability associated with selfing as opposed to outcrossing (e.g. inbreeding depression in *Euclayptus globulus*, Hardner & Potts 1995). To make matters more complicated, lineages and populations of the same species can exhibit variable susceptibility to inbreeding depression (Armbruster & Reed 2005) subsequently exhibiting variable genetic vulnerability. It is difficult to determine, without explicit investigation, the relative importance of genetic and non-genetic factors to the overall viability of a remnant population.

Eucalyptus camaldulensis Dehnh. subsp. *camaldulensis* (henceforth *E. camaldulensis*) is an iconic species of tree in Australia that occurs within all mainland states. In the Hunter Valley region of Eastern NSW, however, it is listed as an endangered ecological community on the *Threatened Species Conservation Act* 1995 (NSW). In this region, the species persists as a series of small, variously isolated patches within a wide range of landscape contexts.

The species exhibits a number of characteristics that may potentially impact upon the sustainability of remnants and the level and structure of genetic variability within them. Firstly, *E. camaldulensis* is a niche specialist in which critical life-stages are dependent upon water availability (Dexter 1970; Cunningham et al. 2007b) hence recruitment patterns, and subsequently genetic variation and structure are intimately associated with the environment. Secondly, reproduction is complicated by a lack of reproductive barriers between closely related species (Griffin et. al. 1988; Moran 1992). This has the capacity to alter the interpretation and impact of genetic variability. Finally, it has been suggested that post-zygotic seed abortion results in the preferential development of zygotes (James & Kennington 1993); potentially impacting on the selection of progeny that is resource and mate dependant.

This thesis explores the genetic diversity of remnant stands of *E. camaldulensis* in the Hunter Valley. It investigates where and how genetic variability is located, and what impact genotype has on individual fitness and growth. It also considers the types of habitat the species occupies and how habitat relates to recruitment processes. By investigating a range of genetic and non-genetic factors, the thesis aims to present a genetic assessment of an endangered population that is both temporally and environmentally relevant, and hence of immediate benefit to the conservation of genetically and ecologically viable populations.

1.2 Thesis outline

The introductory chapters of this thesis (chapters two and three) provide background information to enhance the interpretation of the genetic data. Chapter Two explores the reproductive biology and the ecological requirements of *E. camaldulensis* and reviews the body of research reporting on the distribution and structure of genetic

variability within natural populations of eucalypts. Chapter Three introduces the study region, outlines the current extent of *E. camaldulensis* and investigates historical sources to estimate the pre-European distribution of *E. camaldulensis* in the Hunter Valley region.

Chapters Four through to Seven (Figure 1) have been structured as individual papers. Consequently there is some overlap, particularly in the species' description and certain components of the methodology.

The fourth chapter explores the level and distribution of genetic diversity within thirteen remnant stands of *E. camaldulensis* in the Hunter Valley. Microsatellite diversity is quantified to determine the levels of genetic variability, how it is distributed and how the levels detected compare with the levels of genetic diversity in other eucalypt species. Temporal comparisons of genetic variability across age-classes (based on diameter at breast height measurements) are undertaken to determine if age cohorts exhibit different levels of genetic variability or structure. Temporal trends are assessed to ascertain the direction and strength of genetic change.

Chapter Five explores a range of non-genetic remnant attributes in nine remnant populations to determine the condition (age-class structure, density, and health) and landscape context (isolation, shape) of remaining stands. To investigate how non-genetic factors relate to genetic factors the level of correlation between non-genetic and genetic population attributes is assessed.

Certain attributes of remnant populations inferred from chapters Four and Five indicated habitat types within populations exhibited different levels of genetic diversity. To further explore the interaction between habitat and genetic variability, Chapter Six assesses the level and structure of genetic variability within newly recruited seedlings/saplings within two habitat types (riparian and floodplain). It also assesses the level of spatial genetic structure in established populations to explore whether early established genetic structure is persistent in the adult phase.

Finally, chapter Seven investigates the impact of genetic diversity on the performance of individual seedlings and on the mean reproductive output of parent trees. Genetic parameters within this study include both individual outcrossing rates and the degree of heterozygosity. The aim of this chapter is to assess the possibility of selection

against inbred or outbred individuals and to estimate the mating system of individual trees within remnant populations.

The concluding chapter incorporates all of the results from all chapters to discuss how the results of this research contribute to the effective conservation of *E. camaldulensis* remnants. The chapter will also discuss how the information and experience gained relate to the conservation of remnant populations of niche specialist species in which environmental factors are intimately linked to critical life-stages.

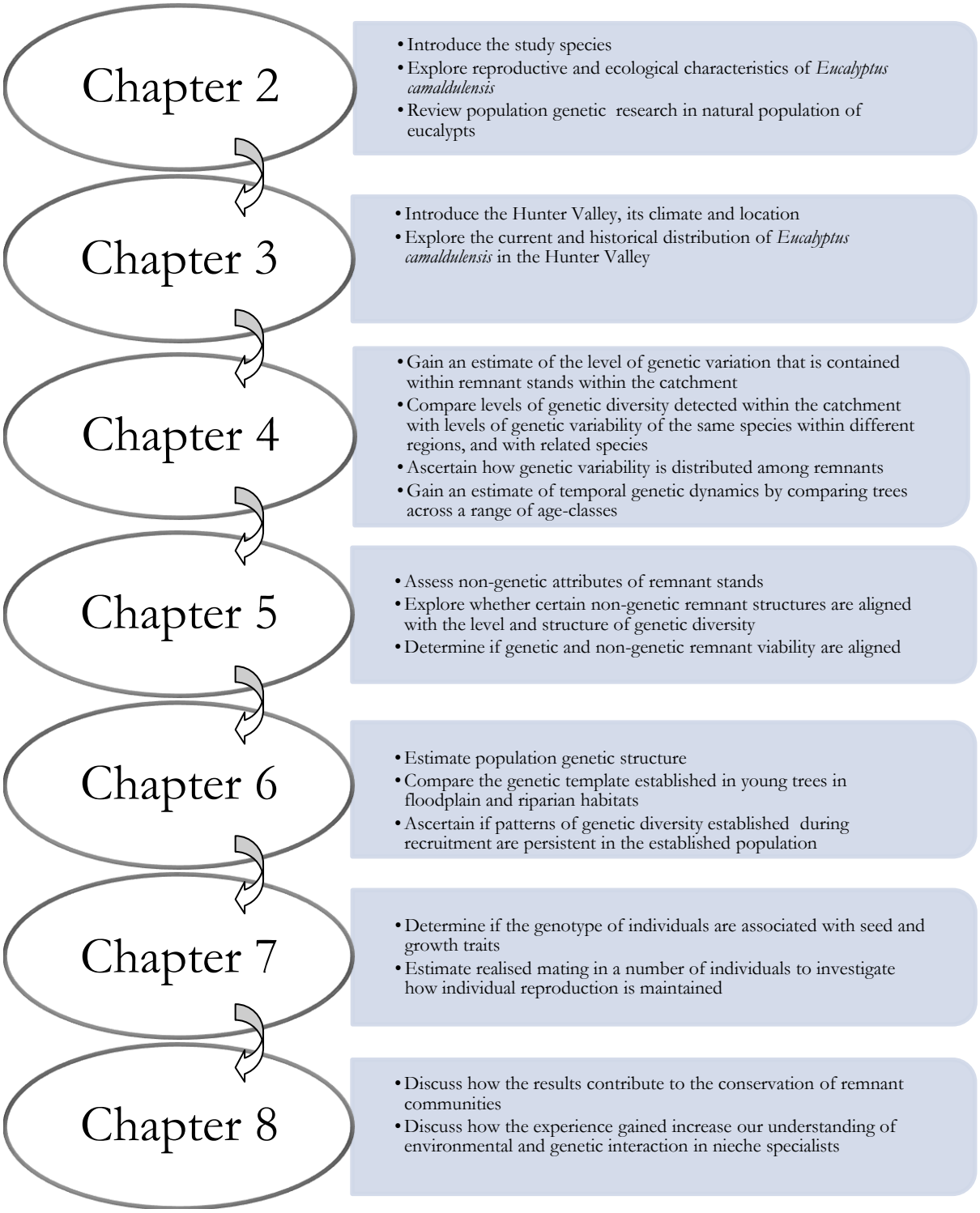


Figure 1. Chapter overview

2 Study Species

2.1 The Species

Eucalyptus camaldulensis (River Red Gum) is one of Australia's most well-known and well-loved species. It is a magnificent tree that attains massive proportions in old age and as such is a memorable feature of the Australian landscape. It is found in all mainland states of Australia in flood prone areas and along river margins (Di Stefano 2002; Butcher et al. 2009). Within its range and habitat it is usually the dominant species and is critical in the provision of habitat and stream bank stability (Butcher et al. 2009). *Eucalyptus camaldulensis* has been planted extensively for pulpwood, particularly in Thailand and India, and it has also been utilized for sleepers and other hard wood products (Boland et al. 2006). It is a member of the *Symphyomyrtus* subgenus of *Eucalyptus* that encompasses many economically important species (e.g. *Eucalyptus globulus, E. nitens*).

Marked phenotypic variation is observed across the range of *E. camaldulensis* and this has resulted in a relatively complex taxonomic history (see Butcher et al. 2009). The recent review of the taxonomy of *E. camaldulensis* recognises seven infraspecific taxa (McDonald et al. 2009) that conform to the morphologically and genetically congruent data detected in the study conducted by Butcher et al. (2009). The species within the Hunter Valley is *E. camaldulensis* Dehnh. subsp. *camaldulensis* and is differentiated from other *E. camaldulensis* by its strongly beaked operculum (McDonald et al. 2009), the cap that protects the developing flower bud.

2.2 Taxonomic description

The most recent taxonomic description of *E. camaldulensis* Dehnh. subsp. *camaldulensis* is provided by Butcher et al. (2009). The same description is incorporated into a recent review of the infraspecific taxonomy of *E. camaldulensis* (McDonald et al. 2009). The taxonomic description is as follows:

> "*Trees* up to 40m tall. Bark mostly smooth, variegated various shades of grey, brown, pinkish-red, cream, creamy-yellow or white, or with a rough, dark grey basal stocking; granular on saplings. *Seedling leaves*

lanceolate to narrowly elliptic, 7.5–16 x 1.3–4.2 cm, green or blue-green, growing tips usually pruniose. Juvenile leaves lanceolate to broad-lanceolate 6.5–16.5 x 2–5 cm, green, blue-green or grey-green, ±pruniose towards apices, stems and lamina sometimes pruniose. *Adult leaves* lanceolate to narrow-lanceolate, or often falcate, 10–30 x 0.8–2.5 cm, green or blue-green, dull; venation sparse to moderately reticulate; *apical branchlets* reddish or dark red-maroon or yellow. *Inflorescences* auxiliary, simple or sometimes racemose, 7, 9, or 11 flowered; *penduncles* 0.8–1.8 cm long. *Mature buds* pedicellate, *pedicels* 0.3–1 cm long; *mature inner opercula* prominently beaked, 0.3–0.7 cm long (beak up to 0.4 cm long); *stamens* inflexed with anthers at base of bud cavity. *Fruit* (0.4)0.5–1 cm wide, valves (3–)4 or 5/ *Seeds* smooth, yellow to yellow-brown, double-coated" (Butcher et al. 2009).

2.3 Hybridisation between *Eucalyptus camaldulensis* and its relatives

Although varietal diversity within *E. camaldulensis* is attributed to the broad geographic range of the species, the results of genetic and phenotypic research have also suggested that introgression of genes from closely related species may have contributed to diversification (Butcher et al. 2002). Hybridisation between members of the *Symphyomyrtus* group often produces viable offspring (Bisht et al. 1999) and eucalypt species are known to hybridize in the field (Griffin et al. 1988; Barbour et al. 2002; Potts et al. 2003; Barbour et al. 2005). However fertile hybrids are limited to those that result from crosses between species occurring within the same subgenus and hybridisation increases with taxonomic proximity (Griffin et al. 1988). Where the distribution of two or more species from the same subgenus overlap, therefore, hybridisation should be considered a possible cause of phenotypic and genetic variation. Butcher *et al* (2002) found restriction fragment length polymorphism (RFLP) evidence of hybridisation between *E. camaldulensis* and *Eucalyptus tereticornis* in the eastern division of their east and west population groupings in Australia. Eastern populations of *E. camaldulensis* shared genetic traits with *E. tereticornis* occurring within the same region. Introgressed RFLP variation from *E. tereticornis* contributed a significant portion of the overall genetic differences between the eastern and western groups. These results suggested (Butcher et al. 2002) that eastern populations had a hybrid origin and this may have played a significant role in regional diversification.

The occurrence of fertile hybrid progeny in *Eucalyptus* species has several important implications. First, hybridisation makes problematic the delineation of species and thus, their conservation status. Second, introduced species of the same subgenus must be considered as potentially threatening to the maintenance of natural genetic variation (Potts et al. 2003). Genetic pollution of natural stands have been documented in Tasmania, where introduced *Eucalyptus nitens* was demonstrated to have hybridised with *Eucalyptus ovata* in natural stands on the edge of plantations (Barbour et al. 2005; Barbour et al. 2002). Hybrids were found a significant distance (1.6km) into native stands. In natural populations of *E. camaldulensis* in the Hunter Valley, genetic pollution via hybridisation is most likely to occur between *E. camaldulensis* and one of several other closely related co-occurring species (e.g. *E. tereticornis* and *Eucalyptus blakelyi*).

Although it is apparent natural hybrids of eucalypts pre-date European settlement (Rhymer & Simberloff 1996; Potts et al. 2003), several authors have posed the question as to whether anthropogenic disturbance increases the frequency of hybridisation (e.g. Levin et al. 1996; Rhymer & Simberloff 1996). Lamont et al. (2003) found anthropogenic disturbance promoted hybridisation in two naturally co-occurring *Banksia* species. This resulted from changes in the flowering duration and intensity that were promoted by disturbance. If anthropogenic disturbance results in a breakdown of evolved separation mechanisms between species, hybridisation may increase.

The outcomes of genetic pollution are uncertain. Hybrid progeny may exhibit increased fitness. This is termed hybrid vigour and has been recorded in several studies, including a study where researchers investigated artificially initiated hybrids of *E. camaldulensis* and *E. tereticornis* (Bisht et al. 1999). Furthermore a vigorous hybrid of *E. camaldulensis* and *E. tereticornis* is grown extensively in India for pulp wood production (Boden 1964 cited in Potts et al. 2003). Where hybrid progenies exhibit increased fitness, introgression of alien genes may result in extinction of the rare form (Potts et al. 2003). Hybridisation may also re-invigorate the gene pool via the introduction of new genetic diversity. It may also, however, induce outbreeding depression – a decline in the fitness of outbred to inbred progeny (Fenster & Galloway 2000). Outbreeding depression has been observed in eucalypts (e.g. reduced fitness of F1 in *E. ovata and E. globulus*, Lopez et al. 2000) and several studies

have detected significant declines in seed set following controlled hybridisation (Drake 1975; Meddings et al. 2003) indicating abortion of hybrid zygotes.

2.4 The Reproductive ecology of *Eucalyptus camaldulensis*

The majority of *Eucalyptus* species exhibit a mixed mating system (Griffin et al. 1987, Pound et al. 2002), however most species develop a disproportionate amount of outcrossed progeny given the opportunities for self-pollination. *Eucalyptus* species produce masses of bisexual flowers (Beardsell et al. 1993) and although they are protandrous many flowers are open at different phases on the same tree at any one time during flowering. Pollination is predominately initiated by insects, although bird species and small mammals have been suggested as possible pollinators (Butcher et al. 2009; Southerton et al. 2004). Pollinator movement has been predicted to result in regular pollen transfer between flowers on the same tree providing ample opportunities for geitonogamous pollination (House & Bell 1986; Beardsell et al. 1993; Moncur et al. 1995; Butcher et al. 2005). However, the observed levels of outcrossing are high (Beardsell et al. 1993) albeit variable (Moran & Brown 1980; Hardner et al. 1996; Butcher & Williams 2002), indicating that there is some barrier to self pollination (Pound et al. 2002).

Evidence of pre-zygotic self-incompatibility mechanisms have been found in some *Eucalyptus* species (Sedgley & Smith 1989; Sedgley & Granger 1996; Pound et al. 2002), however they are noticeably absent in others (Pound et al. 2002; Pound et al. 2003a). Several studies have suggested the high level of outcrossing and outcrossed progeny in some species may result from a post-zygotic self-incompatibility mechanism (James & Kennington 1993; Martin & Lee 1993; Pound et al. 2002; Pound et al. 2003b). In some species, where pollen tube growth is somewhat restricted following self-fertilization, but the mechanism is incomplete, both pre- and post-zygotic self-incompatibility have been suggested (Sedgley & Granger 1996).

The breeding system of a species has a considerable impact upon the level and structure of genetic variability generated within a population. Early meta-analysis of breeding systems and genetic variability within species has indicated highly outcrossing species exhibit higher levels of genetic variability and less population differentiation (Hamrick & Godt 1996), and may be consequently more vulnerable to genetic decline following fragmentation than selfing species (Aguilar et al. 2008).

While obligate outcrossing (self-incompatible) species are particularly vulnerable (as they are not reproductively independent), preferentially outcrossing species may also exhibit high genetic loads (accumulated detrimental alleles), depending on historical selfing rates. *Eucalyptus camaldulensis* is self-compatible, and, while the ability to self-pollinate substantially increases a species' capacity to withstand isolation, if the quality of selfed progeny is inferior, long-term remnant decline may ensue.

The mechanism underlying self-compatibility/self-incompatibility is of particular importance in defining a species' genetic resilience. James and Kennington (1993) suggested that post-zygotic seed abortion is preferential in *E. camaldulensis* indicating that the development of zygotes is dependent upon genotype, the range of genotypes available and the availability of resources. Such a mechanism, operating as a complex method of reproductive assurance, could enhance the sustainability of small remnants by maintaining high-quality progeny.

2.5 Niche habitat, recruitment, and survival in *Eucalyptus camaldulensis*

Eucalyptus camaldulensis populations occur within a narrow niche along river margins and in associated floodplains (Butcher et al. 2009). The restricted distribution of the species results from a strong dependence on water, especially in the early life stages. Seedling establishment has been identified as a critical life-stage in *E. camaldulensis* (Roberts & Marston 2000); both germination of seed and the early growth of seedlings are dependent on water. This is unlike some eucalypts in which recruitment is enhanced following fire. Dexter (1970; cited in Di Stefano 2002) illustrated the complexity of seed germination and consequent seedling establishment in *E. camaldulensis* by listing the key influential factors:

a) Seed supply

b) Incidence of flooding

c) The time of flood recession

d) Duration and depth of flooding in the season following germination

e) Seedbed type

f) Availability of water in the sub-soil

g) Distribution and abundance of summer rainfall

h) Insects, livestock and other animals.

The specific germination and growth requirements of *E. camaldulensis* render populations vulnerable to stochastic variation in flood regimes (both natural and anthropologically initiated). This has been demonstrated in the Murray-Darling basin where the health of *E. camaldulensis* sites is contingent upon flooding, the patterns of which have been altered following river regulation (Bren 1988). This intimate relationship between hydrology and recruitment suggests that hydrology is also likely to impact indirectly upon the pattern and levels of genetic diversity maintained within and between *E. camaldulensis* populations by influencing germination, seedling survival and subsequent age-class structure.

However, not all life-stages exhibit the same level of dependence on water. Adult trees have been demonstrated to withstand dry periods, indicating the dependence on water availability decreases with age (Roberts & Marston 2000). Bacon (1993 and references therein) has attributed this capacity to the root structure and growth in *E. camaldulensis,* that enables adult trees to access ground water. The dichotomy between the water requirements of the seedling and the adult tree is important as it indicates adult reproductive trees have the capacity to persist in habitats that may not be able to support the high water demands of seedlings.

2.6 Level and distribution of genetic diversity in *Eucalyptus* species

The genetic diversity of *Eucalyptus* species has been extensively studied over the last thirty years using a range of methodologies and analyses. From the late 1980s through to early 2000 many studies were conducted within natural populations of eucalypts. A review published by Moran (1992) summarised much of this work in an attempt to determine which factors were influencing the level and distribution of genetic diversity in the genus. Moran (1992) found that widespread *Eucalyptus* species typically exhibit high levels of genetic diversity, with the majority of genetic variation found within rather than between populations. Conversely, species that occurred in isolated populations tended to exhibit greater differentiation and less genetic diversity (e.g. *E. crucis*, Sampson et al. 1988; *E. perriniana*, Rathbone et al. 2007; Byrne & Hopper 2008). The latter group is of particular interest as it includes many of the rare eucalypt species (e.g. *Eucalyptus caesia, E. crucis*, *E. pulverulenta, E. perriniana*). Early

workers in the genus assessed genetic parameters using allozymes (Prober & Brown 1994; Coates & Sokolowski 1989; Moran & Hopper 1983), however, more recently a range of methods directly assessing genotype variation have been employed (e.g. restriction fragment length polymorphism, microsatellites). Although the magnitude of the diversity statistics increases with the variability of the marker system (and the degree of differentiation detected decreases), the trends observed by Moran (1992) continue to arise in the more contemporary studies of the genus (Table 1).

There are exceptions, however, and exploring those species that do not conform to general patterns can give insight into what other factors might be influential in defining genetic diversity and structure. *Eucalyptus parvula* (previously *E. parvifolia*) exhibits low levels of differentiation and relative high levels of genetic diversity despite occurring in small, isolated populations (Prober et al. 1990). The lack of differentiation between populations within this species was attributed to the clearing of the woodlands surrounding populations, indicating that the populations may have been remnants of an historically continuous population. *Eucalyptus albens* also exhibits very low levels of differentiation ($G_{ST} = 0.06$) despite its geographically isolated populations (Prober & Brown 1994). The lack of differentiation in *E. albens* was also attributed to its recent fragmentation. These studies indicate time since isolation is likely to be a critical factor in determining the extent of differentiation between isolated populations of *Eucalyptus* species. This finding has also been documented in other species of tree (Aguilar et al. 2008).

Several atypical genetic patterns have also been attributed to plant-pollinator interactions. *Eucalyptus rameliana* is a restricted species that occurs within discrete populations however it exhibits high levels of genetic diversity (Sampson et al. 1989) that are more akin to widespread species. In this case the atypical results were attributed to bird pollination that was predicted to promote gene-flow over large distances (Sampson et al. 1989). Similarly, high levels of diversity and low levels of differentiation were detected in *Eucalyptus wandoo* and were attributed to the maintenance of pollinator movement between populations. Pollinator movement was suggested as being facilitated by the habitat connectivity provided by small remnants and isolated trees (Byrne et al. 2008).

A number of the rare and restricted *Eucalyptus* species are able to reproduce vegetatively (e.g. *Eucalyptus curtsii* Smith et al. 2003; *Eucalyptus phylacis*, Rossetto et al.

1999*)* and this capacity to reproduce clonally may also influence genetic parameters, although in these situations populations are likely to exhibit accentuated trends already associated with isolation (i.e. low levels of genetic diversity, high levels of genetic differentiation). Extensive clonality detected in *E. curtisii* (Smith et al. 2003) is likely to have contributed to the very low level of genetic diversity (He = 0.54, microsatellites). Smith et al. (2003) found two sites were composed of single clones. Similar low levels of genetic diversity were detected in *E. phylacis* (Rossetto et al. 1999) in which 170 stems were found to be the same genotype.

The extensive research conducted within natural populations of *Eucalyptus* provides a comprehensive bank of information on typical genetic patterns (Table 1). An important finding is that recently fragmented populations of *Eucalyptus* species may appear, superficially, to have genetic diversity and differentiation patterns consistent with their pre-fragmentation distribution. However, the differentiation detected in naturally discrete species suggests long-term isolation can result in higher levels of genetic differentiation than are typical in widespread species. The range of values observed also indicates specific species attributes (e.g. population connectivity, pollinator type, reproductive mode) may be decisive in determining levels and patterns of genetic diversity in natural populations.

Table 1. Genetic diversity parameters for *Eucalyptus* species. np = number of primers or probes, n pops = the number of populations, H_T = total heterozygosity across all populations, He = mean heterozygosity within population, F = the mean inbreeding coefficient within populations. F_{ST}, G_{ST}, R_{ST} and Q_{ST} are alternate measures of genetic differentiation . Table continues over the page

Author(s)	**Species**	**Method**	**np**	**n pop**	**H_T**	**He**	**F**	**F_{ST}**	**G_{ST}**	**R_{ST}**	**Q_{ST}**	**Distribution**
Prober and Brown (1994)	*E. albens*	Allozymes	18	25	0.289	0.289	0.11		0.06			Widespread but fragmented
Coates & Sokolowski (1989)	*E. diversicolor*	Allozymes	16	13	0.299	0.15	0.22		0.092			Regional but continuous
Moran & Hopper (1983)	*E. caesia* subsp. *caesia*	Allozymes	18	7		0.073						Regional with discrete populations
Moran & Hopper (1983)	*E. caesia* subsp. *magna*	Allozymes	18	6		0.062						Regional with discrete populations
Moran & Hopper (1983)	*E. caesia*	Allozymes	18	13	0.176	0.068			0.61			Regional with discrete populations
Peters et al. (1990)	*E. pulverulenta*	Allozymes	16	4	0.1	0.07			0.30			Disjunct
Sampson et al. (1995)	*E. rameliana*	Allozymes	11	9	0.23	0.22			0.092			Regionally restricted with isolated populations.
McDonald et al. (2003)	*E. cladocalyx*	Allozymes		8	0.191	0.148					0.26	Regional & disjunct
Prober et al. (1990)	*E. parvifolia*	Allozymes	10	8		0.21			0.07			Localised & fragmented
Prober et al. (1990)	*E. paliformis*	Allozymes	10	6		0.136			0.04			Localised & undisturbed, discrete
Sampson et al. (1988)	*E. crucis*	Allozymes	8	10	0.32	0.19			0.244			Regional & disjunct
Sampson et al. (1988)	*E. crucis* subsp. *crucis*	Allozymes	8	6		0.19			0.174			Regional & disjunct
Sampson et al. (1988)	*E.crucis* subsp. *lanceolata*	Allozymes	8	4		0.19			0.187			Regional & disjunct
Sampson et al. (1989)	*E. rhodantha /adults*	Allozymes	4	2	0.518		0.18		0.09			Regional & fragmented
Sampson et al. (1989)	*E. rhodantha /pollen*	Allozymes	4	2	0.444		-0.12		0.08			Regional an & fragmented
House and Bell (1996)	*E. pellita*	Allozymes	10	17	0.299	0.249			0.20			Wide range – more or less continuous
House & Bell (1986)	*E. urophylla*	Allozymes	8	25		0.172			0.12			Widespread
House and Bell (1996)	*E. scias*	Allozymes	10	8	0.343				0.15			Widespread & patchy
Jones et al. (2008)	*E. grandis*	cpDNA		15	0.832				0.3			Widespread – continuous
Butcher et al. (2005)	*E. benthamii*	Microsatellites	22	4	0.295	0.696	0.06	0.105				Regional & fragmented
Butcher et al. (2009)	*E. camaldulensis*	Microsatellites	15	22		0.800	0.12	0.06				Widespread
Jones et al. (2005)	*E. morrisbyi*	Microsatellites	6	2	0.77	0.69	0.07	0.19				Restricted & fragmented

Author(s)	Species	Method	n	n pop	H_T	He	F	F_{ST}	G_{ST}	R_{ST}	Q_{ST}	Distribution
Jones et al.(2007)	*E. globulus (4* subspecies)	Microsatellites	8	5	0.87	0.82	0.238	0.08				Widespread
Steane et al. (2006)	*E. globulus*	Microsatellites	8	11	0.83	0.75	0.14	0.09				Widespread
Smith et al. (2003)	*E. curtsii*	Microsatellites	5	12		0.54		0.3		0.22		Regional & naturally isolated
Byrne et al. (2008)	*E. wandoo*	Microsatellites	6	2		0.876				0.018		Regionally extensive but patchy
Rathbone et al. (2007)	*E. perriniana*	Microsatellites	8	9	0.85	0.73	0.12	0.16				Wide range but small isolated pops.
Payne et al. (2008)	*E. urophylla*	Microsatellites	12	19		0.739	0.074	0.031				Naturally disjunct
Nevill et al. (2010)	*E.regnans*	cpMicrosatellites	10	40	0.89	0.18			0.79	0.93		Discontinuous
Byrne et al.(1999)	*E.kochii* subsp. *kochii*	RFLP	38	2	0.468	0.46	0.067	0.017				Continuous within a small range
Byrne et al. (1999)	*E. kochii* subsp. *plenissima*	RFLP	38	4	0.527	0.505	0.022	0.042				Widespread in the ne wheat belt in Western Australia
Byrne et al. (1999)	*E. horistes*	RFLP	38	4	0.501	0.486	0.054	0.029				Widespread in the n wheat belt in Western Australia
Butcher et al. (2002)	*E. camaldulensis*	RFLP	33	31	0.53	0.49	0.019*				0.078	Widespread
Byrne et al. (1998)	*E. nitens*	RFLP	40	8	0.445	0.373	0.044		0.16			Widespread & disjunct
Elliot & Byrne (2003)	*E. occidentalis*	RFLP	25	10	0.373	0.351	-0.036		0.059			Restricted with small isolated pops.
Hines & Byrne (2001)	*E. loxophleba*	RFLP	30	20	0.418	0.337	0.046	0.089				Widespread
Hines & Byrne (2001)	*E. loxophleba* subsp. *loxophleba*	RFLP	30	6		0.368	0.086					Widespread
Hines & Byrne (2001)	*E. loxophleba* subsp. *supralaevis*	RFLP	30	4		0.397	0.016					Widespread
Hines & Byrne (2001)	*E. loxophleba* subsp. *lissophloia*	RFLP	30	4		0.384	0.033					Widespread
Hines & Byrne (2001)	*E. loxophleba* subsp. *gratiae*	RFLP	30	3		0.391	0.031					Widespread
Elliot & Byrne (2004)	*E. angustissima* subsp.*angustissima*	RFLP	30	4	0.31	0.33	0.04		0.106			Restricted
Elliot & Byrne (2004)	*E. foliosia*	RFLP	30	2		0.338	-0.158		0.086			Restricted
Elliot & Byrne (2004)	*E. misella*	RFLP	30	1		0.369	-0.038					Restricted
Byrne & Hopper (2008)	*E. caesia* subsp. *caesia*	RFLP	20	11	0.148	0.078	-0.152		0.40			Clonal & restricted
Byrne & Hopper (2008)	*E. caesia* subsp. *magna*				0.117	0.067	0.063		0.44			Clonal and restricted
Elliot & Byrne (2004)	*E. angustissima* subsp. *quaerenda*	RFLP	30	1		0.331	-0.022					Restricted

3 The Hunter Valley

3.1 Climate and location

The Hunter Valley (Figure 4) is a wide valley on the eastern side of the Great Dividing Range in NSW, Australia. It is located approximately 150km north-west of Sydney. The region experiences a mild to hot summer. Temperatures in summer commonly exceed 40˚C and in winter temperatures can be below 0˚C in the upper Hunter Valley but milder in the eastern valley floor (Spencer et al. 2004). The majority of the rain falls in the summer months. In the centre of the catchment mean annual rainfall is 641.5mm (Jerry's Plains 1804–009). Rainfall increases marginally toward the coast and in the foothills of the surrounding mountains. Two large rivers, the Hunter and the Goulburn (a tributary of the Hunter), dissect the Valley and there are a number of smaller tributaries (Figure 4). The lowland areas are susceptible to flooding due to the size and topography of the catchment and seasonal weather systems (Spencer et al. 2004).

3.2 *Eucalyptus camaldulensis* in the Hunter Valley

There are twenty-eight remnant populations of *E. camaldulensis* currently known in the Hunter Valley some of which incorporate 2–3 sub populations (Umwelt 2007). The populations exhibit variable age-class structures indicating different regeneration patterns (Chapter 5). Typical remnant populations occur along the margins of rivers (riparian) and in floodplain habitat (Table 2). Some remnant populations encompass both habitat types. In this work, the two habitat types are distinguished by the location and arrangement of the trees: riparian stands are arranged in a linear formation along the immediate margin of the river; floodplain stands are non-linear patches located in floodplain habitat removed from the river margin. In the floodplain habitat the eucalypts themselves are the only defining characteristic of the community with the understory dominated by exotic pastures and annual weeds (Figure 2). In the riparian zones, *Casuarina cunninghamiana* often co-occurs with *E. camaldulensis* although the understory is also heavily infested with annual weeds. Riparian zones are characterised by frequent disturbance and the vegetative assemblage reflects this, with dramatic changes in understory vegetation between

seasons. A single population at Denman in located on a permanent lagoon (Figure 9); at this site the population is linearly arranged along the margins of the lagoon.

The landscape context of remnant populations is variable. A number of remnant populations are found on mining leases and several are on agricultural holdings (Figure 3), while a number are within urban areas. Although several populations are on traveling stock routes and in public parks, none of populations within the catchment are on protected land. In some remnant populations regeneration efforts have been undertaken (Table 2) and have been successful, although planted individuals of *E. camaldulensis* were not locally sourced and other species were planted within habitats where they are not naturally found (Webb & Erskine 2003).

3.3 Historical distribution of *Eucalyptus camaldulensis* in the Hunter Valley

The interpretation of genetic data collected from recently fragmented populations is greatly enhanced when it is accompanied with an accurate understanding of the historical distribution of the species. The purported range of *E. camaldulensis* in the Hunter Valley prior to European settlement is estimated to have been between 10000 and 20000 hectares along the rivers, tributaries and floodplains of the Valley (Umwelt 2007). To 'ground truth' the predicted historical distribution of the species within the study area, I explored a wide range of historical resources, including early explorer's journals, newspapers, artistic descriptions, herbarium specimens and photographs.

Early observations indicate that the riparian vegetation in the eastern part of the catchment was dominated by closed forest. This community, referred to as the 'cedar brushes' or 'brushes', most closely resembles the community described as Lower Hunter Dry Rainforest. Cedar (*Toona ciliate*) and fig trees (*Ficus sp.*) are cited, as are Kurrajongs (*Brachychiton populneus*) and a thick understory of vines. A similar closed forest assemblage was also suggested in a study conducted within the catchment by Bennett and Mooney (2003), who, by identifying pollen in pre-European sediment, detected a clear reduction in closed forest components since European settlement (Bennett and Mooney 2003). The rapid change in the eastern part of the catchment was undoubtedly the result of the logging of cedar, one of the earliest industries in the region. Hickney (1850) related the extent of early exploitation of cedar when he described the state of cedar in the colony;

> "Although the more accessible parts of the flats of the William, as well as of the Hunter, and its other tributaries, may be almost exhausted, we have still the rivers of greater magnitude to the north, and after that an inexhaustible supply in the mountain ranges of these rivers. For where cedar is found on the banks of rivers, we must of necessity find it in the mountain ranges which form the sources of these rivers" (Hickey 1850).

The extent of *E. camaldulensis* in the eastern part of the catchment is thus uncertain. Memory (1877), recounting activities about the Hunter River near Hinton talks of 'gigantic gum trees', 'figs' and the 'huge stumps of cedar trees which had long before 'disappeared', indicating *E. camaldulensis* may have been present, but that it may have possibly co-occurred with other species.

The removal of cedar is likely to have modified the quality of riparian habitat, potentially impacting upon the type of vegetative community that it could support. Although there is no direct evidence for this hypothesis, Hickey (1850) suggested the rehabilitation of cedar was inhibited by the clearing of riparian vegetation:

> I have seen vigorous trees left standing when the native brush about them has been cleared away, but without their natural shelter, which these brushes afford from winds and frosts, they will not live (Hickey 1850).

Historical sources suggest the cedar brushes did not extend into the central part of the catchment. It is possible that, in this region, where the climate was no longer conducive to closed forest, riparian and floodplain habitat may have been occupied by open woodland dominated by *E. camaldulensis*. However, evidence for this is poor. Although a number of early written references refer to gum trees on the river margins in the valley, it is uncertain if they refer to *E. camaldulensis* since the references are non-specific (Figure 10). Photographs taken in the early 1900s show what appears to be evidence of sapping (similar to ring-barking) (Figures 5 and 7) and large trees are noticeably absent. In one of the earliest photographs of the Hunter River, taking in the vicinity of Muswellbrook (Figure 8), there is evidence of riparian gum trees, but no trees of any significant age. This is probably due to clearing. While cedar was cleared in the east, indiscriminate clearing was also occurring in the settled parts of the catchment. At this time the felling of timber and sapping was considered as land improvement. By 1875 compensation could be

claimed to recover the cost of sapping (Anon, 1877a). It is likely therefore, that riparian habitat may have been mostly cleared of vegetation as early as 1900.

Several sources also indicate the frequency and intensity of flood events varied considerably over the last 200 years. Since seed germination and survival is dependent upon water availability (Dexter 1970; Di Stefano 2002; Cunningham et al. 2007b), the frequency of historical flooding events is likely to be important to population establishment, expansion and persistence, especially on floodplains where above ground water is only periodically available (e.g. Bren 1988). Variation appeared to result from both natural climatic variability and anthropogenically-initiated modification (Erskine 1992). Erskine and Warner (1988) identified the period between 1821 and 1856 as a 'Dry Dominated Regime and the second half of the 1800s (1857–1900) as a Flood Dominated Regime. Similarly the first half of the twentieth century was defined as a Dry Dominated regime and the second a Flood Dominated Regime. Erskine and Warner's (1988) accounts match a graph collated by the NSW Department of Water Resources (Figure 11). The graph shows marked variation in flood height and frequency over time. Since we have no climatic records within the area before the 1820s, we do not know how suitable the floodplain habitat may have been for *E. camaldulensis* immediately prior to European settlement, or how extensive floodplain populations of *E. camaldulensis* were. An article from 1877 suggests, however, that the author was not accustomed to seeing *E. camaldulensis* in floodplain habitat in his area:

> "The continued dry weather of the past three years is revealing further evidence, in drying up the deep lagoons on the Hunter flats in this vicinity, (Muswellbrook) and ex-posing to view, in their deepest bed or bottom, numbers of stumps of the red gum (Anon, 1877)"

The author then continues, stating that:

> "We all know well these trees won't grow in water and that if it surrounds them in the least they die. And this being the case, there is only one conclusion we can arrive at, and that is that when these trees (perhaps a century ago) commenced to grow-first as little delicate trees, and then into saplings, and from saplings into trees-this lagoon must have been perfectly dry (Anon, 1877).

The historical investigation here provides ample evidence to suggest that since European settlement, the Hunter Valley has been dramatically modified by the intense development within the region. This is likely to have had a profound impact upon vegetative communities, particularly along the river margins and in floodplains, which were settled first. Although there is limited evidence of the historical distribution of *E. camaldulensis* within the Hunter Valley, the odd reference exists indicating that the species was present, though the extent and continuity of the population remains a mystery. Clearly, whatever community did exist, it is likely to have been severely reduced during the last 200 years. A comparison of the Redbournberry site in the early 1900s (Figures 5 and 7) compared with today (Figure 6) suggests degradation of this site was more pronounced 100 years ago.

The paucity of historical references to the species might reflect either that *E. camaldulensis* was not as widespread as previously thought, or that the historical resources that I explored were inadequate. In the Hunter Valley where rapid population expansion occurred over a brief period of time, an accurate description of the historical distribution of *E. camaldulensis* is complicated first by the temporal variation in habitat suitability, secondly, by the obscurity of the species' early classification and finally by the changes in landscape values; the latter plays a significant role in the historical depiction of landscape in art and written media. Under these circumstances, genetic data may provide a more comprehensive indication of the pre-European species distribution.

Figure 2. *Eucalyptus camaldulensis* understory (*Silybum marianum*) in the floodplain component of the Lemington site, October 2007

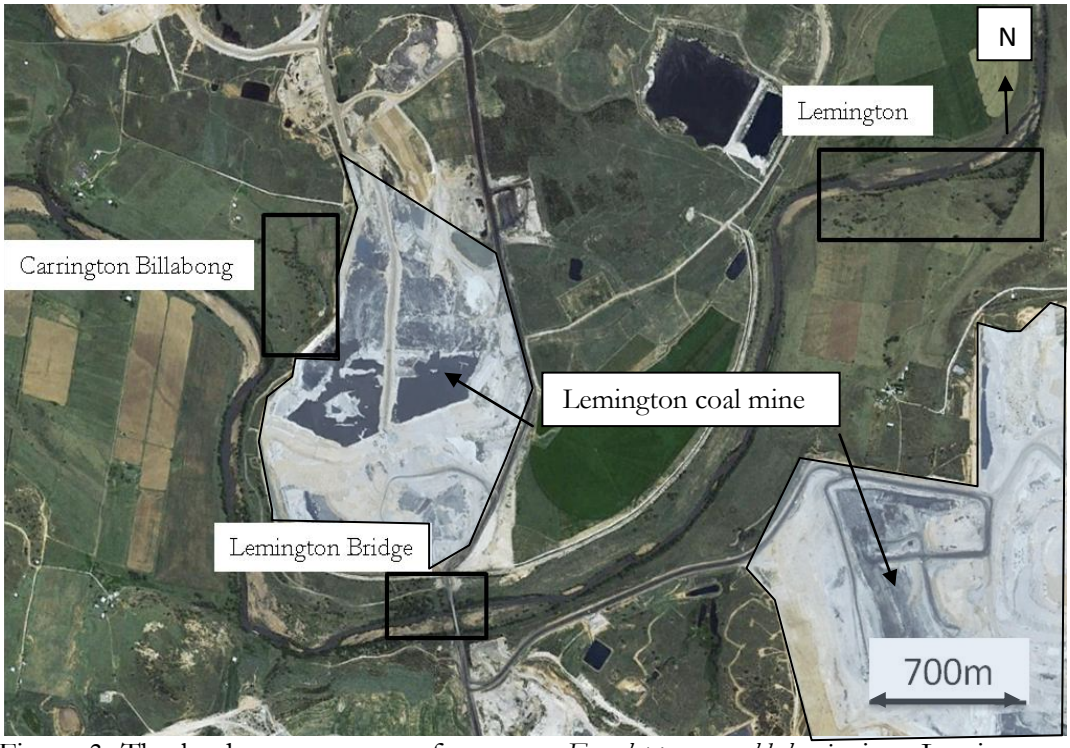


Figure 3. The landscape context of remnant *Eucalyptus camaldulensis* sites: Lemington coal mine and agriculture.

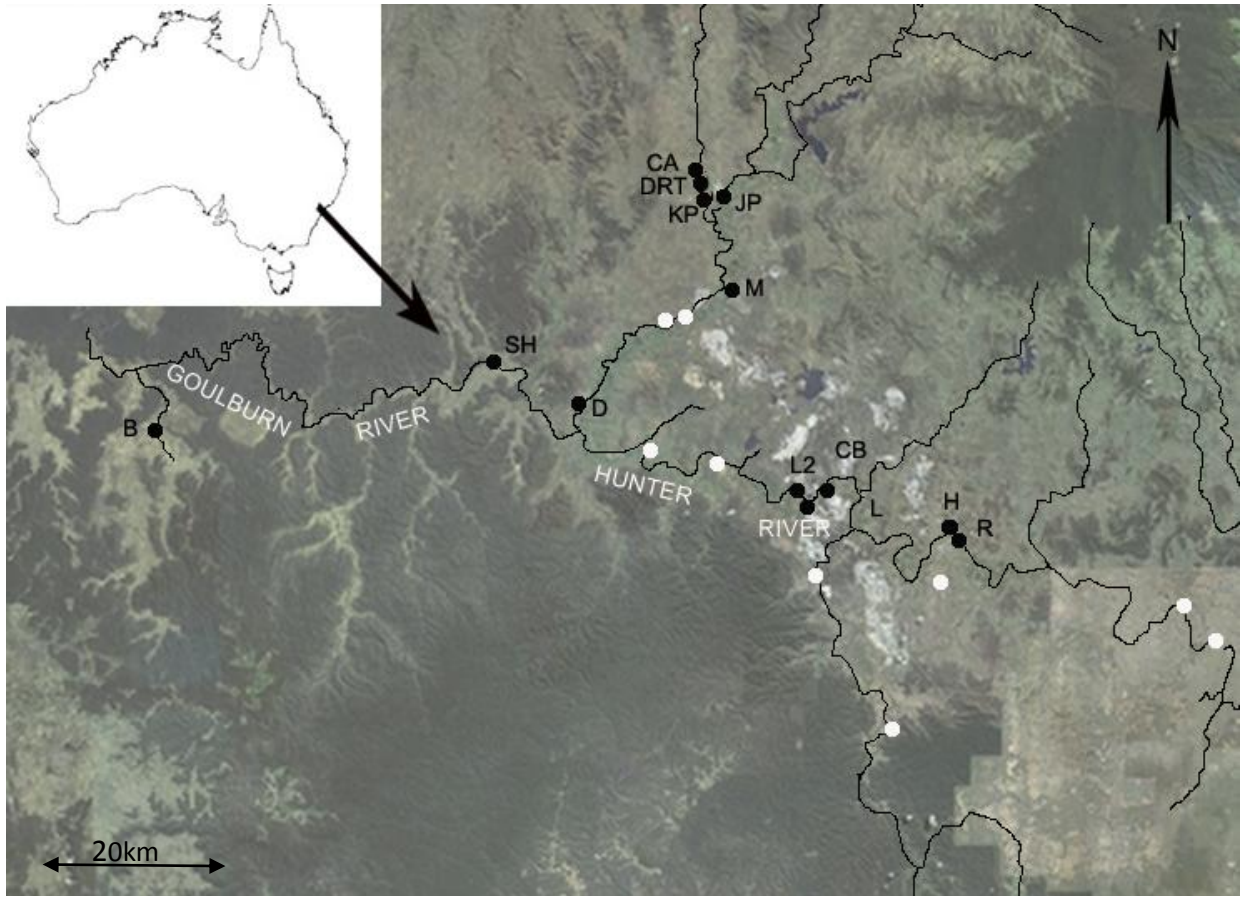


Figure 4. *Eucalyptus camaldulensis* remnants in the Hunter Valley, NSW. Black circles denote study sites. White circles are known remnant populations that were not included in this research. See Table 2 for expanded site titles and descriptions.

Table 2. Attributes of studied remnant populations of *Eucalyptus camaldulensis*, population size = the number of individual plants (all plants with a diameter at breast height (cm) >2.5)

Population	Population Size	Revegetation	Habitat type	Water availability	Context
Denman (D)	122	Y	Disconnected lagoon	Continuous	Urban
Muswellbrook (M)	11	Y*	Riparian	Continuous	Urban
Lemington (L)	52	N	Riparian/floodplain	Continuous/periodic	Rural
Hunterview (H)	152	Y	Riparian	Continuous	Urban
Redbournberry (R)	77	Y	Riparian	Continuous	Urban
Camyr Allyn (CA)	700	N	Floodplain	Episodic	Rural
Lemington Bridge (L2)	15	N	Riparian	Continuous	Rural/mine site
Dartbrook (DT)	61	Y	Floodplain	Episodic	Rural
Bylong (B)	125	N	Riparian	Episodic	Rural
Sandy Hollow (SH)	55	N	Riparian/floodplain	Continuous	Rural
Kingdom Ponds (KP)	23	N	Floodplain	Episodic	Rural
Carrington Billabong (CB)	112	N	Floodplain	Episodic	Rural/mine site
Jefferson Park (JP)	31	na	Golf course	Uncertain	Urban

Revegetation indicates whether *E. camaldulensis* trees had been planted at the sites as part of re-vegetation

Figure 5. Looking upstream (east) from Redbournberry site (July 1906) showing degraded river margin and the extent of land clearing. Reproduced with permission, State Library NSW (accessed via trove: http://trove.nla.gov.au)

Figure 6. A large tree (dbh > 200 cm) at Redbournberry site taken in 2010 surrounded by a number of younger trees, *Casuarina cunninghamiana* and an understory of exotic weeds, October 2007

Figure 7. Redbournberry site, July 1906, showing evidence of sapping and the denuded and degraded river bank. Reproduced with permission, State Library NSW (accessed via trove: http://trove.nla.gov.au)

Figure 8. An early photograph (early 1900s) taken near Muswellbrook in the centre of the study range displaying minimal riparian vegetation. Reproduced with permission, State Library NSW (accessed via trove: http://trove.nla.gov.au)

Figure 9. Remnant population at the Denman site showing mature trees growing along the margin of a permanent lagoon, August 2010

"Glad, indeed, was I to meet the gum trees of the Hunter river in every stage of their existence: they are to me as the primeval forest of the land, silent preachers, reminding the new humanity which hopes and toils beneath their boughs that for long generations they have been the sole denizens of the soil, and that their health-giving properties should at least preserve them from being exterminated root and branch by the greed and short-sightedness of man" (Soltera 1884).

"The flooded gum trees on these brushes were of an immense size; I measured one on the Canningalla farm which was 21 feet in circumference at it base, and 15 feet in circumference six feet from it base. Not having an instrument I could not ascertain its height. This tree was dead, and none of the top limbs had fallen from it" (Gilpin 1887).

"....the flooded gum trees were of so large a circumference that the man who cleared a portion of the brush on it erected stages some ten feet from the ground, to enable him to fell them, as they commenced tapering off considerably from that height" (Gilpin 1887).

The Hunter River is "….as wide as the Thames in the lower part of its course winding slowly towards the ocean, among forests that have never felt the stroke of an axe or seen any face lately but that of the wandering barbarian. On either bank, the lofty gum tree or *Eucalyptus* shoots up its white naked stem to a height of 150 feet from the rich alluvial soil.."(Lang 1834)

Figure 10. Historical references to gum trees in riparian habitat in the Hunter Valley

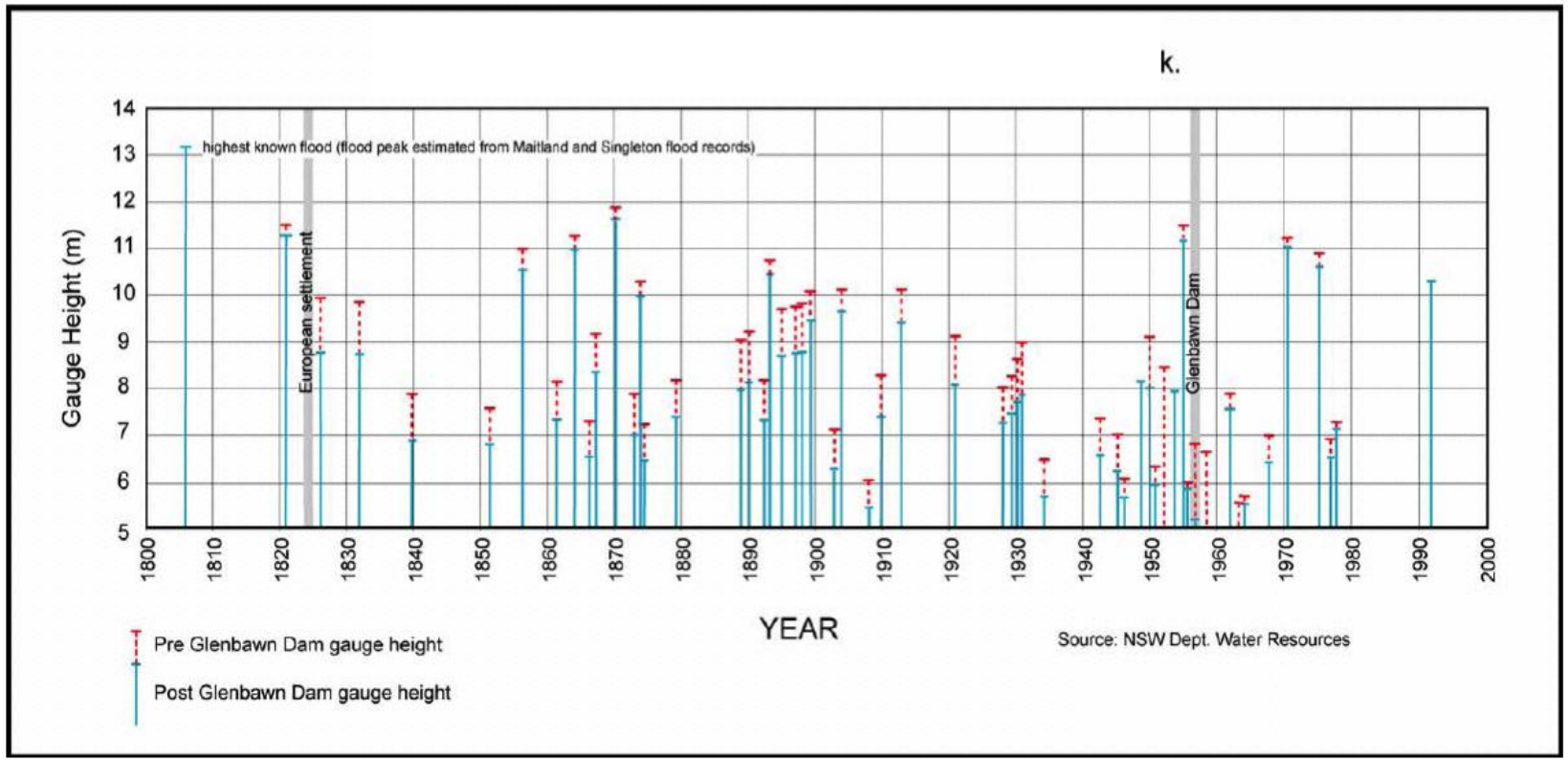


Figure 11. The history of floods recorded at Muswellbrook (cited in Spencer et al. 2004)

4 Genetic decline detected in small disjunct populations of *Eucalyptus camaldulensis* Dehnh. subsp. *camaldulensis* (River Red Gum)

4.1 Abstract

Eucalyptus camaldulensis is a widespread species in Australia but is locally endangered in the Hunter Valley. In this region the species persists in a highly modified landscape within small remnants. This research assessed the level of genetic diversity and temporal changes of genetic diversity and structure within and among thirteen forest remnants using nine microsatellite markers. Populations included were variously fragmented and isolated and restoration had been undertaken at some sites. Analyses revealed a high level of genetic diversity (He = 0.74) and low genetic differentiation between remnants (Dest = 0.196) suggesting an historically more connected population. Analyses of genetic diversity in different dbh cohorts indicated that younger trees exhibited lower levels of genotype variability. This trend was also present within the majority of remnants where temporal comparisons were possible. An exception was evident in a population with restoration planting.

4.2 Introduction

Across the globe deforestation and landscape modification have irreversibly altered forest ecosystems. Remaining forests in many parts of the world are small, spatially isolated and highly disturbed (Young et al. 1996; Broadhurst & Young 2007; Farwig et al. 2008; Aguilar et al. 2008), particularly in areas with valuable natural resources (Saunders et al. 1991). The impact of habitat fragmentation on the viability of remnant populations has been widely studied. Recently, much interest has been directed at determining the impact of fragmentation on the genetic sustainability of single species occupying fragmented habitats. Because genetic variation is critical for the ongoing evolution of a species (Frankham 1995; Frankham et al. 2002; Aguilar et al. 2008) and genetic variability has been linked to relative high fitness outcomes (Leimu et al. 2006), an increasing number of workers seek to determine if forest

remnants display reduced genetic variability or other genetic signals that might indicate the onset of genetic decline. The predictions of how fragmentation may potentially impact upon genetic variability are largely based on the theory of island biogeography (MacArthur & Wilson 1967; Saunders et al. 1991; Lowe et al. 2005; Aguilar et al. 2008) in which the number of species is related to the size and distance between islands. While the island biogeography theory relates to species, the unit of interest in conservation genetics is the allele. The degree of isolation and the size of a remnant population therefore have a potentially significant impact upon allelic structure and diversity.

The genetic consequences of fragmentation include reduced genetic diversity, reduced migration and the subsequent development of genetic differentiation between remnants (Ellstrand & Elam 1993; Young et al. 1996; Lowe et al. 2005; Aguilar et al. 2008). Initially genetic decline may be signaled by reduced allelic diversity as opposed to a decreased expected heterozygosity. Loss of genetic diversity is predicted to occur initially as a result of the initial bottleneck and from genetic drift (the random loss of alleles) (Young et al. 1996; Nason & Hamrick 1997; Aguilar et al. 2008). In the longer term, decreased genetic variability may arise in the form of the decreased heterozygosity associated with inbreeding (Ellstrand & Elam 1993; Young et al. 1996). Increased inbreeding is predicted to occur in remnant populations as a result of reduced mate choice and/or altered pollinator interactions (Aizen & Feinsinger 1994a). Genetic differentiation is predicted to develop between remnant populations following fragmentation via the re-enforcement of the random allelic content residing in the remnant as a result of reproductive isolation (Young et al. 1993).

The literature to date indicates theoretical predictions of genetic decline are realised in many fragmented natural populations (Aguilar et al. 2008). Lower levels of genetic diversity have been detected in research comparing intact habitat with fragmented habitat (e.g. Franceschinelli et al. 2007) and in meta-analyses that compare large and small populations (e.g. Leimu et al. 2006). Aguilar et al. (2008) observed genetic declines become more evident when remnants have been fragmented for substantial periods of time (>100 years) indicating that the degradation of genetic variability is ongoing. The hypothesised development of genetic differentiation between forest remnants over time has also been observed in several studies (Jump et al. 2006;

Franceschinelli et al. 2007) indicating that in some species, reproductive isolation results from fragmentation.

Evidence of altered breeding patterns is less decisive. Because inbreeding coefficients are not largely associated with genetic decline (e.g. reduced number of alleles but no increased inbreeding coefficient in *Acer saccharum*, Young et al. 1993), reduced genetic variability in fragmented habitats has been attributed to initial bottlenecks and subsequent genetic drift rather than reduced genetic diversity from increased inbreeding (Young et al. 1996; Leimu et al. 2006). However, workers directly assessing reproduction have found evidence of altered mating patterns in fragmented habitats (e.g. Aizen & Feinsinger 1994b) and meta analyses have detected reduced outcrossing rates in fragmented compared with continuous habitat (Aguilar et al. 2008). In *Eucalyptus globulus* small remnants and isolated trees produced consistently inferior progeny compared with large intact populations (Borralho & Potts 1996) and lower outcrossing rates were estimated in small compared with large fragments (Mimura et al. 2009). Lowered seed set was also detected in small populations of *Eucalyptus salubris* compared with large populations (Krauss et al. 2007).

There are many species, however, that do not suffer one or more of these impacts and in which the predicted theoretical genetic consequences are not evident (Young et al. 1996). High levels of gene-flow have been detected as occurring between fragmented remnants indicating that pollinators can travel substantial distances (Byrne et al. 2008; Mimunra et al. 2009), thereby potentially mitigating the genetic impacts of isolation. Further, small remnants have been found to exhibit higher levels of genetic diversity (e.g. *Acer saccharum,* Young et al. 1993) and higher levels of outcrossing than larger remnants (Mathiasen et al. 2007). And although meta-analyses have found significant correlations between population size and genetic diversity, the degree of correlation (e.g. approx. $r = 0.42$ between population size and genetic variability, Leimu et al. 2006) indicate a substantial portion of species do not conform to the observed trends.

Eucalyptus camaldulensis is a dominant riparian species in Australia. In the Hunter Valley region in eastern NSW, however, it is listed as an endangered ecological community on the *Threatened Species Conservation Act* 1995 (NSW). It is also one of the dominant species in the endangered Hunter Floodplain Red Gum Woodland (TSC Act 1995). Although the pre-European distribution of the species has proven

difficult to estimate, the community is thought to have been more or less continuous along the river margins in the mid and western component of the Hunter Valley catchment in eastern Australia (Chapter 3). The species is restricted to riparian and floodplain habitat because of early dependence upon inundation for seed germination and seedling growth. In the Hunter Valley it is now known from only twenty-eight degraded and variously isolated remnants, the majority of which have less than one hundred individuals.

In this study nine polymorphic co-dominant microsatellite markers were used to assess the genetic variability and genetic structure of a sample of remnant stands of *E. camaldulensis*. The aim of the work was to determine how genetic diversity was distributed across the Hunter Valley catchment and to ascertain if genetic parameters differed across age classes and hence were variable across time.

4.3 Methods

4.3.1 Study sites

The Hunter Valley in central-eastern NSW is valued for its rich agricultural land and mineral resources. As a result, it has been heavily impacted by widespread clearing and other landscape modification associated with development. Clearing began in the early 1800s and is ongoing. Several dams in the upper reaches of the Hunter River built in the mid 1900s have also changed the flood dynamics in rivers and floodplains. Urban development has also been extensive; several of the larger remaining populations of *E. camaldulensis* exist within urban settlements. Remnant populations are characterised by isolation and the habitat between populations is of variable quality (Table 2).

4.3.2 Material collection and DNA extraction

Leaf material was collected from 216 individuals distributed across 13 remnant populations (Figure 12). Sites were variable in size, shape and proximity to the river margin. A number of sites had been planted with outsourced *E. camaldulensis* individuals as a component of restoration efforts. In Muswellbrook (n = 11) several planted trees were included in sampling. Between six and 30 trees were sampled from each site. Sample number was dependent upon the size of the remnant and stand density. This limited the number of samples collected in small populations, some of

which are less than a hectare in area and contain as few as eleven individual trees. In large populations the distance between sampled individuals was often several hundred meters while in small populations, at least 15m separated sampled trees. The predominant aim of the sampling scheme was to sample across the population while avoiding the possibility of sampling within spatially restricted genetic neighbourhoods. The location of individual trees was plotted on a satellite image to confirm that the on-ground sampling regime had captured the full extent of each population.

Leaf material was dried in silica gel prior to extraction. Deoxyribonucleic acid (DNA) was extracted from 20mg of silica dried leaf material using the DNeasy mini extraction kit (Q_{IAGEN}). The presence of DNA was confirmed by running 10 μL of DNA in agarose gel at 200 volts for one hour.

4.3.3 Polymerase chain reaction

Polymerase chain reaction (PCR) was used to amplify microsatellite regions. Thirty microsatellite primers were screened from the EMBRA (Brondani et al. 2006) and Eg (Benson et al. 2008), marker sets developed for *Eucalyptus* species within the *Symphyomyrtus* group. Primers were screened on a subsample of 10 individuals. Eight primers (Appendix 1) that gave clear, reproducible and polymorphic bands were selected. As in a previous study (Butcher et al. 2008) one primer pair (Embra 17) amplified two regions resulting in a total of nine microsatellite regions. Primers were tagged with florescent markers (Vic, Ned, Fam and Pet). Polymerase chain reaction was performed for each primer separately (for PCR programs see Appendix 1). The reaction mixture per individual per primer pair was: Buffer 2.5 μL, Mg++ 3.0 μL, DNTPs 0.5 μL, FWD primer 0.25 μL, RVSE primer 0.25 μL, Taq polymerase 0.2 μL, template DNA 1 μL and H_2O 17.3 μL to give a total volume of 25 μL. Regions were amplified using a PC-960 Thermo Cycler. Programs are provided in Appendix 2.

Five micro-litres from each PCR were combined and samples were quantified using capillary gel electrophoresis on a ABI 31-30 genetic analyzer. The size standard used was LIZ 500. Results were visualised and scored using the Genemarker software (Softgenetics).

4.3.4 Microsatellite performance

Microsatellite diversity was assessed by calculating the percentage polymorphic loci (P), the total number of alleles resolved (Nt), the mean number of alleles resolved per population (Na), and the mean number of effective alleles per population (Ne). The performance of microsatellite loci was investigated by testing each locus against Hardy-Weinberg equilibrium (HWE) using exact tests performed with GENEPOP 4 (Raymond & Rousset 1995; Rousset 2008). Linkage disequilibrium was also assessed for each pairwise loci x loci combination using GENEPOP 4 (Raymond and Rousset 1995, Rousset 2008). Micro-checker (van Oosterhout et al. 2004) was used to assess the possibility of null alleles at each locus within each population.

4.3.5 Genetic diversity and genetic structure

Genetic diversity within remnant populations was estimated by calculating the number of alleles (Na), the number of effective alleles (Ne), the number of private alleles, Shannon's Diversity Index (I), observed heterozygosity (Ho), the biased and unbiased measures of expected heterozygosity (He and UHe) and the inbreeding coefficient (F). All measures (omitting the number of private alleles which was the total number across all loci) were calculated as the mean (nine loci) within each population. These analyses were performed with the program GenAlEx 6.1 (Peakall & Smouse 2006). Because sample sizes were dependent upon population size, rarefaction methods (Hurlbert 1971) were undertaken to obtain an adjusted measure of genetic diversity (allelic richness, AR) and population divergence (private allelic richness, PAR). Rarefaction has been found to be effective at correcting for uneven sample size, even when sample sizes vary considerably (Pruett & Winker 2008). Rarefaction to estimate allelic variation was calculated with the program HP-rare (Kalinowski 2004; Kalinowski 2005).

Within population genetic structure was assessed by calculating the inbreeding coefficient (F) in each population and by testing for deviations from HWE. Deviation was tested for each locus independently as consistent patterns across loci within populations can give biologically informative results (Dakin & Avise 2004). Linkage disequilibrium was also assessed for each pairwise loci x loci combination. Analyses were calculated in GENEPOP 4 (Raymond & Rousset 1995; Rousset 2008). Fisher's exact test (Fisher 2006) for combining p values was calculated to

determine overall significance values for pairwise linkage disequilibrium and deviations from HWE (combined population pairwise p values).

The partitioning of genetic variability across the catchment and pairwise population differentiation was calculated following the formula outlined in Jost (2008) for the estimate of actual differentiation.

Dest = ((Ht-Hs)/(1-Hs)) * ((n)/(n-1))

The online Jost calculator SMOGD (Crawford 2010) was used for calculation. This measure was selected for its superiority in ranking differentiation when using high variability markers (Jost 2008; Heller & Siegismund 2009). An alternative measure of differentiation (F_{ST}) was calculated in GenAlEx 6.1 (Peakall & Smouse 2006) via AMOVA and overall differentiation and pairwise population differentiation were tested for significance via random permutation (999). Although the differentiation measure F_{ST} can give spurious results under certain circumstances (Jost 2008), the inclusion of the measure was used to allow comparison with the body of literature already calculated for the genus. The contribution of individual remnants to the overall allele diversity within the catchment was estimated by calculating the percentage of total variability (defined here as allele presence) represented within each population relative to the total. Pairwise and three-way combinations were calculated to determine which combination of remnants captured the greatest allelic variation.

Further investigation into the spatial distribution of diversity was undertaken by inferring clusters of K populations throughout the catchment using the program Structure 2.3 (Pritchard et al. 2000). Structure assumes both Hardy-Weinberg and linkage equilibrium in the assembling of clusters. The distribution of admixture patterns between populations can give insight into genetic origin while an estimate of the number of discrete populations can enable a retrospective estimate of fragment connectivity. A burn-in of 10000 followed by 10000 additional generations was undertaken for K ranging from 1–15. Ten iterations for each K value were conducted. I used an admixture model with correlated allele frequencies (Falush et al. 2003) without prior location data (Hubisz et al. 2009). Both the methods described

by Evanno et al. (2005) and suggested by Pritchard et al. (2000) were used to determine the most likely K. The method suggested by Prichard et al. (2000) determines K by selecting the K value with the minimal log likelihood. The method suggested by Evanno et al. (2005) computes K by the rate of change of the log-likelihood probability across K.

To determine if the levels of differentiation between populations were related to geographic distance, correlation analyses were conducted. The Mantel test was applied to determine if the two matrices (Dest & geographic distance) were significantly correlated. The mantel test was performed in Genalex 6.1 utilizing an imported pairwise Dest matrix calculated in SMOGD (Crawford 2010). The results were tested for significance by running 999 permutations.

4.3.6 Temporal analysis

The diameter at breast height (dbh (cm)) was used to estimate the relative age of the sampled individuals across the catchment. Diameter at breast height measurements have been used in other studies of trees (e.g. Rossetto et al. 2004) and in other *Eucalyptus* species as a surrogate for estimating age (e.g. George et al. 2005). While all sites within the study were within the same catchment, so subject to similar climatic variables, individual growth may be dependent on site-specific resource availability. Webb and Erskine (2003) found significant differences in the growth rate of *E. camaldulensis* in the Hunter Valley, however the upper and lower limit for dbh measurements in 14 year-old trees was only 34 cm (calculated from the girth estimate). In this study diameter at breast height categories were broad to counter error associated with growth rate.

The dbh was recorded for all sampled individuals. Individuals were then placed into one of five dbh categories (2.5–30cm, 31–50cm, 51–80cm, 81–120cm and >121cm).

An estimation of temporal genetic variability and structure was undertaken by calculating and comparing the level of genetic diversity in dbh cohorts pooled across the catchment. Because samples came from all remnants, they could not be considered as originating from a single randomly mating population; hence measures based on Hardy-Weinberg assumptions were avoided; the Shannon's Diversity Index and allelic and private allelic richness were calculated. Allelic richness and private allelic richness measures were calculated by rarefaction procedures to adjust for

sample size in the program HP-rare (Kalinowski 2004; Kalinowski 2005). Loci were then investigated independently to test the hypothesis that older age groups contained higher levels of genetic diversity. A sign test was conducted over the nine loci and a significance value (p) was determined from the binomial function n = 9, probability = 0.5.

The same set of analyses was conducted to compare dbh groups within remnants. Grouping within remnants was variable and dependant on the age-class structure within each stand. For example, in Dartbrook, there were no trees with dbh measurement less than 70 cm. Since remnants can be assumed to be in HWE, measures based on the Hardy-Weinberg assumptions were included (unbiased He, inbreeding coefficient). Again sign tests were conducted to explore the hypothesis that older groups would exhibit higher levels of genetic diversity.

4.4 Results

4.4.1 Microsatellite performance

A total of 180 alleles were detected across thirteen populations and 216 individuals. All of the loci were 100% polymorphic. The mean number of alleles per locus was consistently less than the mean number of effective alleles, indicating many of the alleles occurred at low frequency (Table 3). There was substantial variation in the number of alleles amplified between loci (4.4–11.1 mean alleles per population). Although Fischer's exact test for combining p values found significant linkage disequilibrium between 10 pairs of loci, these significant values were largely driven by highly significant results in a small number of populations. No pair of loci exhibited significant linkage disequilibrium in more than half of the remnants in which significance values could be determined. Hence, it is unlikely that any pair of loci studied were linked.

Three of the nine loci (Embra 17b, Eg1067 and Eg1028) exhibited significant p values for deviation from HWE in more than half of the populations. In all cases the deviation was positive, indicating an excess of homozygosity. These results were mirrored by those obtained from the Micro-checker software, which suggested the presence of a null allele at the loci amplified by Embra 17b, Eg1067 and Eg1028 in almost half of the populations. In this study, analysis of genetic structure (inbreeding coefficients) within populations are performed with and without microsatellite

amplified by Embra 17b, Eg1067 and Eg1028 to prevent any bias introduced by a possible overestimate of homozygosity at these loci (Dewoody et al. 2006).

4.4.2 Genetic diversity and genetic structure

The various methods of estimating population level genetic diversity and allelic richness gave similar results. Unadjusted estimates (Shannon's Diversity Index) detected the highest level of diversity in the population located at Bylong (I = 1.93) (Table 4). This outcome reflected both the high number of total alleles in addition to the high number of private alleles detected in this population. Nei's measure of unbiased expected heterozygosity (UHe) was highest in the Sandy Hollow population (UHe = 0.85) (Table 4). This indicated that although Bylong had a higher number of alleles overall, the frequency of alleles in the Sandy Hollow population was evenly distributed over a greater number of alleles. Allelic richness measures calculated with rarefaction tests detected the highest level of allelic richness in the small population at Muswellbrook (Table 4). This indicates that the population at Muswellbrook would have the highest diversity if all remnants were equally large. Muswellbrook also exhibited the highest value for private allelic richness. Overall the Muswellbrook, Sandy Hollow, Bylong and Camyr Allyn populations ranked highly in all measures of diversity and richness while Lemington Bridge, Hunterview, Redbournberry and Jefferson Park consistently ranked poorly (Table 4).

The mean population inbreeding coefficient indicated a homozygote excess in all populations except for L2. The mean inbreeding coefficient calculated with the omission of microsatellite regions amplified by Embra 17b, Eg1067 and Eg1028 detected an excess of homozygotes in all but four remnants (Table 4). There was, however, considerable variability in the inbreeding co-efficient between loci within remnants. Consistent observations of significant ($p < 0.01$) deviations from HWE across loci were detected in three remnants: Denman, Camyr Allyn and Bylong; omission of the three loci exhibiting possible null allele signals did not alter their relatively high inbreeding coefficients.

Overall differentiation (Dest) was 0.196, indicating that the majority of variability within the catchment is shared between populations and there is only a small proportion (Dest = 0.196) that differentiates them. This was also indicated by the low FST (0.05) which was significant ($p = 0.001$). There was a range of

differentiation levels between pairwise comparisons. The least population differentiation (Dest = 0.00) was between Kingdom Ponds (KP) and Jefferson Park (JP) while the greatest differentiation (Dest = 0.33) was recorded between Hunterview (H) and Lemington Bridge (L2) (Table 5).

Camyr Allyn (CA) contained the highest percentage of the total alleles detected within the study (48%) (Figure 13a). The optimum combination of two populations to account for allele composition was the Bylong (B) and Camyr Allyn (CA) populations. Only 27% of alleles found in the catchment were not represented in these combined populations (Figure 13b). A further 7% were captured by including Muswellbrook population (Figure 13c).

The cluster assignment in Structure (Figure 14) detected high levels of admixture and high within population genetic diversity and was consistent with the low overall Dest indicating extensive genetic overlap between most remnants. The optimum number of K determined by the minimal value of the log likelihood probability was eight however, the alternate method (Evanno et al. 2005) calculated K = 7. All individuals displayed multiple clusters indicating genetic admixture. In the majority of populations, admixture patterns were not consistent. However, in a number of populations admixture pattern were consistent in a large number of invidiuals within the population (e.g. Bylong and Hunterview).

The Mantel test detected no significant correlation between geographic distance and genetic distance indicating population proximity accounted for only a minor component of population differentiation (Figure 15).

4.4.3 Temporal analyses

All diversity and allelic richness measures calculated for pooled dbh samples across the catchment revealed that the highest measures were found in the oldest cohort. Rarefied measures, however, also detected relatively high levels of allelic richness in the youngest cohort (Table 6). The sign test applied across loci detected significant increases in genetic variability ($p < 0.005$, Bonferroni adjusted) in a number of pairwise cohort comparisons for all genetic diversity and allelic richness measures (Tables 7). The Shannon's diversity was significantly higher in the 51-80, 81-120 and >120 dbh cohort than the 31-50 dbh cohort and allelic richness was significantly higher in the 81-120 cohort compared with the 31-50 dbh cohort. Allelic richness

was also significantly higher in the >120 dbh cohort compared with the 81-120 dbh cohort. Private allelic richness was significantly higher in the >120 dbh compared with both the 81-120 and 51-80 dbh cohorts.

Within population analyses indicated that several populations (Lemington, Dartbrook, Camyr Allyn, Bylong, Hunterview and Sandy Hollow) exhibited lower levels of genetic diversity in the younger group for all measures (Table 8). Four populations also exhibited higher genetic diversity in the younger cohort for all genetic diversity measures (Muswellbrook, Redbournberry, Lemington Bridge and Kingdom Ponds); however, statistical significance based on the premise of a directional trend was less prevalent among increased measures. Of the 72 comparisons of genetic variability calculated (6 per population), 43 measured detected decreases and 16 of these were significant ($p<0.05$). Of the 29 measurements that detected increases, only five were significant ($p<0.05$).

Seven of the 12 populations studied exhibited increased inbreeding coefficients between the older and younger cohorts, however, no increases were statistically significant. Three populations exhibited both declines in genetic variability over all measures and increased inbreeding coefficients, however, an equal number exhibited decreased inbreeding coefficients in combination with genetic declines. A statistically significant decreased inbreeding coefficient was detected at the Carrington Billabong site (Table 8).

4.5 Discussion

4.5.1 Levels of genetic diversity

The mean level of neutral genetic variability (He = 0.74) detected in the fragmented remnant populations of *E. camaldulensis* was similar to other widespread eucalypt species (e.g. *E. globulus*, Steane et al. 2006) and similar to the levels collected for the same species over a much larger geographic range (He = 0.77 in *E. camaldulensis* subsp. *camaldulensis*, Butcher et al. 2009). This suggests that the pre-European distribution of *E. camaldulensis* within the Hunter Valley may have been historically more extensive and continuous. The high levels of genetic diversity detected also indicate that fragmentation has not resulted in genetic decline. However, when pooled samples were grouped by dbh, the trees with the greatest dbh exhibited the highest diversity. Although not all comparisons were statistically significant the trend

of increasing diversity with dbh cohort was consistent for all of the diversity and allelic richness measures used. The exception, the high level of genetic variability in the youngest cohort, was the result of including young trees sourced from outside the catchments in this group.

Lower levels of genetic variability in the younger cohorts were also evident when intra-population comparisons between dbh cohorts were made indicating that population level genetic diversity was positively impacted by the inclusion of old trees. Although within-population samples were limited by the available data, six of the 12 populations exhibited decreases in all measures of genetic diversity and all of these populations had a significant decline in at least one measure. To gain this result the majority of loci were required to exhibit the same directional trend lending strength to the likelihood that this was a biological phenomenon in these populations rather than a chance statistical artifact.

While no differences in genetic diversity between age cohorts have been detected in some species (e.g. *E. globulus*, Jones et al 2007), higher diversity has been found in older plants in other studies of forest trees (Baucom et al. 2005; Farwig et al. 2008; Rathmacher et al. 2009) and in other plant species (Cruse-Sanders & Hamrick 2004). High diversity in the older cohorts compared with young have been attributed to a range of factors including: the survival of more heterozygous individuals over time (Cruse-Sanders & Hamrick 2004), founder effects (Baucom et al. 2005), fragmentation related genetic drift (Cruse-Sanders & Hamrick 2004; Baucom et al. 2005) altered mating patterns (Farwig et al. 2008) and temporal variability in recruitment sources. Any or all of these factors may potentially interact in fragmented populations. Determining if lower levels of genetic variability result from fragmentation is problematic.

Where low levels of genetic variability result from sampling earlier selection genetic arrays (i.e. more homozygous), low genetic variability in the younger cohort should be associated with higher inbreeding coefficients. No significantly higher inbreeding coefficients were detected in the younger cohort in this study. Increased inbreeding coefficients may also be expected when lower levels of genetic variability result from modified breeding mechanisms. However, the literature indicates that reproductive attributes may be modified in fragmented habitats without impacting upon the inbreeding coefficient.

Direct assessments of one or more reproductive attributes have been demonstrated to differ in fragmented habitats (e.g. Borralho & Potts 1996, Mimura et al 2009), but studies in which the inbreeding coefficients remain unaffected are common (Dayanandan et al. 1999; Baucom et al. 2005). The absence of a significantly increased inbreeding coefficient in fragmented populations may not, therefore, be a reliable indicator that breeding has not been modified. In populations where the inbreeding coefficient is unaffected it is possible that mating patterns have been altered, but that insufficient time has passed for the inbreeding coefficient to be effected. Alternatively post-dispersal mechanisms might reduce the impact of altered mating patterns, for example, an increase in self pollination may occur, but the amount of outcrossing might still be adequate because only a small portion of seeds (outcrossed) succeed (Krauss et al. 2007). In *Eucalyptus* species where many seeds are produced but only a few germinate and survive long enough to become part of the population, this may be particularly relevant.

Lower levels of genetic variability in the younger cohort may also be caused by genetic drift. Unlike altered mating patterns, genetic decline attributable to genetic drift is relatively easy to discern (e.g. Young et al. 1996; Leimu et al. 2006). Genetic drift is additional to the initial bottleneck effect and is signaled by decreases in genetic variability that may take multiple generations to accumulate (Leimu et al. 2006). Genetic drift is more pronounced in small populations and is reinforced by a lack of inter-population gene-flow. Given a sufficient number of generations and adequate isolation, genetic drift should result in increased genetic differentiation (Ellstrand & Elam 1993). Similar to the study conducted by Baucom et al. (2005), many of the remnants studied here are likely to have suffered a large reduction in the number of individuals and declines in genetic variability in the younger cohorts is most likely to result from a smaller number of reproductive trees contributing seed to the next generation. At sites in which a relatively large number of reproductive trees reside, declines in genetic variability are likely to relate to the stochastic availability of safe recruitment sites. A reduced number of safe recruitment sites results in a decline in the number of parent trees that are able to contribute seed to the next generation. In *E. camaldulensis*, safe sites require inundation, but their availability may also be reduced due by competitive interactions. It has been previously demonstrated by Dexter et al. (1970) that competitive interactions between grass and *E. camaldulensis*

seedlings is a significant deterrent of success at the seedling stage for *E. camaldulensis*. All *E. camaldulensis* remnants are characterised by both modified hydrologies and extensive weed infestations.

Of the six populations that did not exhibit reduced genetic variability, four populations indicated increased diversity for all diversity measures (Muswellbrook and Redbournberry, Lemington 2 and Kingdom Ponds). In both Muswellbrook and Redbournberry populations, restoration planting has been undertaken which has included the planting of *E. camaldulensis* seedlings sourced from outside the Hunter Valley. In this study, sampling focused on the naturally recruited population however, outsourced individuals were sampled in the Muswellbrook population. Despite a very small population (n = 11) Muswellbrook exhibited the third highest level of genetic diversity (I = 1.87). This indicates that introducing outsourced trees in revegetation programs will, and has, had a profound impact on the genetic diversity of remnants. There is no doubt the restoration plantings were responsible for the increased genetic diversity at Muswellbrook; all of the private alleles driving inflated diversity measured were detected in the youngest cohort.

At Redbournberry several planted (as opposed to naturally recruited) individuals may have been inadvertently included; it was difficult to discern the planted from the naturally recruited in some parts of the population. Further, the outsourced individuals were much older than those at Muswellbrook, and, although no flowers were observed, it is possible that very young saplings may have eventuated from crosses between outsourced and naturally recruited individuals. Unlike Muswellbrook however, the Redbournberry population had a very low number of private alleles (n = 1) and although the increase in diversity was large, the overall levels of genetic diversity in the population were still low (I = 1.53) given the population size (n = 77). This suggested that outsourced individuals were not included in the census, as they would have increased the number of private alleles. An alternate hypothesis is that increases in genetic diversity at Redbounberry (and Kingdom Ponds and Lemington2) may have resulted from gene-flow.

Gene-flow, offering amelioration of genetic decline might be substantial in the riparian habitat of *E. camaldulensis* (but see Butcher et al. 2002). Pollination may also facilitate gene-flow. Although the species is anecdotally insect pollinated, researchers have highlighted the capacity of insects to move pollen over large distances. A recent

study detected high levels (>50%) of pollen sourced from outside remnant populations in *Eucalyptus wandoo* (Byrne et al. 2008), while hybrid F1 individuals of *Eucalyptus nitens* was detected >1.6km into natural stands (Barbour et al. 2005). Further, birds and bats may contribute to pollen movement over larger distances (Southerton et al. 2004a). These studies warn us that assumptions based on the supposed inability of a pollinator (or pollinators) to move pollen may be unfounded. Researchers are also finding that the response of pollinators to isolated trees may be contrary to what we expect, e.g. higher levels of gene-flow and lower inbreeding in small fragments in *Embothrium coccineum* (Mathiasen et al. 2007), high genetic diversity in fragments due to seed deposition by bats (Aldrich et al. 1998) and high levels of outcrossing in isolated *Eucalyptus leucoxylon* (Ottewell et al. 2009). A more detailed understanding of effective pollinators and their abundance in the landscape would aid our understanding of dispersal in this species.

4.5.2 Distribution of microsatellite diversity

The values calculated here (Dest = 0.19, F_{ST} = 0.05) attributed the majority of variability to within populations, yielding results in agreement with the literature for other woody species (Hamrick et al. 1993). Although not directly comparable the overall level of genetic differentiation was similar to that found in other widespread *Eucalyptus* species, e.g. F_{ST} in *E. globulus*, F_{ST} = 0.08 (Jones et al. 2007) and in previously reported measurements for *E. camaldulensis,* F_{ST} = 0.06 (Butcher et al. 2009). This suggests that, across the catchment, fragmentation of habitat has not resulted in remnant differentiation and that the majority of remnants are not (or were not) reproductively isolated. However, although the global level of differentiation and the between population pair differentiation was low, both the global (F_{ST}) and the majority of population pairwise differentiation values were found to be statistically significant based on F_{ST} pairwise comparisons.

The interpretation of differentiation statistics is problematic because the data incorporate multiple generations and is impacted significantly by the genetic content of population founders (Lowe et al. 2005). A lack of differentiation can be observed but be representative of pre-fragmentation population continuity rather than contemporary gene-flow. Further, if the same source(s) founded multiple populations then detection of contemporary gene-flow would be impossible without age-class

analysis and then, genetic differentiation could only be detected if populations have had sufficient time to become genetically distinct.

High levels of genetic differentiation are also difficult to interpret; particularly when remnants are different sizes. High genetic differentiation is commonly interpreted as reproductive isolation. However, in this study, differentiation was detected between small remnants and large remnants where the smaller remnant has almost all the alleles of a larger remnant, but the larger had many alleles that were not found in the smaller remnant (Appendix 3).

4.5.3 Conservation and management implications

The predominant lack of population differentiation indicates there is only a small amount of genetic variability that is not detected throughout the catchment. The data presented indicate that the protection of a few populations is likely to capture the majority of variation within the catchment. Results show that by combining three of the populations with the highest diversity measures (Bylong, Camyr Allyn and Muswellbrook), 80% of the total variability detected in this study can be captured. However conservation based on such results should be carefully considered.

It has been shown that neutral genetic diversity does not necessarily reflect quantitative variability (Reed & Frankham 2003). This may have serious consequences in the face of rapid environmental change. Also, as Holsinger et al. pointed out (1999), rare alleles, which increase diversity measures, are unlikely to be important in the provision of adaptive traits. Exclusion of rare alleles or the selection of diversity measures that downplay the importance of rare alleles should be considered.

High genetic diversity might also occur in a number of ways, some of which may not be consistent with conservation objectives. The population at Bylong exhibited a very high number of rare alleles relative to other populations. High numbers of private alleles may indicate introgression (Butcher & Williams 2002). This could be indicative of hybridisation between the nearby and closely related *E. tereticornis*. Fragmentation has been demonstrated to affect the rate of hybridization in eucalypt species (Field et al. 2008; Field et al. 2011). I observed that the capsule phenotype at the Bylong site was extremely variable, unlike at other sites (pers.obs. Nov09). *Eucalyptus* species hybridize readily and hybridisation has been documented in

fragmented populations of *Eucalyptus benthamii* (Butcher et al. 2005) and suggested as a possible explanation for atypical outcrossing rates (Peters et al. 1990). In the absence of suitable partners, alternative viable hybrid matches can be made. This reproductive elasticity may increase sustainability in small patches where suitable sister species are available, however such spontaneous hybridization events, occurring as a result of anthropogenic disturbance, throws up some interesting questions in relation to species conservation.

At the Muswellbrook site, where planted trees were sampled, six private alleles were recorded. This was a very high number relative to the other populations studied. The long-term outcomes of the introduction of new genetic material into natural populations are uncertain. Outbreeding depression has been documented in *E. camaldulensis* in the progeny of dissimilar parents (Butcher & Williams 2002). Consequently introduced genetic material (and the increased genetic variability associated with it) may not necessarily increase population sustainability. To adequately assess the benefit of increased diversity resulting from outsourced planted trees, genetic surveys should be accompanied by fitness assessments.

And finally, as indicated by the higher genetic variability in the older cohort in some populations, population sustainability more broadly should also be assessed. In *E. camaldulensis* even aged stands can house relatively high levels of genetic diversity but ground surveys clearly show that long-term self-sustainability in these populations is unlikely due to a lack of regeneration. Alternatively, populations with low diversity may have long-term potential. The population at Redbournberry exhibited low levels of genetic diversity but also exhibited a temporal increase in genetic diversity. Historical photographs taken at this site (See Chapter 3, Figures 5 and 7) show that few trees existed at the site in early 1900, indicating that the site was either founded by a small number of individuals, or individuals in an initial population had been cleared leaving a small number of trees. Either way, a founder effect is likely to have resulted in low levels of genetic diversity in early 1900, however, increases in diversity indicate gene-flow may be occurring and subsequently enriching local genetic diversity in these populations.

4.6 Conclusion

While comparing intact and fragmented habitat may give more definitive results, the results here suggest that although high levels of genetic variability were detected across the catchment, genetic variation may be lower in the younger cohorts of remnant *E. camaldulensis* populations. However there were notable exceptions. Introduction of new genetic material from outside the region has had a detectable influence on neutral genetic variability (e.g. Muswellbrook) while several populations with low levels of genetic variability exhibited higher levels in the younger trees compared with older trees; patterns that are consistent with genetic recovery.

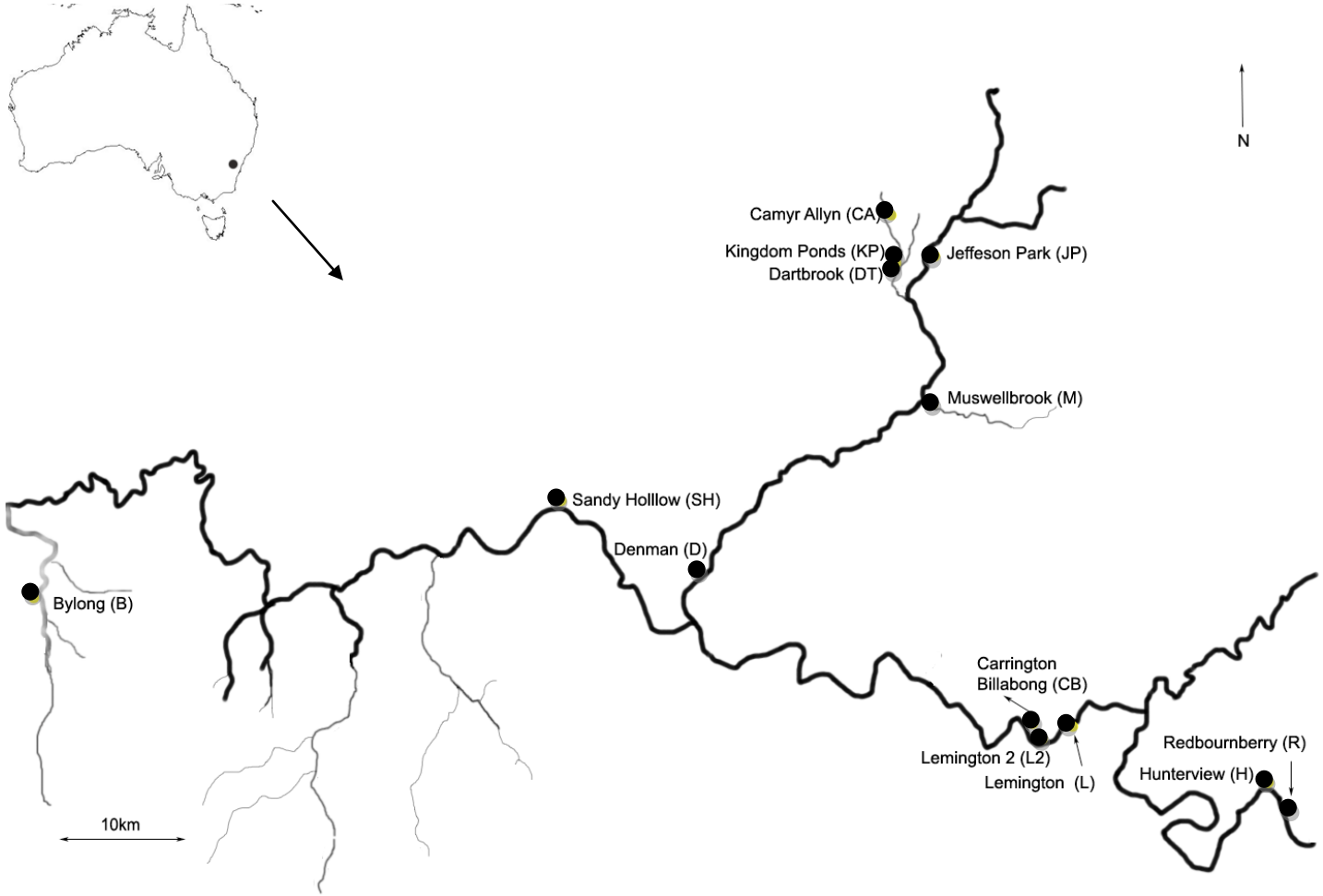


Figure 12. *Eucalyptus camaldulensis* study sites in the Hunter Valley, NSW, Australia

Table 3. Microsatellite performance; all loci were 100% polymorphic

Locus	Nt	Na	Ne
Embra 8	22	10.62	7.36
Embra 20	19	9.00	5.30
Embra 17a	22	9.00	5.42
Embra 17b	32	11.08	6.38
Embra 2	11	4.39	2.48
Eg1062	15	6.31	3.83
Eg1096	10	4.77	2.49
Eg1067	32	11.00	6.50
Eg1028	17	6.69	4.26

Nt = total number of alleles across all populations, Na = mean number of alleles per population, Ne = mean number of effective alleles per locus

Table 4 Genetic parameters for 13 remnant populations of *Eucalyptus camaldulensis* in the Hunter Valley catchment area

Pop	N	n	Na	Ne	Pa	I	He	UHe	AR	PAR	F	F*
D	122	20	8.44	5.36	1	1.79(6)	0.78(4)	0.80(4)	5.26(6)	0.30(8)	0.21	0.21
M	11	10	8.56	5.98	6	1.87(3)	0.79(2)	0.83(2)	6.02(1)	0.91(1)	0.09	0.01
L	52	22	8.89	4.94	1	1.78(7)	0.77(6)	0.79(7)	5.16(8)	0.23(9)	0.14	0.08
H	152	18	7.11	3.73	0	1.44(11)	0.66(11)	0.68(12)	4.28(13)	0.11(13)	0.14	0.08
R	77	18	7.00	4.19	1	1.53(10)	0.70(10)	0.72(10)	4.52(11)	0.15(12)	0.07	-0.03
CA	700	26	11.44	6.07	7	1.92(2)	0.77(5)	0.79(8)	5.47(3)	0.40(4)	0.15	0.13
L2	15	6	4.56	3.16	1	1.21(13)	0.6(13)	0.67(13)	4.33(12)	0.31(7)	-0.13	-0.12
DT	61	18	9.33	5.25	4	1.80(5)	0.77(7)	0.79(6)	5.24(7)	0.32(6)	0.19	0.07
B	125	30	11.78	6.06	10	1.93(1)	0.78(3)	0.79(5)	5.46(4)	0.58(3)	0.18	0.13
SH	55	12	7.78	5.56	6	1.84(4)	0.81(1)	0.85(1)	5.68(2)	0.81(2)	0.16	0.15
KP	23	10	7.11	4.78	0	1.67(9)	0.76(8)	0.80(3)	5.28(5)	0.21(10)	0.02	0.00
C	112	20	8.33	5.00	1	1.72(8)	0.74(9)	0.76(9)	5.06(9)	0.33(5)	0.09	-0.01
JP	31	6	4.89	3.53	0	1.32(12)	0.66(12)	0.72(11)	4.56(10)	0.16(11)	0.12	0.04
Mean	-	-	8.09	0.49	2.92	1.68	0.74	0.77	5.10	0.37	0.11	0.06

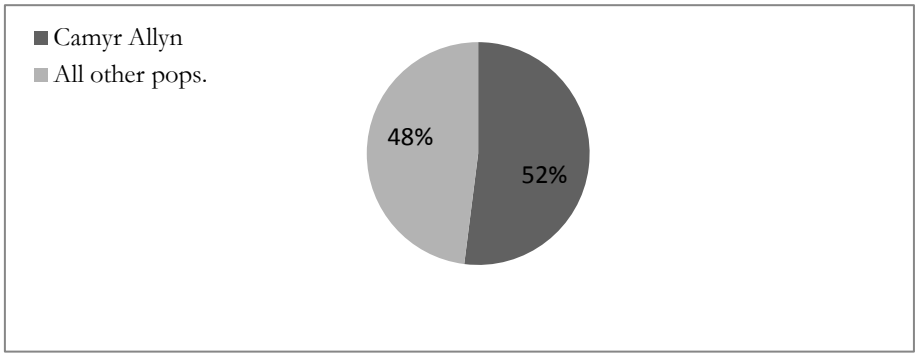
N = population size, n = number individuals sampled, Na = mean number of alleles per locus, Ne = mean number of effective alleles per locus, Pa = total number of private alleles across all loci, I = Shannon's Diversity Index, He = expected heterozygosity, UHe = unbiased expected heterozygosity, AR = allelic richness, PAR = private allelic richness, F = mean inbreeding co-efficient across loci, F* = inbreeding coefficient calculated without loci Embra 17b, Eg1067 and Eg1028. Rankings for diversity statistics follow in brackets. See Table 2 for expanded population titles

Table 5. Genetic differentiation (Dest) between remnant *Eucalyptus camaldulensis* stands in the Hunter Valley calculated following Jost (2008) under diagonal and pairwise FST.

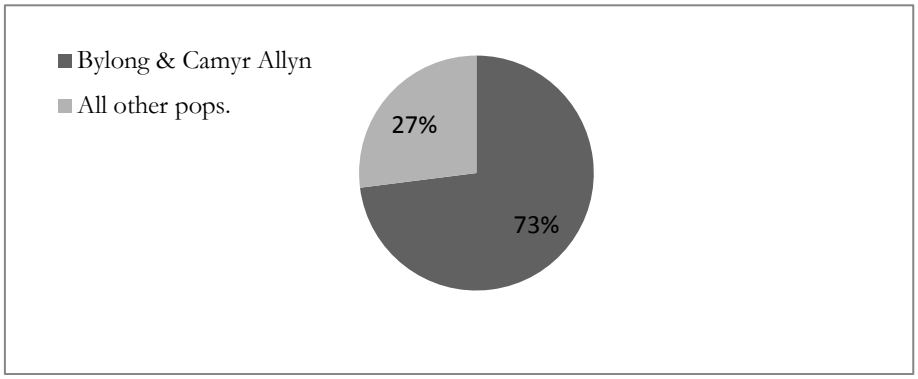
Pop.	D	M	L	H	R	CA	L2	DT	B	SH	KP	C	JP
D	0.00	0.04	0.04	0.07	0.04	0.03	0.09	0.02	0.06	0.06	0.04	0.05	0.05
M	0.15	0.00	0.03	0.07	0.05	0.01	0.07	0.04	0.02	0.04	0.03	0.03	0.03
L	0.12	0.08	0.00	0.08	0.06	0.03	0.11	0.04	0.05	0.05	0.05	0.04	0.03
H	0.16	0.13	0.18	0.00	0.09	0.05	0.18	0.05	0.09	0.10	0.08	0.09	0.08
R	0.10	0.18	0.16	0.23	0.00	0.04	0.13	0.05	0.06	0.10	0.06	0.02	0.06
CA	0.07	0.01	0.07	0.14	0.11	0.00	0.07	0.02	0.03	0.05	0.03	0.03	0.04
L2	0.16	0.12	0.17	0.33	0.31	0.14	0.00	0.10	0.07	0.09	0.07	0.09	0.13
DT	0.04	0.10	0.09	0.12	0.12	0.06	0.24	0.00	0.05	0.04	0.02	0.03	0.02
B	0.21	0.04	0.23	0.27	0.16	0.11	0.26	0.13	0.00	0.06	0.03	0.03	0.05
SH	0.17	0.09	0.10	0.28	0.30	0.08	0.16	0.05	0.14	0.00	0.04	0.07	0.06
KP	0.12	0.04	0.12	0.18	0.12	0.03	0.08	0.02	0.08	0.06	0.00	0.04	0.02
C	0.13	0.09	0.10	0.23	0.03	0.07	0.27	0.05	0.09	0.12	0.06	0.00	0.03
JP	0.11	0.01	0.03	0.06	0.07	0.07	0.25	0.06	0.12	0.06	0.00	0.02	0.00

Pop. = population. See Table 2 for expanded population titles. Shaded squares are those comparisons not significant (P > .05) based on 999 permutations

a) Camyr Allyn (dark grey) and all other populations combined (light grey)



b) Bylong and Camyr Allyn combined (dark grey) and all other populations combined (light grey)



c) Bylong, Camyr Allyn and Muswellbrook combined (dark grey) and all other populations combined (light grey)

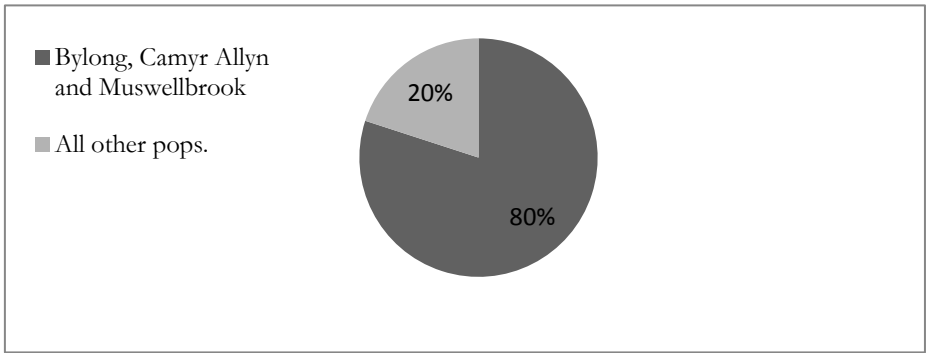


Figure 13. Percentages of detected allelic variability present in combinations of *Eucalyptus camaldulensis* remnants in the Hunter Valley

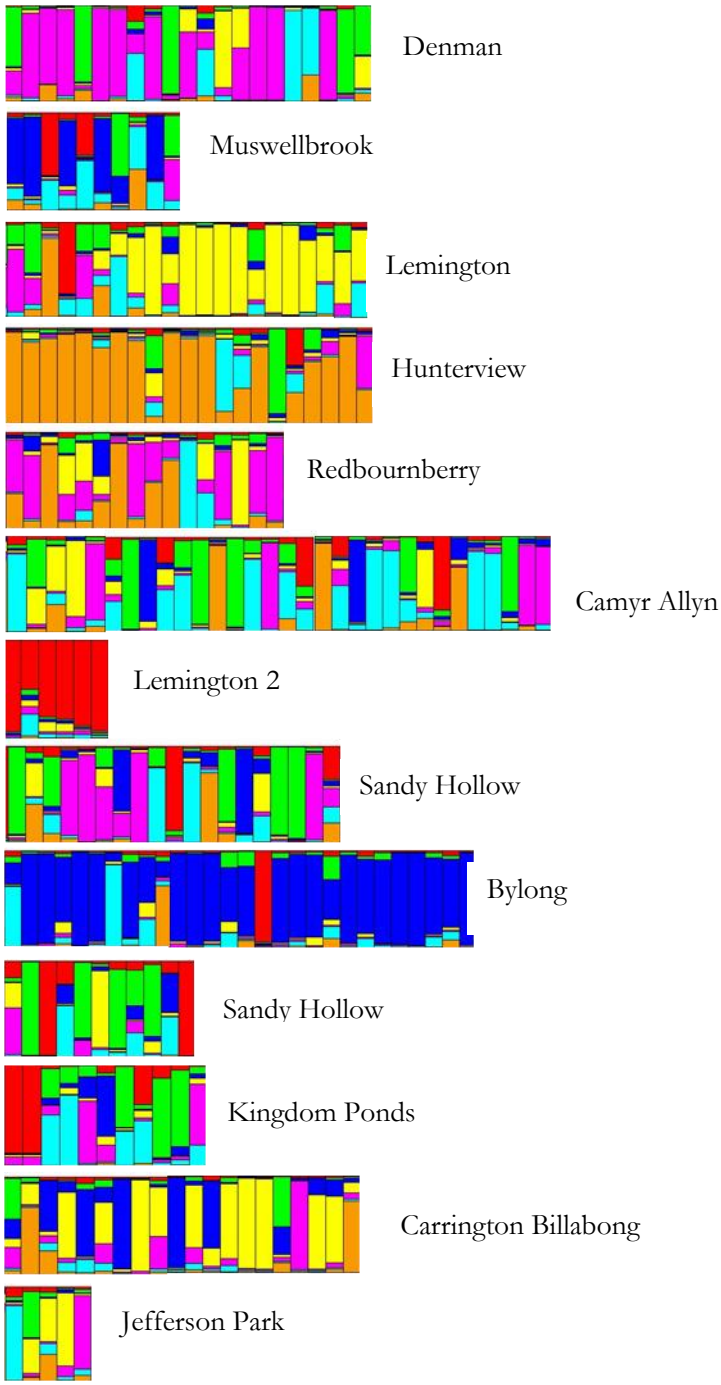


Figure 14. Graphical representation of cluster assignment for K=7. Each bar represents an individual *Eucalyptus camaldulensis* tree and each colour denotes an inferred cluster. Individual trees are grouped by population

Table 6. Genetic diversity and allelic richness measures of *Eucalyptus camaldulensis* trees in the Hunter Valley. Trees were pooled from 13 populations and placed in dbh cohorts. Diveristy measures were averaged over nine loci. Rarified parameters were determined by the lowest sample size (n=30) in each group.

dbh (cm)	n	AR	PAR	I
2.5–30	30	11.46	0.94	1.923
31–50	27	10.05	0.18	1.824
51–80	32	11.44	0.56	1.969
81–120	51	12.27	0.92	2.102
>120	62	13.36	1.24	2.215

n = sample size, AR = rarefied allelic richness, PAR = rarefied private allelic richness, and I = Shannon's Diversity Index. Columns are shaded to indicate high (dark) and low (light) relative values for each measure

Table 7. Significant p vales calculated from the binomial distribution n=9 (loci) for the probability (p=0.5) of increased genetic diversity (a) I = Sannon's Diversity Index,b) AR = allelic richness and c) PAR = private allelic richness) between *Eucalyptus camaldulensis* trees grouped by dbh (cm) pooled from 13 populations across the Hunter Valley. Bonferroni adjustment was made for multiple comparisons within each genetic parameter.

a)

dbh (cm)	2.5–30	31–50	51–80	81–120	>120
2.5–30	0				
31–50	ns	0			
51–80	ns	0.002	0		
81–120	ns	0.002	ns	0	
>120	ns	0.002	ns	ns	0

b)

dbh (cm)	2.5–30	31–50	51–80	81–120	>120
2.5–30	0				
31–50	ns	0			
51–80	ns	ns	0		
81–120	ns	0.002	ns	0	
>120	ns	ns	0.002	ns	0

c)

dbh (cm)	2.5–30	31–50	51–80	81–120	>120
2.5–30	0				
31–50	ns	0			
51–80	ns	ns	0		
81–120	ns	ns	ns	0	
>120	ns	ns	0.002	0.002	0

Table 8. A comparison of genetic diversity between *Eucalyptus camaldulensis* trees grouped by diameter at breast height in recently fragmented populations in the Hunter Valley

Pop	n (dbh cm)	Ne	I	He	UHe	AR	APR	F
Bylong	11(15-80)	4.25(0.59) ↓	1.57(0.14) ↓	0.716(0.046) ↓	0.75(0.049) ↓	5.21 ↓	2.14 ↓	0.09(0.09) ↓
	11(>81)	6.09(1.03)	1.88(0.16)	0.791(0.036)	0.831(0.04)	6.3	3.23	0.21(0.07)
Carrington	8 (15-100)	4.58(0.81) ↑	1.54(0.18) ↓	0.70(0.07) ↓	0.75(0.07) ↓	4.59 ↑	1.87 ↑	0.00(0.10) ↓
Billabong	12(>100)	4.57(0.66)	1.62(0.15)	0.74(0.04)	0.77(0.04)	4.45	1.72	0.08(0.07)
Camyr Allyn	13(30-100)	4.90(0.80) ↓	1.69(0.18) ↓	0.73(0.06) ↓	0.76(0.06) ↓	6.03 ↓	2.28 ↓	0.10(0.07) ↓
	13 (>100)	6.28(1.08)	1.88(0.19)	0.79(0.04)	0.82(0.05)	6.61	2.86	0.12(0.05)
Denman	12 (15-80)	4.51(0.53) ↑	1.61(0.14) ↑	0.77(0.04) ↑	0.77(0.05) ↓	4.12 ↓	1.83 ↓	0.24(0.09) ↑
	7(>80)	4.47(0.55)	1.59(0.12)	0.75(0.03)	0.81(0.03)	4.38	2.09	0.13(0.12)
Dartbrook	5 (70-100)	3.46(0.45) ↓	1.31(0.12) ↓	0.68(0.04) ↓	0.75(0.05) ↓	3.13 ↓	1.45 ↓	0.14(0.09) ↑
	13 (>100)	5.53(0.65)	1.81(0.17	0.77(0.04)	0.80(0.05)	3.57	1.89	0.12(0.06)
Hunterview	8 (20-70)	3.13(0.43) ↓	1.21(0.17) ↓	0.61(0.07) ↓	0.65(0.08) ↓	3.63 ↓	1.28 ↓	0.15(0.086) ↑
	9(>100)	3.76(0.52)	1.43(0.15)	0.68(0.05)	0.72(0.05)	4.08	1.73	0.07(0.08)
Kingdom	4(70-100)	4.02(0.42) ↑	1.41(0.09) ↑	0.73(0.03) ↑	0.86(0.04) ↑	3.25 ↑	2.1 ↑	-0.07(0.11) ↓
Ponds	5(>100)	3.43(0.34)	1.36(0.10)	0.68(0.04)	0.76(0.04)	2.86	1.71	-0.02(0.09)
Lemington	11(20-100)	3.67(0.40) ↓	1.45(0.12) ↓	0.70(0.04) ↓	0.74(0.04) ↓	4.52 ↓	1.53 ↓	0.02(0.08) ↓
	10(>100)	5.23(0.62)	1.77(0.14)	0.78(0.04)	0.81(0.04)	5.45	2.46	0.17(0.05)
nMuswellbrook	6 (5-70)	4.85(0.62) ↑	1.62(0.14) ↑	0.75(0.05) ↑	0.82(0.05) ↑	3.14 ↑	2.26 ↑	0.04(0.08) ↑
	4 (>100)	0.368(0.55)	1.31(0.14)	0.67(0.05)	0.77(0.06)	2.93	2.06	-0.09(0.18)
Redbournberry	13 (15-50)	4.28(0.60) ↑	1.51(0.17) ↑	0.71(0.06) ↑	0.74(0.06) ↑	3.23 ↑	1.54 ↑	0.06(0.08) ↑
Bridge	5(>80)	2.94(0.34)	1.15(0.15)	0.60(0.07)	0.66(0.08)	2.93	1.23	-0.16(0.07)
Sandy Hollow	5 (60-100)	3.74(0.40) ↓	1.42(0.10) ↓	0.71(0.03) ↓	0.80(0.03) ↓	2.99 ↓	1.87 ↓	0.13(0.05) ↑
	5(>100)	4.53(0.48)	1.56(0.10)	0.76(0.03)	0.86(0.02)	3.24	2.13	-0.01(0.11)
Lemington	3 (20-60)	2.90(0.40) ↑	1.10(0.14) ↑	0.59(0.06) ↑	0.68(0.07) ↑	1.68 ↑	0.95 ↑	-0.21(0.07) ↑
Bridge	4(>100)	2.03(0.30)	0.64(0.12)	0.42(0.01)	0.63(0.13)	1.63	0.9	-0.35(0.25)

n (dbh) = sample number in dbh (cm) class, Ne = number of effective alleles, I = Shannon's diversity index, He = expected heterozygosity, UHe = Nei's unbiased measure of heterozygosity, AR = rarefied allelic richness and APR = rarefied private allelic richness, F = inbreeding coefficient. Shaded values are significant ($p < 0.05$). Arrows indicate if the measure is increasing (arrow up) or decreasing (arrow down) between the younger and older dbh category

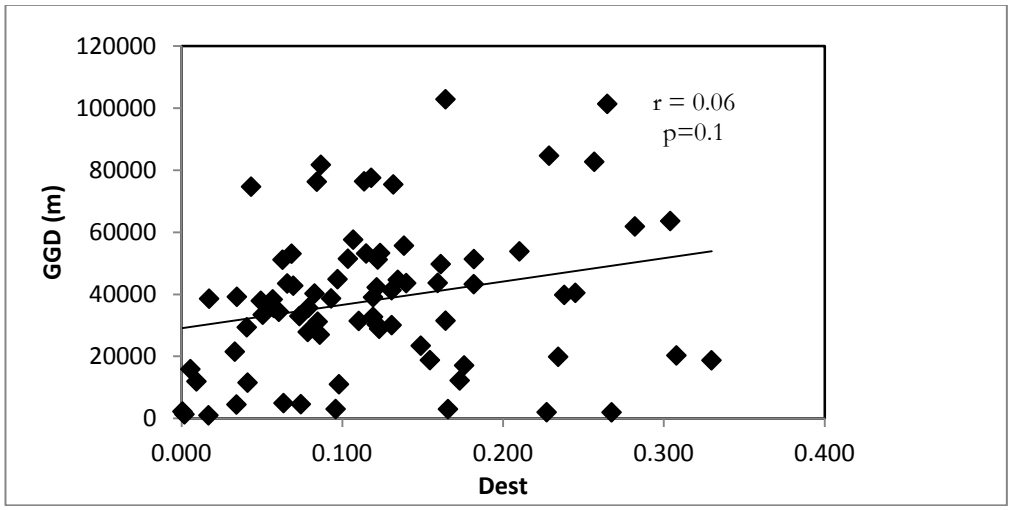


Figure 15. The correlation between the level of differentiation (Dest) between remnant *Eucalyptus camaldulensis* populations and geographic distance (GGD = geographic distance in meters) via the Mantel test. p = significance of correlation based on 999 permutations.

5 Population viability in episodic recruiters: exploring the correlation between genetic and non-genetic attributes of *Eucalyptus camaldulensis* Dehnh. *camaldulensis* (Myrtaceae) populations

5.1 Abstract

The aim of this research was to compare genetic and non-genetic factors in remnant populations of *Eucalyptus camaldulensis*, a dominant riparian and floodplain species in Australia whose seed germination and seedling survival is dependent upon habitat inundation. I used nine microsatellite markers to characterise genetic diversity and genetic structure within nine remnant populations in the Hunter Valley region of Australia. I also incorporated a range of non-genetic parameters including population size, tree density, demographic structure, stand condition and the distance between each population and their closest neighbour. Contrary to meta-analyses to date, population size was not significantly correlated with any measure of genetic diversity. Rather, expected heterozygosity was negatively correlated with the edge to area ratio ($r = -0.56$, $p = 0.06$) and density ($r = -0.76$, $p = 0.01$). Density and edge to area ratio were also significantly positively correlated with the number of individuals in the 2.1-30 age class ($r = 0.64$, $p = 0.03$) and negatively correlated with older age classes. This suggests that populations with high edge to area ratios, high densities, and higher numbers of young trees may be less genetically variable than populations with opposite traits.

Key words: genetic diversity, fragmentation, age-class structure, population viability

5.2 Introduction

The maintenance of genetic variability is critical for population viability (Fisher 1930; Frankel & Soulé 1981); however, the link between genetic variability and population viability is rarely confirmed. Population viability is an inclusive term describing the sustainability of a population based on a range of genetic and non-genetic (environmental, demographic) factors that indicate the likelihood that the population will be able to persist into the future. Although the importance of genetic factors in

the viability of fragmented populations is not contested (i.e. higher evolutionary potential, the relative high fitness of genetically heterozygous progeny) in some species, non-genetic factors may supersede genetic factors (Lande 1988; Menges & Dolan 1998) resulting in an incongruity between environmental and genetic aspects of population viability. This incongruity may be especially pronounced in species in which critical life stages are dependent on particular environmental cues, such as flooding.

Populations of species with a strong dependence on environmental cues occur within habitats where these requirements are met. However, the suitability of particular habitats is likely to vary over time (Lande 1988; Menges & Dolan 1998), both as a result of natural climatic stochasticity and anthropogenic landscape modification (Saunders et al. 1991; George et al. 2005; Honnay & Jacquemyn 2007). In some cases modification (e.g. disturbance) may promote regeneration (e.g. *Macadamia integrifolia*, Neal et al. 2010); in others, landscape modification may render once suitable habitat unsuitable. Where suitable conditions are not maintained, recruitment failure may ensue. Such populations are no longer environmentally viable and are characterised by skewed or even-aged age-class structure (e.g. *Jupipus communis*, García et al. 1999) with scant occurrences of recruitment. Oostermeijer et al. (1994) termed such populations 'regressive'.

Regressive population structure, however, may also be caused by genetic depauperacy, particularly in small populations where restricted receipt of pollen impacts upon recruitment (Lamont et al. 1993; Groom 1998). Thus regressive population structure may be caused by genetic, rather than environmental factors. Moreover, genetic and non-genetic factors may act synergistically, resulting in ever decreasing overall population viability; termed the extinction vortex, this has been widely discussed in the literature (Gilpin & Soule 1986; Lamont et al. 1993; Tanaka 2000; Scott & Gross 2004). Alternatively, synergisms between non-genetic and genetic factors may result in greater population viability (e.g. the effect of population growth on inbreeding depression, Mills & Smouse 1994; the variable impact of environment on the extent of inbreeding depression, Falk & Holsinger 1991; Schemske 1996; Reed et al. 2002). The complicated nature of the interaction between non-genetic and genetic factors is of particular interest to conservation biology. The endless unique interactions between a particular species and its environment, and the

wide range of fragmentation effects possible, however, render predictions of these interactions difficult (Young et al. 1996). Hence exploring these correlations explicitly, particularly in species with high environmental dependency, is critical for comprehensive conservation of populations.

Eucalyptus camaldulensis is a widespread species in Australia occurring along river margins and adjacent floodplains (Boland et al. 2006). Several of its life cycle stages are dependent upon particular environmental variables. Specifically, recruitment and survival is tempered by water availability (Roberts & Marston 2000); the adults are relatively drought tolerant, probably due to their capacity to access groundwater reserves (Bacon et al. 1993; Roberts & Marston 2000). This dichotomy results in the possibility of residual populations in which recruitment is all but absent. In such populations, extant trees are likely to have been recruited when conditions were favorable but subsequent recruitment has been limited due to environmental constraints. In the Hunter Valley region of Australia, extensive land clearing has resulted in the widespread fragmentation of *E. camaldulensis* habitat. This has been accompanied with significant hydrological modification resulting from the construction of dams and the extraction of water for mining and agriculture. These perturbations may potentially have modified the ability of the existing habitat to support *E. camaldulensis* populations.

Correlations between genetic and non-genetic factors have been explored in many species, although the number of non-genetic factors addressed is limited. Several researchers have conducted meta-analyses and reported significant correlations between population size and genetic diversity (Young et al. 1996; Leimu et al. 2006; Honnay & Jacquemyn 2007), while an increasing number of researchers are investigating isolation and patch size in combination with genetic diversity yielding some interesting (and sometimes conflicting) results (Foré et al. 1992; Young et al. 1993; Prober & Brown 1994; Mathiasen et al. 2007). In species where critical life-stages are dependent upon environmental cues, elucidation of genetic diversity and population viability based on meta-analyses of generalist species may not be informative. The interaction between environment and survival is complex (Hobbs & Yates 2003b). Strong dependency on environmental variables has the capacity to alter predictions of population viability based on patterns detected in other species. These

species are likely to account for a significant proportion of those in which typical correlations do not occur.

This study aimed to assess a range of questions to help inform the larger question; what impacts do habitat and environmental factors have on remnant population structure and how does this relate to remnant genetic diversity and structure? This question was answered by assessing the degree of correlation between genetic and non genetic factors. Explicitly the questions addressed were; are populations that have a more sustainable age class structure (i.e. those populations still recruiting) more genetically variable? Is density (which reflects recruitment processes and the availability of suitable conditions for recruitment) correlated with high levels of genetic variability? How does the shape of the population (which reflects the proportion of floodplain to riparian habitat) relate to the level of genetic variability contain within? Does the shape of a population reflect density and age-class structure and is the health of the population related to genetic variability or environmental/ population attributes?

5.3 Methods

5.3.1 Site assessment

Nine sites representing a range of population sizes and contexts were selected within the Hunter Valley (Figure 16). The dimensions and area of each population were calculated using Google Earth Pro. Area was calculated by constructing a polygon over the vegetated region. Edge to area ratio (E/A) was then calculated by dividing the total area (m^2) by the perimeter (m). Edge to area ratio was calculated to estimate the area of edge habitat relative to internal habitat.

Remnant isolation was estimated by calculating a nearest neighbour distance. Near neighbour distance was measured as the distance (km) between a specific remnant and the nearest neighbouring remnant.

The age-class structure of each population was estimated by placing all trees at each site into one of five diameter at breast height (dbh (cm)) classes (2.5–30cm, 31–50cm, 51–80cm, 80–120cm, >120cm) based on either a direct measurement (where access allowed) or an estimation averaged over two independent observations. In the large population at Camyr Allyn, where it was not possible to assess all individuals,

the age-class structure of the population was estimated by calculating the number of individuals in each dbh class in two 100m x 100m quadrats and calculating a mean.

The percentage of individuals within each assigned dbh class was calculated for each remnant to enable a comparison of age-class structure independent of population size. Although dbh categories were somewhat arbitrary, historical photographs indicate only the trees in the largest class are likely to pre-date European settlement. Further, previous work conducted at multiple sites within the Hunter catchment indicated the maximum variation of dbh between 14-year-old trees was 34 cm, but that most varied far less (data from Webb & Erskine 2003, measurement adjusted from girth).

The size (the number of individuals) of each remnant population was assessed by direct counts in all populations except Camyr Allyn; in Camyr Allyn population size was estimated by counting individuals from an aerial image using Google Earth Pro. Tree density was calculated as the number of trees/total hectares to give either an exact count or an estimate of the number of trees per hectare.

Stand condition was estimated by calculating crown vigour (Cunningham et al. 2007b). Canopy vigour was determined by estimating the percentage of branches that exhibited live foliage. Crown vigour was assessed by visual inspection. This measure is semi-qualitative (Cunningham et al. 2007b), however, it is simple to assess and has been found to be highly correlated with live basal area and plant area index (Cunningham et al. 2007b). Although all three measures combined are likely to give more accurate results, canopy vigour alone is likely to give a reasonable estimate of stand condition.

5.3.2 Material collection and DNA extraction

Leaf material was collected from 184 individuals distributed across nine remnant stands of *E. camaldulensis* within the Hunter Valley Catchment area (Figure 16). Between 11 and 30 samples were collected from each remnant population. The amount of material sampled was dependent upon population size. Sampled individuals were separated by at least 15m to minimise the risk of sampling closely related individuals. In large populations the distance between sampled individuals was often greater than one hundred metres. Leaf material was dried in silica gel prior to extraction.

Deoxyribonucleic acid (DNA) was extracted from 20mg of silica dried leaf material using the DNeasy mini extraction kit (Q_{IAGEN}). The presence of DNA was confirmed by running 10 μL of DNA in agarose gel at 200 volts for one hour.

5.3.3 Polymerase chain reaction

Eight microsatellite primers were selected from the EMBRA (Brondani et al. 2006) and Eg (Benson et al. 2008) primer sets (Appendix 1), which previously yielded clear, reproducible and polymorphic bands. Primers were tagged with florescent markers. Polymerase chain reaction (PCR) was performed separately for each primer (for PCR programs see Appendix 2). The reaction mixture per individual per primer pair was: Buffer 2.5 μL, Mg++ 3.0 μL, DNTPs 0.5 μL, FWD primer 0.25 μL, RVSE primer 0.25 μL, Taq polymerase 0.2 μL, template 1 μL and H_2O 17.3 μL to give a total volume of 25 μL. Regions were amplified using a PC-960 Thermo Cycler. Five micro-litres from each PCR were combined and samples were quantified using capillary gel electrophoresis. Results were visualised and scored using the Genemarker@ software (Softgenetics).

5.3.4 Genetic parameters

A range of genetic diversity measures were included in the analysis because of their capacity to explain different aspects of genetic diversity (e.g. evenness, richness). Genetic diversity was estimated by calculating the total number of alleles (Na), the number of effective alleles (Ne), the number of private alleles (PA), Shannon's Diversity Index (I), observed heterozygosity (Ho), the biased and unbiased measured of heterozygosity (He and UHe) and the inbreeding coefficient (F). The program GenAlEx 6.1 (Peakall & Smouse 2006) was used for the above calculations. All measures (omitting the total number of alleles and the number of private alleles) were calculated as the mean across loci within population. Because sample sizes were uneven, rarefaction measures were also included. Allelic richness (AR) and private allelic richness (PAR) within populations were calculated in program HP-Rare (Kalinowski 2004; Kalinowski 2005).

5.3.5 Statistical analysis

Pearson's product moment correlation coefficients were calculated to explore the level of association between variables. Variables were categorised as either genetic or

non-genetic, and correlations were performed between variables within categories and between categories. Within category analyses were conducted to determine the extent of colinearlity between variables. All pairwise correlations were calculated using the software PASW statistic ver. 18 (Norusis 2010). Correlations were tested for significance using a two-tailed significance test.

5.4 Results

5.4.1 Site assessment

All populations were relatively small (< 700 individuals) with the largest population studied having approximately 700 individuals (Camyr Allyn), however this was unusually large and the next largest (Hunterview) had 152 individuals. The smallest population (Lemington) had 60 individuals.

Populations in the eastern and northeastern part of the catchment (Figure 16) were less isolated that in the western part of the catchment, where the Goulburn River comes close to the eastern escarpment of the Great Dividing Range.

The edge to area ratio varied widely between populations. A high edge to area was detected in populations that formed linear assemblages. These populations occurred along the river margins and were distinct from populations in floodplain habitat which were characterised by a relatively low edge to area ratio. Populations exhibiting intermediate measures incorporated both river margin and floodplain areas. Density was also highly variable between populations. The maximum density was recorded at Hunterview (130 tree/ha) and the lowest at Lemington with only 12 trees per hectare.

The proportion of the population assigned to different dbh classes (Figure 17) followed two distinct patterns. In two populations (Dartbrook and Carrington Billabong), the proportion of individuals in the larger dbh classes far exceeded those in the smaller dbh (cm) classes (Figure 17). However, in several populations the reverse was observed (e.g. Redbournberry, Sandy Hollow, and Bylong) (Figure 17).

Canopy vigour ranged between 90% in the population at Redbournberry and 52% in the Carrington Billabong population, in which evidence of dieback was considerable.

5.4.2 Correlation analysis between genetic parameters

Correlations between different measures of genetic diversity were strong throughout; however, there were subtle differences that reflected how allele frequency (evenness) affected the measure. The number of alleles was not correlated with either the biased or unbiased measure of heterozygosity or the measure of private allelic richness indicating the number of alleles included a substantial portion of rare or private alleles. The number of effective alleles was highly correlated with the Shannon's Diversity Index ($r = 0.98$, $p = 0.00$) indicating that either one of these measures alone may be sufficient (Table 9). The same was true for the unbiased and biased estimates of expected heterozygosity ($r = 0.99$, $p = 0.00$) (Table 9).

The inbreeding coefficient (F) was positively correlated with the number of effective alleles, Shannon's Diversity Index, and both the biased and unbiased measures of expected heterozygosity.

5.4.3 Correlations analyses between non-genetic parameters

Density was positively correlated with canopy condition ($r = 0.57$, $p = 0.05$) and the proportion of the population in the 2.5–30 dbh (cm) class (Figure 18a). Negative correlations were detected between density and the 81–120 dbh (cm) class ($r = 0.77$, $p = 0.01$) (Table 9). Canopy condition was also negatively correlated with the number of individuals in the 81–120 dbh (cm) class ($r = 0.64$, $p = 0.03$) (Table 9).

Area was positively correlated with population size ($r = 0.90$, $p = 0.00$); this was the strongest correlation between non-genetic variables (Table 9). The edge to area ratio was significantly and positively correlated with density ($r = 0.76$, $p = 0.01$) (Figure 18b) and the proportion of the population in the 2.5–30 dbh (cm) class ($r = 0.64$, $p = 0.03$) (Figure 18c). It was also negatively correlated with the proportion of the population in 51–80 and 81–120 % dbh (cm) classes ($r = 0.67$ $p = 0.03$, $r = 0.61$ $p = 0.04$) (Table 9).

5.4.4 Correlations analyses between genetic and non-genetic factors

Nearest neighbour distance, density and the edge to area ratio were the only non-genetic factors significantly correlated with genetic factors. Near neighbour distance was significantly and positively correlated with the number of alleles ($r = 0.61$ $p = 0.04$), the number of effective alleles ($r=0.58$ $p=0.05$), and the number of private

alleles (r = 0.75, p = 0.01) (Figure 19a). Density was negatively correlated with expected heterozygosity (r = -0.76 p = 0.01) (Figure 19b), allelic richness (r = -0.70, p = 0.02) (Figure 19c), and the Shannon's Diversity Index (r = -0.063, p = 0.03) (Table 9). The edge to area ratio was also negatively and significantly correlated with the number of effective alleles (r = -0.059, p = 0.05), the Shannon's Diversity Index (r = 0.60, p = 0.05) and allelic richness (r = 0.62, p = 0.04).

5.5 Discussion

5.5.1 Correlation analysis

This study demonstrates predictions based solely on single attributes, either genetic or non-genetic, can give an insufficient characterisation of populations viability in this species. It also demonstrates genetic diversity can be correlated with specific non-genetic variables.

Although not quantified in this study the type of habitat (floodplain or riparian) was largely described by the edge to area ratio because river margin populations were typically linear strips with a high edge to area ratio, whereas floodplain populations were characterised by a lower edge to area ratio. Populations in which both floodplain and river margin habitats were included exhibited intermediate measures. Edge to area ratio was correlated with a number of different genetic and non-genetic factors, indicating riparian and floodplain populations promoted different genetic patterns. The different habitats (riparian and floodplain/woodland) occupied by *E. camaldulensis,* and the quality of the different habitats have been discussed previously (George et al. 2005; Wen et al. 2009). In this study, age-class structure correlated with edge to area ratio, (e.g. 2.5–30 dbh (cm) x edge to area, r = 0.64) indicating that riparian populations had higher levels of contemporary recruitment. The reverse was true for populations with a low edge to area ratio (remnants which were predominantly floodplain) in which typical arrays showed a high proportion of old trees with little contemporary recruitment (e.g. 81–120 dbh (cm) x edge to area ratio, r = -0.67). This pattern is likely to be due to the continuous availability of water along river margins enabling more opportunities for recruitment and seedling survival (George et al. 2005; Wen et al. 2009). These findings indicate that habitat type is linked to age-class structure, most likely because the environment promoted regualar recruitment.

Density was also highly correlated with edge to area ratio and age-class structure. High densities occurred in habitats with a high edge to area ratio (r = 0.68) and a relative high proportion of young trees (mostly riparian zones). Density is likely to be higher when seed is able to germinate and available resources are adequate to support a high number of individuals. This suggests that riparian zones may have a higher carrying capacity than floodplain zones, and the potential to support more individuals over smaller areas. Since a high carrying capacity may decrease a population's risk of extinction (Lande 1993) and continual recruitment enables replacement, recruitment in riparian populations may exceed floodplain populations.

Interestingly, the Shannon's Diversity Index, the number of effective alleles and allelic richness were all significantly negatively correlated with edge to area ratio. Other genetic diversity measures were also correlated (unbiased and biased measures of heterozygosity) but not significantly. This indicates that, despite more continuous recruitment, remnants with a high edge to area ratio also exhibited low levels of genetic diversity relative to populations with a low edge to area ratio, particularly those populations with low density and a high proportion of old trees. This has significant implications for management. First, it indicates relatively low levels of genetic diversity may not limit population growth if environmental factors are not limiting. Further, it indicates less dense populations have higher levels of genetic diversity; correlations between density and genetic diversity were very strong (He, r = -0.76). Relative to densely populated populations (e.g. Hunterview) sparsely populated remnants had the greatest proportion of the population in the 81–120 dbh category and relative high genetic diversity (e.g. Lemington). This indicates that, although little recruitment and negligible population growth may occur in some floodplain habitats, significant genetic diversity resides in the old trees therein.

The correlation between genetic diversity and non-genetic factors was complicated by the significant positive correlation between the nearest neighbour distance and genetic diversity, and near neighbour distance and the number of private alleles. Contrary to population genetic theory (Young et al. 1996), the correlation between genetic diversity and near neighbour distance was positive, indicating genetic diversity was higher in geographically isolated populations. The number of alleles and the number of private alleles exhibited the strongest correlations, followed by the number of effective alleles and the Shannon's Diversity Index. This suggests that

private alleles and alleles with low frequency were driving the correlation. This assertion is supported by the lack of correlation between near neighbour distance and expected heterozygosity measures that do not weight heavily those alleles with low frequency. Population genetic theory predicts genetic diversity in small, isolated populations should decline and low frequency alleles should be lost first (Maruyama & Fuerst 1985; Garza & Williamson 2001). In *E. albens* isolated populations were found to be relatively genetically depauperate (Krauss et al. 2007), but this situation was not observed in this study. One possible explanation could be the introgression of alleles via hybridisation. Several studies have reported spontaneous hybridisation between closely related species of *Eucalyptus* (e.g. *E. nitens x E. ovata*, Barbour et al. 2002; *E. benthamii x E. viminalis*, Butcher et al. 2005); high numbers of private alleles have been previously attributed to introgression in *E. camaldulensis*, in which spontaneous hybridisation between closely related species can produce fertile hybrids (e.g. *E. camaldulensis x E. teriticornis*, Butcher et al. 2002). Spontaneous introgression could offer an escape path for isolated populations. While strongly influenced by the high number of private alleles and the geographic isolation of the Bylong population, the pattern of increasing rare alleles with increasing isolation may indicate that the influence of hybridisation increases with population isolation. Although not formally studied variable capsule phenotypes were observed in the isolated Bylong population (pers.obs. Nov09) which could have be indicative of hybridization. This hypothesis warrants further investigation, as only a small number of isolated populations were included in this study.

The low number of private alleles in proximal populations is less surprising given gene-flow is more likely over smaller distances, and increased gene-flow should reduce the occurrence of private alleles. The Hunterview and Redbournberry sites are separated by only 1.9km. Several studies have indicated gene-flow across these distances in *Eucalyptus* species (e.g. *E. nitens*, Barbour et al. 2005; *E. wandoo*, Byrne et al. 2008). Alternatively, the relative low levels of private alleles (and overall genetic diversity) in proximal populations may result from the small number of founding trees (indicating more severe bottleneck effect) in the two remnants closest to each other (Redbournberry and Hunterview). This combined with the high number of private alleles in more isolated populations may impact positively on the overall strength of correlation between geographical isolation and genetic diversity measures.

Finally positive correlations were detected between the inbreeding coefficient and a range of genetic diversity measures indicating that an increase in the number of alleles within a population remnant was not accompanied by a subsequent increase in heterozygosity. This result may be due to substantial population substructure (Wahlund effect, Lowe et al. 2004) in large populations with high genetic variability.

5.5.2 Uncorrelated variables

Population size has been found to be significantly and positively correlated with genetic diversity in meta-analysis (Young et al. 1996; Leimu et al. 2006) however, in this study no correlation was found between population size and genetic diversity. Although some large populations exhibited high levels of genetic diversity, low levels of genetic diversity were found in other large populations. The lack of correlation between size and genetic diversity in this species may arise because populations are not at equilibrium (Ellstrand & Elam 1993). The concept of population equilibrium may not be applicable to *E. camaldulensis* populations in which habitat is characterized by disturbance; for example, large populations exhibiting low levels of genetic variability typically contain a small number of old trees indicating that the population may have suffered population constriction and is in a phase of re-colonization.

Various aspects of plant fitness have been correlated with genetic variability (Young et al. 1996; Reed & Frankham 2003). Here canopy vigour was used to assess stand condition, and no correlation between genetic diversity and stand condition was established. Canopy vigour was, however, correlated with the proportion of the population in the 81–120 cm dbh class ($r = -0.64$, $p = 0.03$). This supports that view that canopy vigour was more affected by age-class structure as opposed to genetic variability.

5.5.3 Conservation and management implications

Correlation analyses indicated riparian habitat with a high edge to area ratio (a large riparian component) exhibited greater age-class structure with a significant portion of individuals in the younger dbh categories. Riparian habitat may be more conducive to ongoing recruitment. Since many previous studies have investigated floodwater retention and germination and growth in *E. camaldulensis* (Dexter 1970; Wen et al. 2009) indicating the duration and frequency of inundation are important, ongoing recruitment in riparian habitat is likely to be due to the frequency of water

availability. Conversely, the lack of recruitment in some floodplain habitat is most likely to be at least partly due to a lack of water. Management of the species within the catchment may require habitat enhancement (via increased water). Water retention has been introduced at the Dartbrook site; after inundation I observed a substantial number of seedlings. I also observed seedlings at the Camyr Allyn site following flooding. These observations were made after the census data were collected and no follow up has been conducted.

Habitat enhancement alone may not, however, instigate successful recruitment. The genetic quality of progeny might be insufficient, or pollination services may be limited. Moreover, in some sites, habitat enhancement may not be possible. Where population structure is clearly regressive, (and habitat enhancement is not possible) it might be wise to consider collecting seed for re-introduction into more viable habitat. Transfer of seed between populations confers the risk of outbreeding depression if genotypes are adapted to specific habitats. Although the genetic differentiation between most populations was found to be minimal (Chapter 4), the markers used in the study were neutral and thus inadequate for the detection of adaptive variation. Further investigation into the adaptive variation within populations and possibly controlled crosses should be considered prior to seed transfer.

5.6 Conclusion

This study found that relatively high levels of genetic variability could be detected in populations of *E. camaldulensis* that had age class structures that indicated a lack of recruitment. Diverse age-class structures were detected in populations with high edge to area ratio, and these populations were predominantly found in riparian areas rather than floodplains. However, these populations, especially when proximal to other populations, were relatively genetically depauperate. This is an important finding, as it indicates that in assessing *E. camaldulensis* stands, assessment on environmental and population attributes alone might not be consistent with maintaining genetic variability and hence a concept of population viability that incorporates genetic concerns. *Eucalyptus camaldulensis* populations in floodplain habitat are likely to some extent to have always been ephemeral due to oscillating climatic patterns, however, the restriction of genetic variability along riparian zones indicates that, in the future, should extant floodplain populations become extinct; the genetic diversity of future floodplain populations will be restricted to the genetic diversity maintained along the

river margins. Although at present relatively low levels of genetic diversity are not restricting recruitment, long-term genetic variability may be threatened if regressive floodplain populations become extinct.

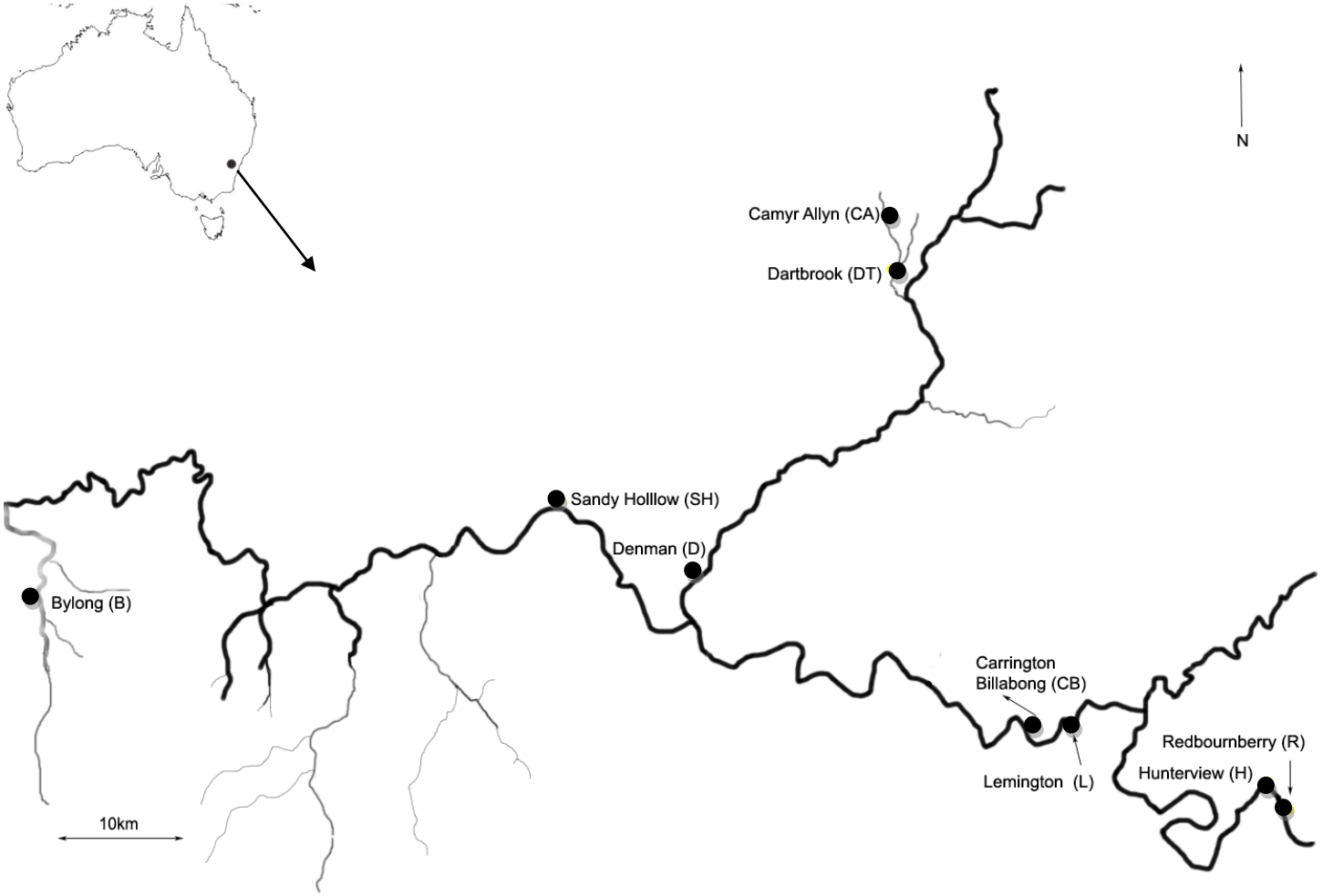


Figure 16. The location of remnant *Eucalyptus camaldulensis* sites studied in the Hunter Valley catchment

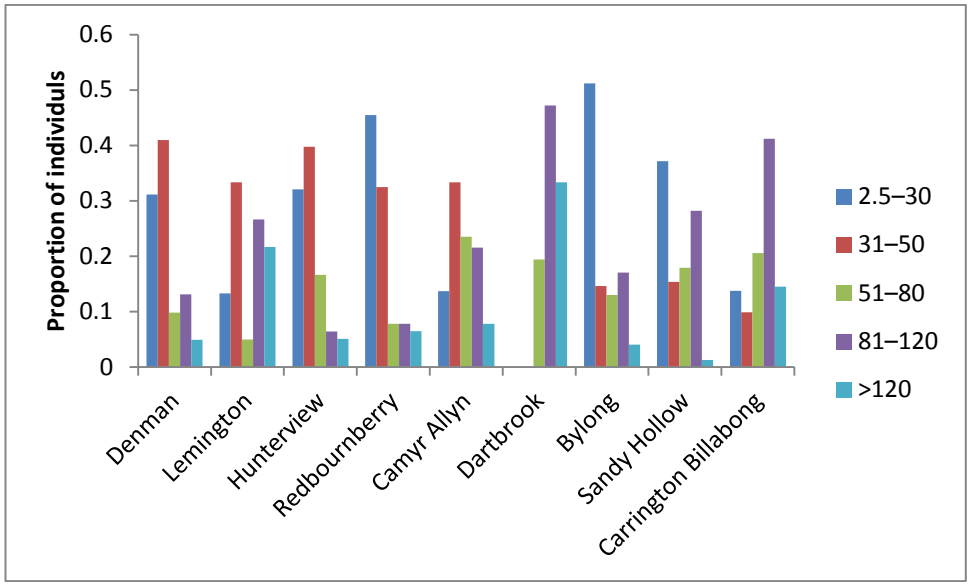
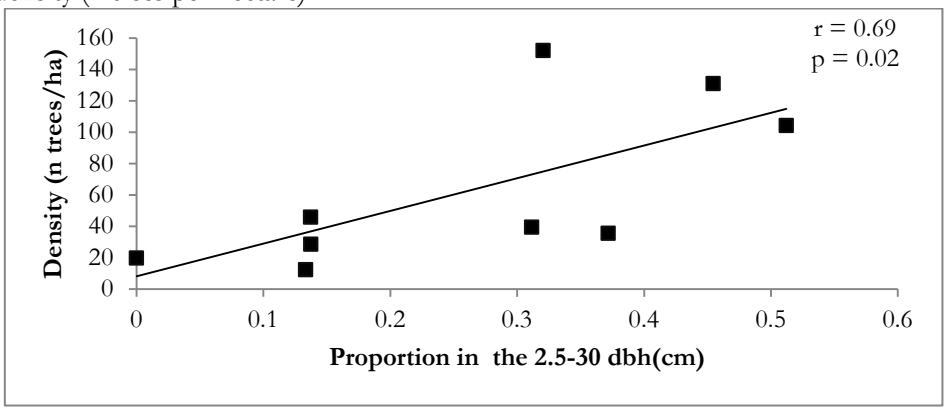
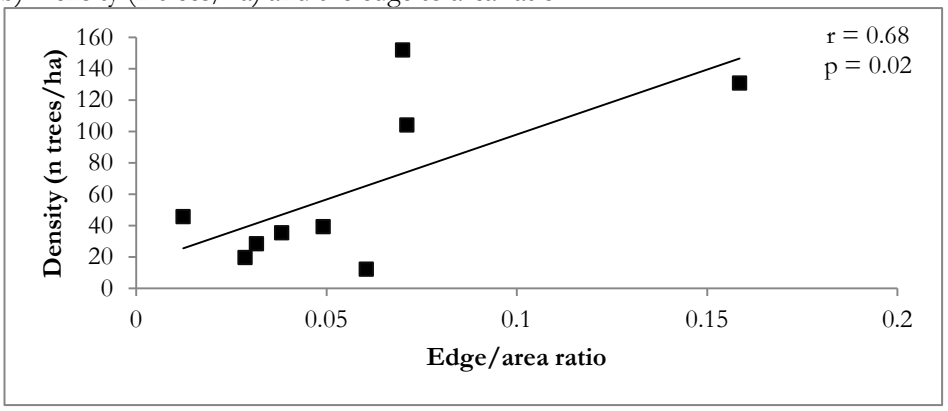


Figure 17. Variable age-class structure in *Eucalyptus camaldulensis* remnant populations estimated by diameter at breast height (dbh cm) classes

a) The proportion (0–1) of individuals in the 2.5–30 dbh (cm) class and tree density (n trees per hectare)



b) Density (n trees/ha) and the edge to area ratio



c) The proportion (0–1) of individuals in the 2.5–30 dbh (cm) class and the edge to area ratio

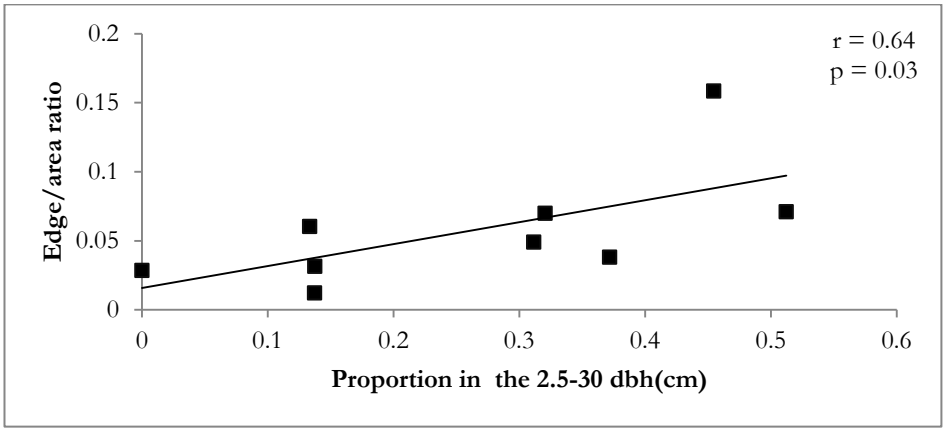
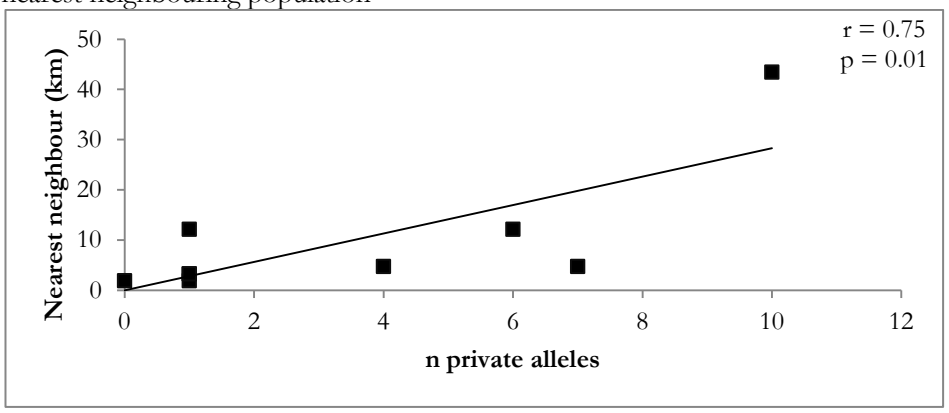
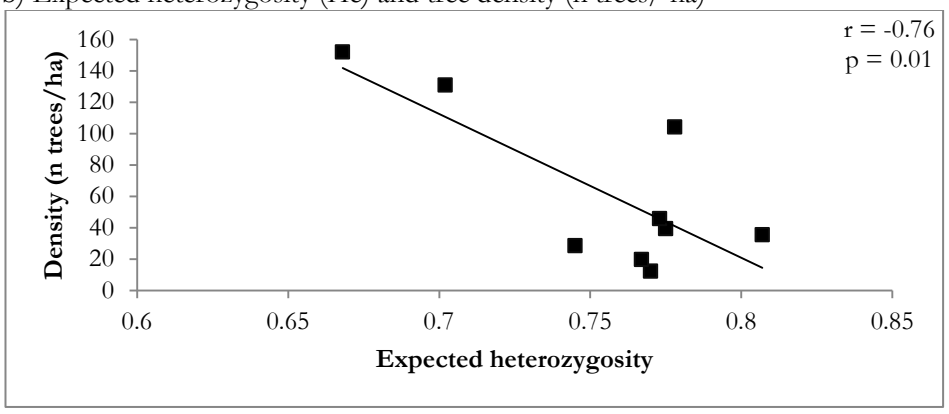


Figure 18. Correlation (Pearson's r) between the edge to area ratio and structural characteristics (age-class distribution, density) measured in nine remnant populations of *Eucalyptus camaldulensis*

a) The number of private alleles and the distance between the population and the nearest neighbouring population



b) Expected heterozygosity (He) and tree density (n trees/ ha)



c) Allelic richness and tree density (n tree/ha)

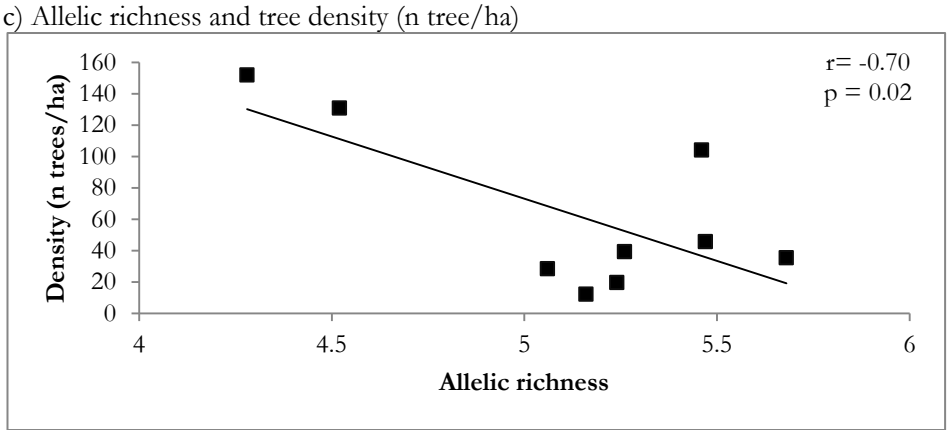


Figure 19. Correlation (Pearson's r) between genetic and non-genetic parameters in remnant populations of *Eucalyptus camaldulensis*

Table 9. Pearson's correlation (r) (below diagonal) and p values (above diagonal) for genetic, geographic and stand structure parameters in *Eucalyptus camaldulensis* remnants. Genetic diversity was correlated with edge to area ratio, density and nearest neighbour distance.

	N	**Ne**	**PA**	**I**	**UHe**	**He**	**F**	**AR**	**PAR**	**Area**	**n**	**E/A**	**D**	**CC**	**NN**	**2.5-30**	**31-50**	**51-80**	**81-120**	**>120**
N		0.00	0.01	0.01	0.16	0.08	0.13	0.04	0.17	0.07	0.08	0.11	0.28	0.28	0.04	0.39	0.27	0.22	0.32	0.42
Ne	0.82		0.00	0.00	0.00	0.00	0.07	0.00	0.01	0.08	0.15	0.05	0.07	0.27	0.05	0.42	0.18	0.19	0.19	0.45
PA	0.80	0.82		0.01	0.07	0.04	0.15	0.02	0.01	0.25	0.19	0.22	0.44	0.49	0.01	0.27	0.14	0.18	0.38	0.31
I	0.80	0.98	0.77		0.00	0.00	0.06	0.00	0.02	0.10	0.22	0.05	0.03	0.19	0.07	0.34	0.17	0.28	0.14	0.42
UHe	0.38	0.81	0.55	0.86		0.00	0.07	0.00	0.00	0.28	0.50	0.07	0.01	0.24	0.19	0.37	0.16	0.39	0.10	0.45
He	0.50	0.87	0.60	0.92	0.99		0.07	0.00	0.01	0.23	0.45	0.06	0.01	0.18	0.14	0.35	0.15	0.39	0.10	0.42
F	0.43	0.53	0.39	0.55	0.53	0.54		0.07	0.17	0.48	0.45	0.07	0.20	0.42	0.11	0.40	0.43	0.43	0.40	0.41
AR	0.61	0.93	0.71	0.96	0.96	0.98	0.54		0.00	0.16	0.33	0.04	0.02	0.21	0.11	0.36	0.13	0.25	0.10	0.48
PAR	0.36	0.72	0.76	0.70	0.81	0.78	0.36	0.83		0.46	0.49	0.14	0.19	0.37	0.06	0.27	0.10	0.20	0.23	0.21
Area	0.54	0.50	0.26	0.47	0.23	0.29	0.02	0.37	0.04		0.00	0.07	0.15	0.50	0.26	0.09	0.34	0.10	0.33	0.42
n	0.52	0.40	0.34	0.30	-0.01	0.05	-0.05	0.18	0.01	0.90		0.21	0.49	0.21	0.37	0.32	0.22	0.08	0.34	0.28
E/A	-0.46	-0.59	-0.29	-0.60	-0.53	-0.56	-0.53	-0.62	-0.40	-0.54	-0.31		0.02	0.17	0.50	0.03	0.20	0.03	0.04	0.24
D	-0.22	-0.53	-0.06	-0.63	-0.75	-0.76	-0.32	-0.70	-0.33	-0.40	0.01	0.68		0.05	0.34	0.02	0.16	0.34	0.01	0.07
CC	-0.23	-0.24	0.01	-0.34	-0.27	-0.35	0.08	-0.31	-0.13	0.00	0.31	0.37	0.57		0.35	0.16	0.10	0.49	0.03	0.13
NN	0.61	0.58	0.75	0.54	0.34	0.41	0.45	0.46	0.56	-0.25	-0.13	0.01	0.16	-0.15		0.05	0.26	0.40	0.36	0.18
2.5-30	-0.11	-0.08	0.24	-0.16	-0.13	-0.15	-0.10	-0.14	0.24	-0.50	-0.18	0.64	0.69	0.37	0.58		0.23	0.15	0.01	0.00
31-50	-0.24	-0.35	-0.41	-0.37	-0.38	-0.39	-0.07	-0.42	-0.47	0.16	0.30	0.32	0.38	0.48	-0.25	0.29		0.12	0.00	0.09
51-80	0.30	0.33	0.35	0.23	0.11	0.11	0.07	0.26	0.33	0.48	0.51	-0.67	-0.16	0.01	-0.10	-0.39	-0.44		0.11	0.45
81-120	0.18	0.34	0.12	0.41	0.47	0.48	0.10	0.47	0.28	0.17	-0.16	-0.61	-0.77	-0.64	-0.15	-0.74	-0.82	0.45		0.01
>120	0.08	-0.05	-0.20	0.08	0.05	0.08	0.09	0.02	-0.31	0.08	-0.22	-0.27	-0.53	-0.42	-0.34	-0.81	-0.49	0.05	0.73	

Yellow shading indicates significance ($p < 0.05$) N = total number of alleles in a population, Ne = effective alleles, PA = private alleles, I = Shannon's Diversity Index, UHe = unbiased expected heterozygosity, He = expected heterozygosity, F = inbreeding coefficient, AR = allelic richness, PAR = private allelic richness, Area = m^2, n = the number of individuals in the population, E/A = edge to area ratio, D = n tree/ha, CC = canopy condition, % cover, NN = nearest neighbour distance, 2.5–30, 31–50, 51–80, 81–120, >120cm = the proportion of total trees in each dbh (cm) class

6 Habitat type promotes variable genetic structure in a water dependant tree, *Eucalyptus camaldulensis* Dehnh. subsp. *camaldulensis* (River Red Gum).

6.1 Abstract

Eucalyptus camaldulensis populations occur along river margins and within floodplain habitat in the Hunter Valley in eastern Australia. This research assessed the level of genetic diversity and the degree of spatial genetic structure in seedlings and saplings in these two distinct habitats. By sampling across populations the research also investigated how the genetic template established during recruitment influenced population-wide spatial genetic structure. Neighbourhoods within floodplain habitat exhibited low levels of diversity (He = 0.62) and patterns of genetic structure indicative of limited seed dispersal; recruitment neighbourhoods in riparian habitat exhibited higher levels of genetic diversity (He = 0.72) and less genetic structure, indicating random dispersal of unrelated seeds. Variable levels of genetic structure were detected at the population level, ranging from high ($Sp = 0.05$) to low ($Sp = 0.004$), and the results suggest that the degree of genetic structure established at the recruitment phase is reflected at the population level in remnants that contain only one habitat type. Populations encompassing both river margins and floodplain habitat exhibited unpredictable levels of spatial genetic structure. The results of this study are discussed in relation to the environment of floodplain and riparian habitat, hydrochory and the resolution of spatial autocorrelation.

6.2 Introduction

Fine scale spatial genetic structure is the nonrandom distribution of genotypes within populations resulting from limited dispersal, mating between proximal individuals (Wright 1943; Malécot 1948; Epperson 1992), and adaptation to microhabitats (Heywood 1991; Epperson 1992; Alvarez-Buylla et al. 1996). When the required conditions are met, spatially restricted patches of similar genotypes accumulate rapidly. Consistent patterns are maintained when a population is at equilibrium

(Turner et al. 1982). The presence, absence or degree of genetic structure in natural populations gives substantial insight into the reproductive history of a population. Although it may be difficult to discern which factor(s) (i.e. pollen, seed or microhabitat) promote genetic structure within a species, strong genetic structure indicates frequent close, or self mating (Hamrick et al. 1993). Spatial genetic structure can also be used to infer the size of the breeding neighbourhood (Wright 1943), a unit within which breeding should theoretically be viable. This is important for species conservation, particularly in fragmented habitat because the degree to which mating patterns are affected by habitat fragmentation is likely to relate to the extent to which reproductive processes are disrupted (Epperson & Alvarez-Buylla 1997).

Many studies have detected spatial genetic structure in forest trees, including within *Eucalyptus* (Skabo et al. 1998; Jones et al. 2007). However, the spatial genetic structure of a species can vary across time and space (Loveless & Hamrick 1984; Jones & Hubbell 2006). Studies have reported variation of genetic structure between different age-classes (Epperson & Alvarez-Buylla 1997; Jones et al. 2006), successional stages (Epperson & Alvarez-Buylla 1997; Chung et al. 2002), habitat types (Premoli & Kitzberger 2005; Hu et al. 2009), and as a function of different establishment patterns (Knowles et al. 1992; Parker et al. 2001). Temporal and spatial variability of genetic structure is usually driven by an environmental factor (e.g. gap colonization, fire disturbance, and thinning due to resource availability) interacting with dispersal characteristics and modifying recruitment processes and survival.

Eucalyptus camaldulensis is a dominant forest tree restricted to river margins and floodplains because of its strong dependence on inundation for germination (George et al. 2005; Wen et al. 2009). Recent research (Chapter 5) found the level of genetic variability within populations was associated with environmental attributes. Specifically, the level of genetic variability was associated with habitat type. Genetic variation was negatively correlated with edge to area ratio, indicating linear populations along river margins were more likely to exhibit low levels of genetic variability compared with floodplain populations (or populations with a high floodplain component) which exhibited low edge to area ratios. These results suggested intrinsic characteristics of the habitat promote specific genetic patterns. A key characteristic which varies between riparian and floodplain habitat is the availability of water. As in other systems (e.g. Cunningham et al. 2007a) water in

riparian habitat is more frequently available while water in floodplain habitat occurs only during high flows. *Eucalyptus camaldulensis* seeds have been demonstrated to float for up to ten days and seeds of *E. camaldulensis* have been detected in flood debris (Pettit & Froend 2001); hence hydrochory (water mediated seed dispersal) is predicted to be an important dispersal mechanism (Cremer 1977). Recruitment patterns promoted by different water regimes in different habitat might, therefore, drive differences in the levels of genetic variability. The predominant impact of water mediated seed dispersal is increased levels of genetic variability, due to the increased movement of seeds (e.g. Kudoh & Whigham 2001; Shimamura et al. 2007), and the breakdown of genetic structure due to movement of seed away from the seed source (parent tree) in accordance with floodwater and local topography (Hu et al. 2009).

This study aimed to address two questions: does the level and structure of genetic diversity vary between different habitat types and do patterns of genetic variability within different habitats influence the level and structure of genetic variation at the population level? I addressed these questions by studying amplified microsatellite regions in groups of individuals sourced from naturally clustered seedlings/saplings in different habitat. I then analysed these data in conjunction with individuals sampled across each population to determine if patterns established early on were persistent in the population at large.

6.3 Methods

6.3.1 Genetic structure sampling

Neighbourhoods in riparian and floodplain habitats were opportunistically sampled in four populations of *E. camaldulensis*: Camyr Allyn (CA), Lemington (L), Sandy Hollow (SH) and Bylong (B) (See Table 2 for site details). In *E. camaldulensis* populations, young trees are naturally clustered together in patches. Neighbourhoods in this study were defined by the natural limit of recruitment patches and varied in size between 11 and 30 trees. All individuals within neighbourhood patches were sampled. Leaf material was collected from seedlings/saplings and dried in silica gel. In total five recruitment neighbourhoods were sampled (Figure 20). Three of these five neighbourhoods were located within riparian habitat (B/Nr, SH/Nr, and L/Nr) and two within floodplain habitat (L/Nf and CA/Nf). The diameter at breast height

of individuals (dbh) was measured (cm) to characterise seedling/sapling size variation within each neighbourhood.

In floodplain habitat, the neighbourhoods (L/Nf and CA/Nf) consisted of patches of seedlings recruited following a flood event. These seedling were unequivocally the same age (<2 cm dbh). Clustered neighbourhoods within riparian habitat were characterised by young trees but dbh of these trees was variable. This suggests that the young trees may not have been recruited at the same time, although a variation in size estimated by dbh (34 cm) has been detected between trees of the same age in this species within the Hunter Valley (Webb & Erskine 2003). Individuals in the SH/Nr riparian neighbourhood exhibited a dbh ranging between 7–10 cm, in B/Nr between 5–14 cm and in L/Nr between 10–23 cm.

All individuals in neighbourhoods were mapped (Figure 20), and key locations were recorded on a Garmin GPS device (accuracy of ± 10m). To assign each individual a geographical co-ordinate, maps of each neighbourhood were overlaid on Google Earth satellite imagery and aligned with key locations.

To estimate population-wide genetic structure, leaf samples were also taken from trees throughout the population at a range of distances that characterised the range of interplant distances within the population. Population scale samples (B/P, SH/P, L/P, and CA/P) were taken from all four populations. The locations of sampled individuals were recorded on a GPS device.

6.3.2 Seed sampling

To enable a comparison between the level of genetic diversity within neighbourhoods and the level of genetic diversity of seed sourced from a single parent tree, ten capsules from each of three trees were collected in the Camyr Allyn population. Seeds were collected from the lower canopy. Ten seeds from each of ten capsules were placed on moist paper in a Petri dish and germinated in an incubation chamber (24°C, 12 hours light/dark) (Bell 1999). After two weeks seedlings were transferred to the greenhouse. After approximately six weeks leaf material (approx. 2 cm^2) was collected from each individual. Not all seeds germinated successfully. After collection leaf material was placed in silica gel for drying.

6.3.3 DNA extraction and polymerase chain reaction

Total genomic DNA was extracted from 20 mg of dried leaf material using the DNeasy plant mini kit. The presence of DNA was confirmed by running 10 μL of DNA in 1% agarose gel at 200 volts for one hour. Eight primers, which yielded clear, reproducible and polymorphic bands, were selected from the EMBRA (Brondani et al. 2006) and Eg (Benson et al. 2008) primer sets (Appendix 1). Primers were tagged with florescent markers. Polymerase chain reaction (PCR) was performed for each primer separately (for PCR programs see Appendix 2). In the seed analysis only four microsatellite regions were amplified (Embra 17, Eg1062, Eg1096, and Eg1028). The reaction mixture per individual per primer pair was: Buffer 2.5 μL, Mg++ 3.0 μL, DNTPs 0.5 μL, FWD primer 0.25 μL, REV primer 0.25 μL, Taq polymerase 0.2 μL, template DNA 1 μL and H_2O 17.3 μL to give a total volume of 25 μL. Regions were amplified using a PC-960 Thermo Cycler. Five micro-litres from each PCR were then combined and samples were quantified using capillary gel electrophoresis. Results were visualised and scored using the Genemarker@ software (Softgenetics).

6.3.3.1 Spatial autocorrelation

Three levels of spatial autocorrelation analyses were performed. The first was confined to within neighbourhoods (neighbourhood scale), the second analysed population scale samples (population scale) and the third combined both neighbourhood and population scale samples from the same population. In the Lemington population, where recruitment neighbourhoods included both a neighbourhood in a floodplain habitat and a neighbourhood in a riparian habitat, two analyses were performed. The first combined population scale samples with riparian neighbourhood samples (L/P + L/Nr), and the second combined the population scale samples and the floodplain neighbourhood samples (L/P + L/Nf).

Spatial autocorrelation analyses were performed by calculating the mean pairwise kinship (Loiselle et al. 1995) between individuals within distance classes. To compare genetic structure between different sites, the same distance classes were used in all analyses (neighbourhood, population and population + neighbourhood) (Tables 11, 12 and 13). Permutations (20000) were run to determine if the level of relatedness differed from that expected if the spatial distribution of individuals was random. Jackknife estimates were calculated to obtain a standard error for the mean kinship in

each distance class. Intra-individual kinship within each group was also calculated; this measure is an approximation of the inbreeding coefficient (Hardy & Vekemans 2009).

To compare the degree of genetic structure between sites and with other species, the *Sp* statistic was calculated (Vekemans & Hardy 2004). The *Sp* statistic assesses the degree of genetic structure by relating the mean kinship coefficient of the first distance class (which is assumed to contain most neighbours) to the overall slope of the regression (r^2) of individual pairwise kinship regressed against log distance. All spatial autocorrelation analysis was conducted in the program SPAGedi (Hardy & Vekemans 2002).

6.3.4 Neighbourhood & population genetic similarity

The genetic similarity between population scale and neighbourhood scale samples were estimated via principal co-ordinate analysis. These analyses were undertaken to explore if genotypes of recruitment groups exhibited genetic arrays that indicated that they have been sourced within the population. In each population an individual pairwise genetic distance matrix was constructed by calculating the mean genetic distance between individuals. Individuals were grouped by sampling unit (neighbourhood versus population). Both the genetic distance matrix and PCA were calculated in GenAlEx 6.1 (Peakall & Smouse 2006).

6.3.5 Levels of genetic diversity

The genetic diversity within hierarchical levels (population, neighbourhood, and seed) was calculated for all groups using four microsatellite loci. Measures calculated were: the mean number of alleles per locus (Na), the mean number of effective alleles (Ne), the Shannon's Diversity Index (I), observed heterozygosity (Ho), the biased and unbiased measure of expected heterozygosity (He and UHe), the inbreeding coefficient (F) and the mean pairwise genetic distance (D). Genetic distance was calculated following the multi-locus method described in Smouse and Peakall (1999). Analyses were conducted in the program GenAlEx 6.1 (Peakall & Smouse 2006).

6.4 Results

6.4.1.1 Neighbourhood scale

With the exception of the floodplain neighbourhood CA/Nf in the Camyr Allyn population (in which a high level of spatial genetic structure was detected $Sp = 0.07$), very little genetic structure was detected within recruitment neighbourhoods indicating that isolation by distance processes (where geographic distance is associated with genetic distance) were not prevalent at the neighbourhood scale (Table 10). The inter-individual kinship was highest in CA/Nf, and significant values were also detected in L/Nr and B/Nr (Table 10). Low and non-significant intra-individual kinship values were detected in L/Nf and SH/Nr.

6.4.1.2 Population scale

Spatial autocorrelation analyses detected variable levels of spatial genetic structure at the population scale. The Sandy Hollow population scale (SH/P) analysis revealed a high level of spatial genetic structure ($Sp = 0.05$), however, although positive, the pairwise kinship in the first distance class was not significantly higher than the mean pairwise kinship for the sample (Table 11). Conversely, a significantly higher mean kinship was detected in individuals in the first distance class in the Camyr Allyn population scale (CA/P) analyses but the Sp statistic was lower ($Sp = 0.3$) indicating less genetic structure overall (Table 11). Weak or absent spatial genetic structure was detected in the Lemington (L/P) and Bylong (B/P) population scale samples (Table 11). In Bylong the pairwise kinship was negative in the first distance class indicating neighbouring individuals exhibited below average (based on the total sample) pairwise kinship values (Table 11). All intra-individual kinship values at the population scale were significant and high (Table 11).

6.4.1.3 Combined population and neighbourhood scale spatial autocorrelation analysis

Negligible spatial genetic structure was detected when neighbourhood and population scale samples were combined (Table 12). In the first distance class only the Camyr Allyn population (CAP + CA/Nf) exhibited the highest mean kinship. In all other populations, individuals separated by greater distances (>10m) exhibited higher kinship. This was particularly pronounced in the Bylong population in which individuals in the 100m distance class exhibited a much higher (0.0086) mean kinship than those in the first distance class (0.0026) (Table 12).

6.4.2 Neighbourhood & population genetic similarity

The principal co-ordinate analysis indicated that the majority of individuals within recruitment neighbourhoods were genetically similar to those sampled in the established populations (Figure 21). A single exception occurred in the B/Nr recruitment neighbourhood (Figure 21c) where a small group of five individuals from within the recruitment neighbourhood exhibited genotypes that were dissimilar compared with the main population sample.

6.4.3 Hierarchical statistics

All progeny arrays exhibited low levels of genetic variability (e.g. Shannon's Diversity Index) and low levels of genetic distance as would be expected given common parentage (Table 13). Floodplain recruitment neighbourhoods exhibited levels of genetic variability that were similar to the levels of diversity detected in the progeny arrays (Table 13) and genetic distances that ranged between D = 4.72 and D = 7.59. Recruitment neighbourhoods within riparian habitat exhibited higher levels of genetic variability than floodplain neighbourhoods and genetic distances between D = 6.00 and D = 7.48. The levels of variability in some riparian recruitment neighbourhoods (SHf and BNf) were similar to those detected at the population scale; population scale samples exhibited the highest levels of genetic diversity (Table 13) and genetic distances between D = 7.14 and D = 8.26.

Negative inbreeding coefficients were detected within all progeny arrays indicating an excess of heterozygotes. In neighbourhood and population scale sampling units the inbreeding coefficient was idiosyncratic. A negative inbreeding coefficient was detected in the SH/Nr sample; a measure not far from Hardy-Weinberg equilibrium was detected in the L/P sample. All other riparian and population samples exhibited positive inbreeding coefficients.

6.5 Discussion

6.5.1 Neighbourhood level genetic diversity and genetic structure

Patterns of recruitment provide the initial genetic template upon which population genetic structure depends (Kalisz et al. 2001; Chung & Epperson 2003; Hampe et al. 2010). The genetic attributes of the recruitment neighbourhoods investigated in this

research indicated that seed dispersal may be restricted under some circumstances, but not others and patterns of dispersal may be associated with habitat type.

The level and structure of genetic variation in floodplain neighbourhoods suggests that seed dispersal may be limited despite germination being preceded by floodwater. In the Lemington floodplain neighbourhood (L/Nf), no genetic structure was detected; however, this was accompanied with very low levels of genetic distance between individuals. The mean level of genetic distance between individuals within the floodplain recruitment neighbourhood at Lemington was less than the genetic distance between seeds sourced from the same tree. Individuals within the group are therefore, possibly the progeny of a single parent tree. The lack of genetic structure suggests closely related individuals are dispersed randomly over the neighbourhood area (approx. $10m^2$). The level of genetic variability indicates limited dispersal, and the absence of spatial genetic structure within the neighbourhood is likely to relate to the small sampling area (i.e. within genetic neighbourhood). The episodic recruitment neighbourhood at Camyr Allyn exhibited a much higher mean genetic distance than the floodplain neighbourhood at Lemington suggesting that multiple seed sources may have contributed to the neighbourhood but closely related individuals were spatially cohesive. In combination, these results suggest episodic recruitment in floodplain habitat may result in the establishment of patches of closely related individuals. As with studies of other species (e.g. Loiselle et al. 1995; Parker et al. 2001), patches of related individuals are likely to have accumulated because of limited seed dispersal.

Neighbourhoods located in riparian habitat exhibited relatively high levels of genetic variation and the *Sp* statistics were low indicating weak or absent genetic structure. This suggests the absence of isolation by distance. Various attributes of riparian habitat may interact to reduce the accumulation of genetic structure. First, the proximity of riparian neighbourhoods to the river is likely to increase exposure to more variable seed sources (migration), subsequently increasing genetic diversity. Second, since the diameter at breast height (cm) exhibited some variability, individuals within riparian neighbourhoods might represent continual (as opposed to episodic) recruitment. The saturation of floodplains requires a flood event in which floodwaters exceed the river bank (Bren 1988); the strong water dependence of *E. camaldulensis* indicates recruitment within the floodplain will be limited to these

rare events. However, in riparian habitats suitable recruitment sites are likely to be more continuously available, offering ongoing recruitment opportunities. This may promote both the mixture of seed (via their movement in water) and recruitment by multiple adults. Third, the density of trees in riparian habitat may reduce genetic structure. Riparian habitat is characterised by high densities when compared with floodplain habitat (Chapter 5). High densities have been demonstrated to reduce genetic structure because of the high number of overlapping seed shadows. An absence of spatial genetic structure attributed to high density was reported in *Jacaranda copaia* (Jones & Hubbell 2006) and in established populations of *Picea sitchensis* (Gapare & Aitken 2005). Finally, linear population arrays have been predicted to accumulate spatial genetic structure less rapidly because of the limitation of dispersal along a two dimensional plane (Hardy & Vekemans 1999). This may be relevant in riparian habitat because of the linear population structure.

Thinning has also been documented to reduce genetic structure in some (e.g. Hamrick et al. 1993), but not other (e.g. *Quercus robur*, Hampe 2004), species. In this study, the recruitment neighbourhoods within riparian neighbourhoods are older than those in the floodplain neighbourhoods (this was unavoidable as the different habitat promotes different patterns of recruitment). Riparian neighbourhoods may, therefore, exhibit reduced genetic structure due to earlier thinning. The relatively high density coupled with high genetic diversity in riparian neighbourhoods, however, indicates this is unlikely to be the case.

6.5.2 Population genetic structure

In Vekemans and Hardy's (2004) reanalysis of spatial genetic structure in a wide range of species, the *Sp* statistic for predominantly outcrossing tree species ranged from 0.039 in *Vouacapoua americana* to 0.002 in *Quercus robur*. Population level spatial genetic structure in the *E. camaldulensis* remnants sampled here ranged between $Sp = 0.051$ in the Sandy Hollow population to $Sp = 0.004$ in the Bylong population. At the population scale (50m distance classes) the degree of genetic structure detected in Sandy Hollow and Camyr Allyn was high relative to other outcrossing species. However in the Bylong population (and to a lesser degree the Lemington population), there was no pattern of declining kinship with distance class (the mean kinship coefficient in the first distance class was negative).

In Camyr Allyn the significantly higher mean kinship in the first distance class (50m) at the population scale was consistent with the high degree of kinship detected within the floodplain neighbourhood. Similarly, the absence of genetic structure in the riparian neighbourhood at Bylong was mirrored at the population level, where no genetic structure was detected. This suggests that genetic patterns established early-on were persistent in these populations.

In Sandy Hollow, the riparian neighbourhood exhibited a total absence of genetic structure, (Sp = -0.002), however strong genetic structure was detected at the population level (Sp = 0.05). Although the majority of the Sandy Hollow population is located along the river margin, a number of trees are also located on an adjoining floodplain. Genetic structure within the floodplain component of this remnant may have resulted in spatial genetic structure at the population level despite riparian neighbourhoods exhibiting negative Sp values. In the Lemington remnant, negligible spatial genetic structure was detected. However, this population also incorporates floodplain and riparian habitat. It is possible, therefore, that remnants encompassing multiple habitats exhibit variable genetic structure. To explore this hypothesis, it would be valuable to sample exhaustively several population remnants that incorporate both habitat types.

The different level and structure of genetic variability within floodplain and riparian neighbourhoods suggests divergent processes are influencing genetic structure. However, the results are not congruent with those reported in previous work (Chapter 5). In Chapter 5, significant negative correlations were found between the levels of genetic diversity and the edge to area ratio indicating that riparian habitat exhibited less genetic variability than floodplain habitat. Given the results here, it does not appear that these patterns are initiated by divergent recruitment strategies. It is likely that other, unmeasured aspects of the habitat type, and/or the demographic structure of the remnants within them may promote the observed patterns of genetic diversity.

6.5.3 Population and neighbourhood scale genetic structure

When population scale and neighbourhood scale samples were combined, all populations exhibited low Sp statistics. In this analysis, mean pairwise kinship was investigated at ten metre intervals; most neighbourhood samples were in the first and

second distance classes. In the combined analyses the mean kinship within distance classes was idiosyncratic and often higher in larger distance classes (e.g. in the Sandy Hollow population, individuals in the 50 m distance class exhibited the highest mean kinship). When the sampling is broken down into 10 m intervals it becomes apparent that individuals separated by distances up to 100 m may exhibit at least the same degree of kinship as individuals in smaller distance classes. This demonstrates the degree of spatial genetic structure detected by autocorrelation analysis is relative to the total sample (Vekemans & Hardy 2004).

6.5.4 Spatial genetic structure and genetic diversity

The degree of genetic structure detected in spatial autocorrelation is influenced by the level of genetic variability within a population (Loveless & Hamrick 1984). The results from a previous research (Chapter 4) indicate that Sandy Hollow exhibited the highest number of private alleles with frequencies greater than a single occurrence. This resulted in the highest expected heterozygosity (He = 0.81) among thirteen populations. The detection of a high level of spatial genetic structure at the population level in Sandy Hollow may be due to the high level of genetic variability. High levels of genetic variability increase the capacity of molecular markers to detect local mating. If there is a high degree of homoplasy, or at least a high degree of similarity between initial founders, spatial genetic structure within populations might be underestimated because of our incapacity to differentiate between recent relations and historical founders i.e. spatial genetic structure might be accumulating due to restricted mating but the wide dispersion of common alleles makes unrelated individuals appear related, thus obscuring genetic structure. The mean level of genetic distance was considerably higher in the Sandy Hollow population and this higher relative genetic distance (enabled by higher diversity) provided a more pronounced comparison with the least related pair – this is perhaps an important phenomenon to consider when the objective is to compare spatial genetic structure between populations with divergent histories.

6.5.5 Inbreeding coefficient

The positive inbreeding coefficient and the positive intra-individual kinship measures detected here indicate that, both at the neighbourhood scale (with the exception of SHr and L/Nf) and at population scale, the standing population exhibited higher

levels of homozygosity than would be expected if the population were mating at random. Although the seed analyses only measured a single reproductive event and should be assessed with caution it was surprising that the inbreeding coefficient of the seed cohort was negative and/or near to zero. This is similar to that found in tree seeds (distinct from seedbank) in *Cecropia obtusifolia* (Alvarez-Buylla et al. 1996) but the opposite of what has been detected in other studies (e.g. increased inbreeding coefficient in progeny from fragmented populations, Aguilar et al. 2008). In numerous other studies the inbreeding coefficient is high in seed cohorts and decreases throughout the lifecycle (e.g. between seed bank and adults in *Cercropia obtusifolia*, Alvarez-Buylla et al. 1996), presumably due to differential selection against less fit genotypes. The change between a negative inbreeding coefficient in the seed, to a positive inbreeding coefficient in the recruitment neighbourhood may occur by chance, and this is not unlikely given the wide range of outcrossing estimates detected among individual eucalypt trees (Griffin et al. 1987; Butcher & Williams 2002; McDonald et al. 2003; Byrne et al. 2008). Earlier research in *E. camaldulensis* populations reported a nearly significant positive correlation between the level of genetic diversity within populations and the inbreeding coefficient (Chapter 5). This result suggested an increase in the number of alleles within a population was not accompanied by a corresponding increase in heterozygosity; this pattern could be explained by the Wahlund effect (Lowe et al. 2004). Another possible explanation for these discordant results could be that selection is occurring against heterozygous progeny. Such filtering of heterozygous genotypes might result from outbreeding depression. Outbreeding depression has been reported in this species to explain negative correlations between fitness and family outcrossing rates (Butcher & Williams 2002). Butcher and Williams (2002) suggested outbreeding depression in *E. camaldulensis* might result from the low fitness value of heterozygous combinations that included alleles with hybrid origins.

6.6 Conclusion

While additional recruitment neighbourhoods in each habitat type would be required to test for statistically significant differences, the data presented indicate that floodplain and riparian neighbourhoods may exhibit different levels of genetic structure and diversity and this may impact upon genetic structure at the population

level. The genetic structure and restricted dispersal in floodplain neighbourhoods was persistent in the Camyr Allyn population (floodplain) while the absence of genetic structure within riparian neighbourhoods was also reflected at the population scale in the population at Bylong (Sp = 0.0042). However, consistent results were not detected in all remnants populations. Although genetic patterns indicate limited dispersal in the floodplain recruitment neighbourhood at Lemington, the predominantly floodplain population exhibited limited genetic structure while at Sandy Hollow (that is predominantly riparian) a high level of genetic structure was detected. Both the Sandy Hollow and Lemington sites include both a floodplain and a riparian component, indicating spatial genetic structure may be variable within populations. While remnant populations that incorporate multiple habitat types gave inconsistent results, the preliminary results from Camyr Allyn (floodplain) and Bylong (riparian) indicate that the type of habitat may be a driving force in the generation of spatial genetic structure in *E. camaldulensis*. If so, management of populations in alternate habitat may require alternative management strategies. For example maintaining suitable recruitment conditions in the seed rain zone in floodplain habitat where seed does not migrate very far may be a priority. Where suitable recruitment conditions are not able to be met, seed collection could be considered.

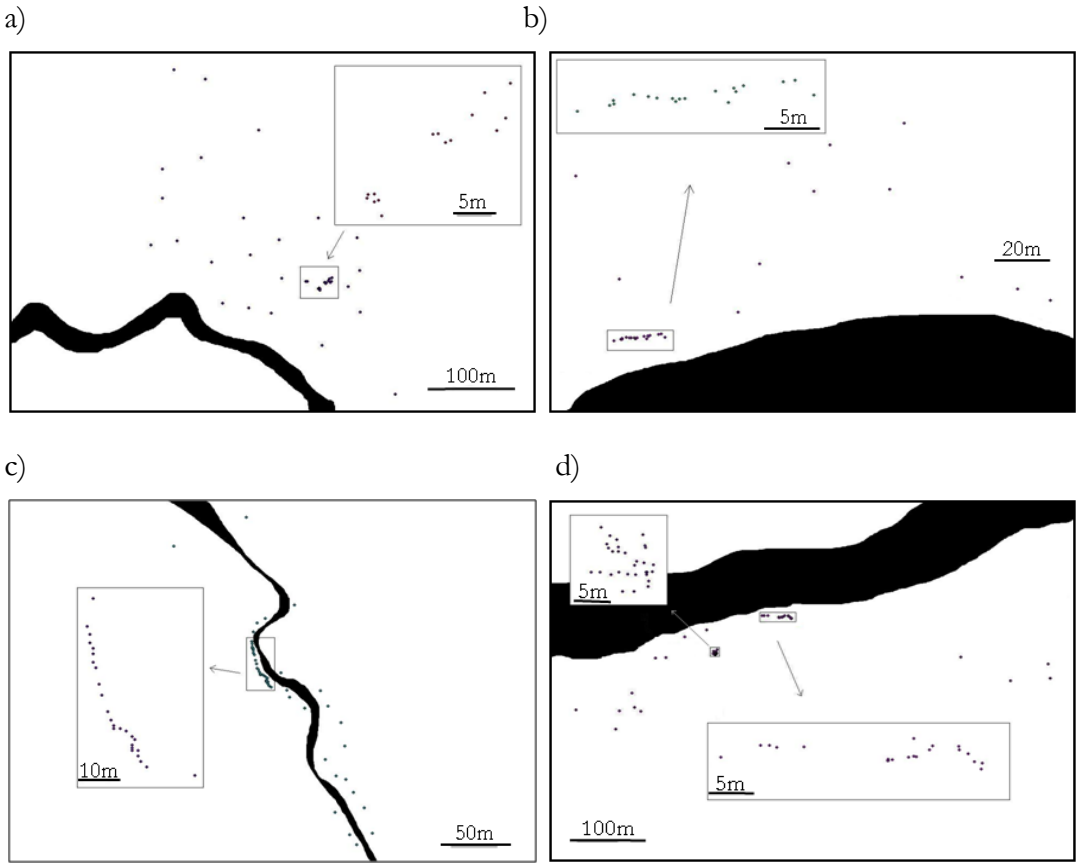


Figure 20. Maps of neighbourhood and population scale sampling units in four remnant populations of *Eucalyptus camaldulensis*: a) Camry Allyn, b) Sandy Hollow, c) Bylong, and d) Lemington. Dark areas depict the river and enclosed areas are the mapped individuals in the neighbourhood scale sampling units

Table 10. The mean pairwise kinship coefficient (Loiselle et al. 1995) and standard error (SE) within eight distance classes in riparian and floodplain recruitment neighbourhoods (Nf = floodplain, Nr = riparian) within remnant populations (CA = Camyr Allyn, L = Lemington, B = Bylong and SH = Sandy Hollow) of *Eucalyptus camaldulensis* in the Hunter Valley. The number of sample and the number of pairwise comparisons are provided in brackets

Pop/sample	Distance class (m)									
	2	4	6	8	10	12	14	16	Intra	*Sp*
CA /Nf (15)	0.0951* (13)	0.0470 (9)	0.0500 (7)	0.0477 (7)	-0.029 (6)	0.1059 (5)	0.0126 (9)	-0.0712 (18)	0.3267*	0.0695
SE	0.0637	0.0509	0.0374	0.1033	0.0711	0.0382	0.0448	0.0267	0.0807	
L/Nf (30)	-0.0049 (104)	-0.0002 (186)	-0.0048 (122)	0.0213 (22)	0.0867 (10)	na	na	na	0.07233	0.0017
SE	0.0105	0.0061	0.0093	0.0169	0.0766	na	na	na	0.1222	
L/Nr (21)	0.0345 (13)	-0.0208 (6)	-0.0039 (14)	-0.0422 (16)	0.0368 (3)	-0.0328 (15)	0.0237 (10)	-0.0644 (8)	0.2725*	0.0023
SE	0.0342	0.0292	0.0328	0.0211	0.0615	0.0216	0.0414	0.0307	0.1010	
B/Nr (26)	0.0784 (9)	-0.1083* (21)	0.064* (21)	0.0422 (20)	-0.0626 (22)	-0.0232 (13)	0.0228 (18)	-0.0169 (12)	0.2884*	0.0056
SE	0.0301	0.0454	0.0201	0.0332	0.0347	0.0341	0.0427	0.0712	0.1039	
SH/Nr (19)	-0.0016 (18)	-0.0008 (18)	-0.0028 (21)	-0.013 (27)	-0.0467 (20)	0.0204 (10)	0.0609 (14)	0.033 (16)	0.0789	-0.0020
SE	0.0143	0.0401	0.0211	0.0203	0.0335	0.068	0.0367	0.0293	0.1032	

*denote significant values (those above and below the upper and lower 95% confidence limits based on 999 permutations), Intra = intra-individual kinship coefficient, *Sp* = the *Sp* statistic (Vekemans & Hardy 2004)

Table 11. The mean pairwise kinship coefficient (Loiselle et al. 1995) and standard error (SE) within five distance classes at the population scale (P) in remnant populations (CA = Camyr Allyn, L = Lemington, B = Bylong and SH = Sandy Hollow) of *Eucalyptus camaldulensis* in the Hunter Valley. The number of sample and the number of pairwise comparisons are provided in brackets

Pop/sample	Distance class (m)						
	50	100	150	200	250	Intra	*Sp*
CA/P (26)	0.1057* (10)	-0.0184 (33)	0.0084 (58)	-0.012 (54)	-0.0178 (43)	0.1495*	0.0286
SE	0.0493	0.0181	0.0176	0.0116	0.0118	0.0510	
L/P (22)	0.0278 (24)	0.0102 (24)	0.0112 (30)	-0.0112 (30)	-0.0123 (16)	0.1794*	0.0151
SE	0.0251	0.0182	0.0209	0.0212	0.0200	0.0441	
B/P (23)	-0.0087 (52)	0.0077 (57)	0.0067 (50)	-0.0159 (38)	-0.01 (26)	0.2087*	0.0042
SE	0.0098	0.0101	0.0169	0.0098	0.0271	0.0525	
SH/P (12)	0.0538 (10)	-0.022 (17)	-0.0002 (24)	-0.0472 (10)	-0.1087 (3)	0.1850*	0.0513
SE	0.0191	0.0187	0.0089	0.0150	0.0469	0.0429	

*denote significant values (those above and below the upper and lower 95% confidence limits based on 999 permutations), Intra = intra-individual kinship coefficient, *Sp* = the *Sp* statistic (Vekemans & Hardy 2004)

Table 12. The mean pairwise kinship coefficient (Loiselle et al. 1995) and standard error (SE) within distance classes in remnant populations (CA = Camyr Allyn, L = Lemington, B = Bylong and SH = Sandy Hollow) of *Eucalyptus camaldulensis* in which recruitment neighbourhoods in floodplain (Nf) and riparian habitat (Nr) have been analysed in conjunction with population scale samples (P). The number of sample and the number of pairwise comparisons are provided in brackets

Pop/sample	Distance class (m)											
	10	20	30	40	50	60	70	80	90	100	Intra	*Sp*
CA/P + CA/Nf	0.0741*	-0.0039	-0.0815*	0.0211	-0.0045	-0.0059	-0.0287	-0.0003	-0.0328	-0.0128	0.2143*	0.0080
(41)	(44)	(35)	(42)	(19)	(19)	(9)	(18)	(29)	(22)	(22)		
SE	0.0257	0.0169	0.0384	0.0508	0.0445	0.0340	0.0324	0.0280	0.0293	0.0415	0.0471	
L/P +L/Nr (44)	0.0279	-0.0122	0.0402	0.0013	-0.0131	0.0038	0.0293	0.0598	-0.0475	-0.0016	0.228*	0.0046
	(78)	(91)	(59)	(48)	(55)	(54)	(24)	(12)	(9)	(6)		
SE	0.0139	0.0081	0.0177	0.0224	0.0264	0.0224	0.0231	0.0222	0.0331	0.0297	0.0675	
L/P + L/Nf	0.027*	-0.0142	0.0084	0.1043	-0.0275	-0.0264	-0.0663	0.0116	0.0481	-0.0256	0.154*	0.0093
(52)	(464)	(57)	(17)	(6)	(47)	(25)	(29)	(9)	(7)	(62)		
SE	0.0085	0.0133	0.0425	0.0373	0.0217	0.017	0.0329	0.0291	0.0396	0.0124	0.0815	
B/P +B/Nr	0.0026	0.0113	0.0046	-0.0056	0.0037	-0.0209	0.0119	0.0286	-0.0022	0.0086	0.2763*	0.0057
(49)	(109)	(97)	(98)	(99)	(75)	(55)	(60)	(37)	(37)	(47)		
SE	0.0098	0.0132	0.0115	0.0097	0.0146	0.0109	0.0105	0.0138	0.0081	0.0152	0.0736	
SH/P+ SH/Nr	0.0068	0.0515*	0.0353	0.0746*	0.1076*	-0.025	0.0136	-0.0432	-0.0193	0.0468	0.1531*	0.0155
(31)	(104)	(54)	(17)	(19)	(9)	(12)	(14)	(8)	(13)	(5)		
SE	0.0109	0.0208	0.0045	0.0104	0.0513	0.0147	0.0287	0.0298	0.0169	0.0355	0.0715	

*denote significant values (those above and below the upper and lower 95% confidence limits based on 999 permutations), Intra = intra-individual kinship coefficient, *Sp* = the *Sp* statistic (Vekemans & Hardy 2004).

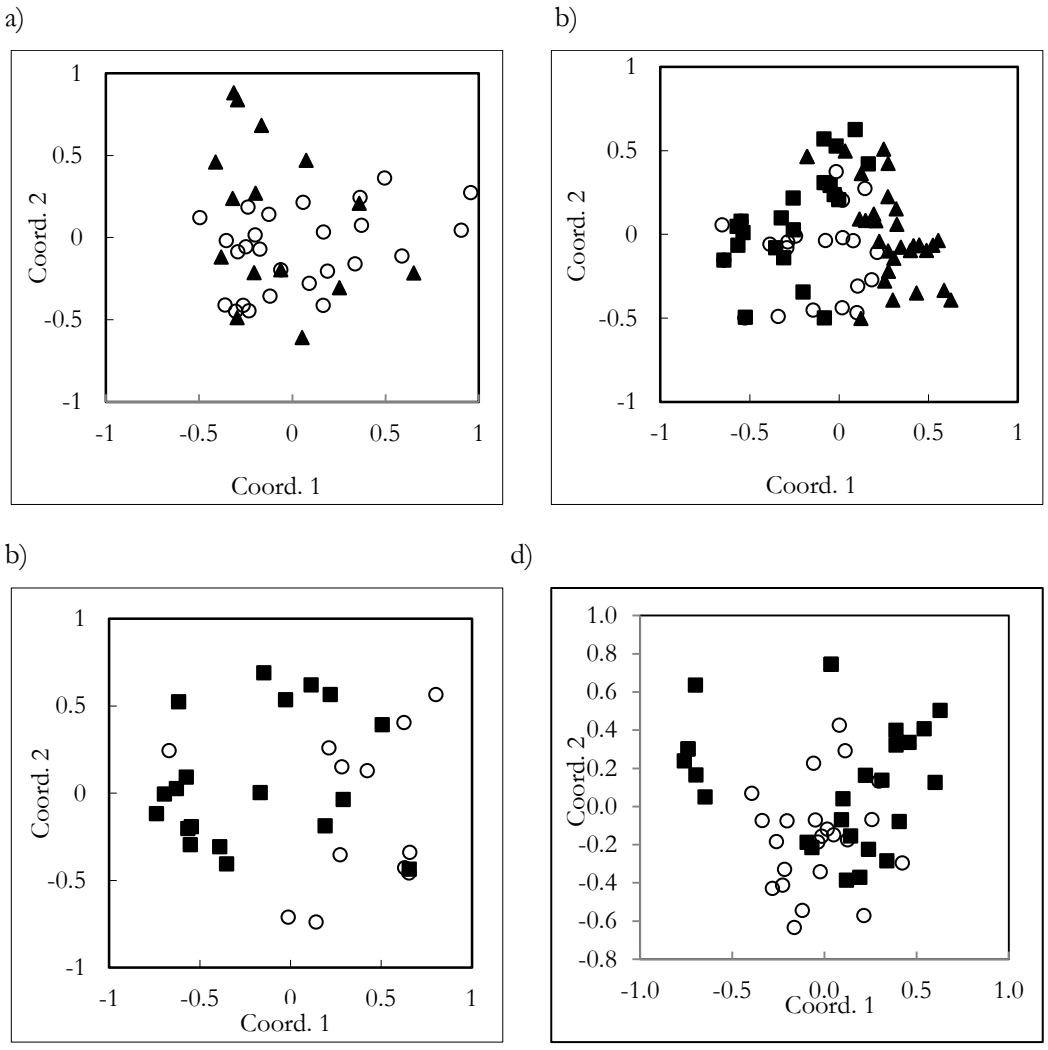


Figure 21. Principal co-ordinate analysis of genetic proximity of population scale samples and neighbourhood scale samples in four remnant populations of *Eucalyptus camaldulensis* **a)** CA/P (empty circle) and CA/Nf (filled triangle) samples (axis 1 = 21%, axis 2 = 20%), **b)** L/P (empty circle), L/Nr (filled square) and L/Nf (filled triangle) samples (axis 1 = 27%, axis 2 = 21%), **c)** B/P (empty circle) and B/Nr (filled square) samples (axis 1 = 24%, axis 2 = 21%) and **d)** SH/P (empty circle) and SH/Nr (filled square) samples axis1 = 30%, axis 2 = 20%)

Table 13. Genetic diversity measures calculated from four microsatellite primers in four populations (Camyr Allyn = CA, Lemington = L, Sandy Hollow (SH), and Bylong = Bylong) of *Eucalyptus camaldulensis*. Samples were at three scales. Diversity measures were calculated from seeds (a,b,c), from within recruitment neighbourhoods in floodplain (Nf) or riparian (Nr) habitat, and from samples taken across established populations (P). Above = mean value, below = Standard error

Pop/ sample	N	Na	Ne	I	Ho	He	UHe	F	D
CAa	21.25	6.00	2.76	1.23	0.71	0.63	0.65	-0.12	5.20
	0.75	0.41	0.17	0.05	0.01	0.02	0.02	0.05	
CAb	22.00	6.00	2.26	1.05	0.54	0.52	0.53	-0.06	5.82
	0.91	1.47	0.36	0.20	0.09	0.08	0.08	0.12	
CAc	30.00	5.75	2.26	1.03	0.55	0.54	0.55	-0.03	5.51
	0.00	1.03	0.25	0.08	0.04	0.06	0.06	0.04	
L/Nf	30.00	6.25	2.97	1.23	0.62	0.59	0.60	-0.04	4.73
	0.00	1.03	0.64	0.25	0.14	0.11	0.12	0.11	
CA/Nf	14.25	5.00	3.25	1.27	0.51	0.64	0.66	0.26	7.33
	0.25	0.71	0.66	0.21	0.14	0.10	0.10	0.13	
L/Nr	19.75	7.50	3.36	1.51	0.59	0.70	0.72	0.14	7.59
	0.63	0.29	0.24	0.04	0.10	0.02	0.03	0.17	
SH/Nr	19.00	7.50	3.95	1.59	0.78	0.74	0.76	-0.05	6.00
	0.00	1.71	0.26	0.13	0.04	0.02	0.02	0.07	
B/Nr	25.50	8.00	4.08	1.59	0.58	0.72	0.74	0.23	7.48
	0.29	0.82	0.79	0.18	0.12	0.06	0.06	0.12	
CA/P	26.00	8.25	4.28	1.59	0.56	0.71	0.72	0.23	7.14
	0.00	1.32	0.99	0.25	0.11	0.09	0.09	0.07	
B/P	21.50	8.25	4.04	1.61	0.64	0.71	0.73	0.11	7.30
	0.96	0.85	0.79	0.20	0.11	0.08	0.08	0.08	
L/P	21.00	7.75	4.22	1.65	0.69	0.75	0.77	0.08	7.33
	0.41	1.03	0.60	0.14	0.04	0.04	0.04	0.05	
SH/P	11.75	7.25	5.31	1.80	0.70	0.81	0.85	0.14	8.26
	0.25	0.63	0.30	0.07	0.05	0.01	0.01	0.05	

N = sample number, Na = mean number of alleles per locus, Ne = the mean number of effective alleles, I = Shannon's Diversity Index, Ho = observed heterozygosity, He = expected heterozygosity, UHe = the unbiased expected heterozygosity, F = the inbreeding coefficient and D = mean pairwise genetic distance within sampling unit. Dark shading indicates high values while light shading denotes low values

7 Heterozygosity associated with superior growth traits in *Eucalyptus camaldulensis* Dehnh. subsp. *camaldulensis* (River Red Gum): an evaluation of open pollinated seeds

7.1 Abstract

One hundred and sixty-eight genotyped seedlings were monitored over a sixteen-month period to investigate the correlation between genotype and seed/seedling traits. Four microsatellite loci were amplified and the genotype of seedlings was described by estimating the individual outcrossing rate (tm), paternal origin (selfed versus outcrossed) and the degree of heterozygosity (proportion of heterozygous/ homozygous loci). No seed traits (seed weight, germination) were significantly associated with genetic traits. Both the mean individual outcrossing rate (tm) and the degree of heterozygosity were positively associated with a number of growth traits; however associations were strongest and more frequent between seedling traits and the degree of heterozygosity. Individual outcrossing rates of parent trees was highly variable (tm = 0.31–0.98) and the degree to which genotype reflected growth traits varied within the progeny of individual trees. Combined the results indicated that inbreeding may be operating in natural populations but the extent of inbreeding depression was highly variable. Inbreeding depression was detected both within selfed progeny and within less heterozygous outcrossed progeny indicating that genetic load of some mate-ships may induce inbreeding depression.

7.2 Introduction

Inbreeding depression is defined as the relative low fitness of inbred versus outcrossed progeny (Charlesworth & Charlesworth 1987). Inbreeding depression is a central concern in the conservation of small plant populations in which elevated levels of self and close sibling mating can occur. The reduced fitness of inbred progeny is thought to result from either the coming together of recessive deleterious alleles and/or the superiority of the heterozygote genotype (overdominance) (Keller & Waller 2002; Slate et al. 2004). However, outbreeding may also negatively impact upon progeny fitness (Young 2000); this is termed outbreeding depression.

Outbreeding depression is defined as the relative poor performance of individuals that are the result of distant crosses and crosses between different species (Fenster & Galloway 2000). Outbreeding depression results from the breakdown of adapted genotypes and/or the disruption of co-adapted gene complexes (Fenster & Galloway 2000). In many species pre-mating (e.g. pollinator specialization) and/or pre-zygotic (pollen incompatibility) mechanisms prevent disadvantageous and hybrid crosses (Scopece et al. 2010) subsequently avoiding outbreeding depression.

Eucalyptus camaldulensis is a long lived forest tree common in riparian habitat in Australia. In *Eucalyptus* reproductive barriers between species within subgenera are often weak (Griffin et al. 1988); it is not uncommon for closely related species to produce viable offspring (Griffin et al. 1988; Barbour et al. 2002; Potts et al. 2003; Barbour et al. 2005; Butcher et al. 2005). The ability to prevent, or select against hybrid or disadvantageous crosses (when flowering time and phenology do not prevent it) depends upon the breeding system. In *Eucalyptus camaldulensis* the combination of mass flowering, self-compatibility and insect pollination offers substantial opportunities for geitonogamous pollination (Moncur et al. 1995; House 1997) however; relatively low levels of self pollination have been reported. This has been observed repeatedly in eucalypt studies and hence most species are described as exhibiting preferentially outcrossing mating systems (Griffin et al. 1987; Pound et al. 2003b). Since few species exhibit pre-zygotic self-incompatibility systems (but see Sedgley & Smith 1989; Sedgley & Granger 1996; Pound et al. 2002), the trend of predominant outcrossing is thought to result from a post-zygotic incompatibility mechanism(s) (James & Kennington 1993; Martin & Lee 1993; Pound et al. 2003a). Several authors have suggested that selective seed abortion may be operating in some eucalypt species (Griffin et al. 1987; James & Kennington 1993; Sedgley & Granger 1996; Pound et al. 2003a) and two theories, which potentially operate simultaneously (Griffin et al. 1987; Martin & Lee 1993), have been offered to explain the mechanism. The first suggests that post zygotic abortion of seeds results from late acting inbreeding depression and is, consequentially independent of the relative abundance of selfed versus outcross progeny (Martin & Lee 1993). The alternative, that resource allocation or sequestration of resources depends upon the genetic quality of seed (Martin & Lee 1993) is compelling. It indicates that those seeds with a higher likelihood of successful germination and growth should be favoured

irrespective of paternity. This mechanism could potentially buffer a population from the negative effects of both inbreeding and outbreeding depression.

Inbreeding depression is well documented in eucalypt species (Potts et al. 1987; Griffin & Cotterill 1988; Hardner & Potts 1995) but previous research indicates that outbreeding depression may also occur within *Eucalyptus*. In a plantation that contained a random arrangement of *E. regnans* trees sourced from two provenances, Burczyk et al. (2002) found that pollination was three times more likely between individuals from the same origin. Although the authors attributed their results to flowering phenology, substantial overlap in flowering time could indicate that lower rates of outcrossing between provenances had a genetic cause. This could be indicative of intra-species outbreeding depression. Post zygotic seed abortion has also been demonstrated to occur at higher rates following hybridisation in a range of *Eucalyptus* species (Drake 1975) indicating the performance of hybrid genotypes was inferior and they were subsequently selected against. Evidence of outbreeding depression has also been detected in a study conducted by Butcher and Williams (2002) who reported a negative correlation between the family outcrossing rate and fitness in *E. camaldulensis,* indicating that high outcrossing rates could be associated with inferior fitness.

Throughout my research, some unusual genotypic patterns were detected that could be indicative of outbreeding depression. Genetic variability in *E. camaldulensis* populations was positively (although not statistically) correlated with the inbreeding coefficient (Chapter 5) indicating a higher proportion of homozygotes in relatively genetically variable populations compared with genetically depauperate populations. Homozygosity of this nature might be promoted by strong genetic structure (i.e. inbreeding within close neighbourhoods, Wahlund effect), the presence of null alleles or a high number or rare alleles. Alternatively, homozygous excess might be promoted where there is selection occurring against heterozygotes. Another component of my research detected low inbreeding coefficients in the progeny arrays from single parent trees than in the established population (Chapter 6). This is the opposite of what is usually detected and could indicate that heterozygous progeny are selected against throughout the life-cycle.

In this study seed and seedling traits of open pollinated seed in fragmented remnants of *E. camaldulensis* were tracked and assessed against their genotype. The aim of the

research was to determine the degree to which selfed progeny were homozygous, and how the degree of homozygosity was related to the various growth traits of the seedlings. The genotype of parent trees was also assessed against the mean performance of their seedlings to ascertain how adult genotype impacted upon reproductive success. The results are discussed in relation to the contribution of individual genetic variability to remnant population viability.

7.3 Methods

7.3.1 Sampling

Nine mature trees were selected from three remnants of *Eucalyptus camaldulensis* (three trees per remnant) within the Hunter Valley catchment area in New South Wales: Camyr Allyn, Dartbook and Denman. A subsample of capsules (7–14) was selected from the lower canopy (2–6m) of each tree to give a total of 94 capsules. Selected capsules were mature but not dehisced. Capsules were placed into individual bags. Leaf material from the maternal trees was also collected and dried in silica gel for DNA extraction. Seeds were dehisced from capsules in paper bags over a two-week period.

7.3.2 Tree, capsules and seed measurements

7.3.2.1 Capsules

Capsules from each tree were emptied and the seed was separated from the chaff. The total number of seeds was counted and chaff and seed components were weighed separately. Chaff was defined as ovulodes, and seeds included all seeds with embryonic material and included seeds that appeared to have been prematurely aborted. All seeds (and chaff component) from the capsules were placed on labeled Petri dishes and placed in a growth chamber. The growth chamber had a consistent temperature of 24°C and 12 hours light/dark (Bell 1999). The number of seeds germinated per capsule was calculated by determining the proportion (n germinated seeds/total seeds) per capsule. Although ovulodes were easily differentiated from chaff via visual inspection, chaff was also placed in the growth chamber. If any of the 'chaff' germinated it was added to the number of seeds germinated for that capsule since initially it had been inaccurately identified. After 40 days seeds that had not germinated were counted and discarded

7.3.2.2 Individual seed and seedling measurements

From each of the above capsules up to ten seeds (some capsules contained less than 10 seeds) were randomly selected and weighed independently (herein the labeled seeds). Additional data was collected for labeled seeds (689 seeds). Time to germination was calculated by checking seeds daily. Seeds were considered to have germinated when both the radical and the cotyledons had emerged. Labeled seeds were grown in the growth chamber for a two-week period after germination. After two weeks seedlings were transferred into the greenhouse where they were grown under controlled conditions (constant temperature of 25°C, watered once daily) in a randomised arrangement. The height, the number of leaves and the length of the longest leaf were measured twice-monthly for four months, leaf number and plant height were then collected at six-month intervals. At ten months, a qualitative assessment of condition was conducted which gave a value (0–5) based on the inspection of the plants. Low values were given to seedlings that exhibited reduced vigour including: defoliation, stunted growth, reduced leaves and discoloration. After the 16-month monitoring period saplings were removed from pots and above and below ground biomass was separated. Samples were dried in an oven over a six-day period at 70 degrees centigrade. Following drying above and below biomass was weighed.

7.3.2.3 Offspring fitness of individual parent trees

The reproductive success of parent trees was calculated by determining the mean value of seed (e.g. weight) and seedling growth (e.g. height) traits of all progeny from parent trees. The standard error of all seed and seedling growth traits was also calculated to quantify the level of variation in the progeny produced by individual trees. To provide a measure of the total performance of seedlings sourced from a parent tree, individual trait measurements were combined to give a mean seedling per parent tree value. Since variation in leaf number and leaf length were independent of plant height, a combined measure was used to summarise plant growth attributes.

Mean seedling = (n leaves x mean leaf length) + height

This formula was designed to enable relative comparisons of vegetative traits of seedlings between parent trees. In the six monthly assessments, where leaf length was omitted, height and leaf number were summed.

Finally, since the variation between the reproductive successs of trees was a product of both the quality and quantity of progeny, an *ad hoc* measurement of total reproductive success was estimated by multiplying the mean total fitness of progeny by the number of progeny produced per parent tree divided by the number of capsules sampled.

Reproductive success = fitness x (n progeny/n capsules)

7.3.3 Material collection and DNA extraction

Total genomic DNA was extracted from parent trees and their progeny. Throughout the monitoring period seedlings from weighed seeds that were failing to grow were removed and placed in silica gel. A small sample (approx. 2 cm^2) of leaf material was also collected from live saplings in the greenhouse and dried in silica gel in preparation for DNA extraction; therefore genotyping of seedlings included only those seeds that germinated, although they may not have survived the entire sampling period. Total genomic DNA was extracted from 20mg of silica dried material of both maternal trees and progeny using the DNeasy plant mini kit. The presence of DNA was confirmed by running 10 μL of DNA in 1% agarose gel at 200 volts for one hour.

7.3.4 Polymerase Chain Reaction

Four primers (Appendix 1) were selected from the EMBRA (Brondani et al. 2006) and Eg (Benson et al. 2008) marker sets developed for *Eucalyptus* species within the *Symphomyrtus* subgenus. Primers were selected that had previously yielded high quality and variable product. Primers were tagged with fluorescent markers. Polymerase Chain Reaction (PCR) was performed for each primer separately. The reaction mixture per individual per primer pair was: Buffer 2.5 μL, Mg++ 3.0 μL, DNTPs 0.5 μL, FWD primer 0.25 μL, RVS primer 0.25 μL, Taq polymerase 0.2 μL, template DNA 1 μL and H_2O 17.3 μL to give a total volume of 25 μL. Regions were amplified using a PC–960 Thermo Cycler. For program details see Appendix 2. Five micro-litres from each PCR were combined and samples were quantified using capillary gel electrophoresis. Results were visualised and scored using the Genemarker@ software (Softgenetics).

7.3.5 Estimation of individual and family outcrossing rates and genetic variability

Three individual genotypic measures were calculated: mean multi-locus outcrossing rate, self/outcross and heterozygosity. Individual multi-locus estimates of outcrossing (tm) were calculated for all individuals using the method of moments method calculated in the program MLTR version 3.2 (Ritland 2002) with known maternal geneotypes. Individuals were then designated as either selfed or outcrossed. Individuals that exhibited tm values greater than or equal to one were considered as outcrossed while individuals with an outcrossing estimate less than one were considered to be the product of self fertilization. Individuals with tm values less than one contained no alleles that were not also detected in the parent. Although occasionally a designated 'self' individual may actually be the result of mating between very similar partners (i.e. close relative) this is likely to be rare. Heterozygosity was calculated as the ratio of heterozygous loci divided by the total number of loci amplified.

Family outcrossing rates of the parent tree were also estimated using the method of moments method (Ritland 2002) while the inbreeding coefficient and the level of genetic diversity (He) were calculated in the program GenAlEx 6.1 (Peakall & Smouse 2006).

In trees which produced more than five selfed progeny, and in which the parent trees exhibited heterozygosity at one or more loci, the single locus expected heterozygosity was calculated based on Mendelian inheritance of the parent genotype. The expected heterozygosity was then compared with the observed heterozygosity to determine if the heterozygosity within selfed individuals was greater than would be expected if the development of progeny were random.

At the population level, maximum likelihood procedures were used to estimate single and multi-locus estimates of outcrossing (ts and tm respectively). The difference between tm and ts (tm–ts) was calculated to estimate levels of biparental inbreeding while single and multi-locus estimates of correlated paternity were also calculated (rps and rpm). Population level measurements were calculated in the program MLTR version 3.2 (Ritland 2002).

7.3.6 Fitness and genotype interaction

7.3.6.1 Individuals

The survival of seedlings was monitored and a percentage survival rate was calculated for individuals designated as outcrossed and those designated as selfed. Survival was defined as being alive at the end of the 16 month sampling period.

Correlation analyses were conducted to measure the association between individual genetic attributes (outcrossed versus selfed, and the proportion of heterozygous loci) and seed/seedling traits. Three approaches were used. The first analyses assessed the correlation (Pearson's r) between all seed/seedling attributes against genotype (the multi-locus outcrossing rate (tm), proportion of heterozygous loci). This analysis pooled individuals from all remnants (Camyr Allyn, Dartbrook, and Denman). The second set of correlation analysis was conducted with seedlings grouped by parent trees. This analysis was performed to estimate the level of correlation (Pearson's r) between genotype (outcrossing rate (tm), proportion of heterozygous loci) and seed/seedling traits within parent trees. The third set of correlation analyses was conducted with individuals grouped by paternity (selfed versus outcrossed). These analyses assessed the degree of correlation between genotype (heterozygosity) against seed/seedling traits within selfed and outcrossed progenies. Inter-trait correlation (Pearson's r) was also assessed to determine if early seedling traits were predictive of later stage seedling performance (e.g. correlation between the first height measurement and subsequent height measurements taken at over time). The significance of correlation was tested by a two tailed significance test. All correlation analyses were conducted in the statistical program JMP version 8.02 (SAS Institute, Cary, NC). T-tests were performed to test for significant differences between mean growth traits of selfed versus outcrossed seedlings.

7.3.6.2 Parent trees

Correlation (Pearson's r) analysis was conducted to explore the hypothesis that the outcrossing rate (tm) of parent trees influenced the mean seed and growth traits, mean seedling fitness, and mean reproductive success. The significance of correlation was tested by a two tailed significance test. Correlation analysis was conducted in the statistical program JMP version 8.02 (SAS Institute, Cary, NC).

The difference between seed and growth traits between parent trees were compared via a fixed effect one-way ANOVA. Differences were tested for significance using the Tukey Kramer test. All statistical analyses were calculated using the program JMP version 8.02 (SAS Institute, Cary, NC).

7.4 Results

7.4.1 Individual level analyses

One hundred and sixty-eight seedlings were genotyped from eight individual trees (DRT3 tree failed to produce any viable seed). A substantial portion (43%) of germinated seed did not survive the sampling period. Complete data sets were only available for 95 seedlings from eight parents.

Of the 168 seedlings genotyped 122 were designated outcrossed (tm ≥1) while 46 were estimated to be the result of self-fertilization (tm <1). Throughout the trial period a greater proportion (45%) of outcrossed seedlings died before the end of the sampling period compared with selfed seedlings (28%).The range of heterozygosity was between 0 (no loci heterozygous) to 1 (all loci heterozygous) in both selfed and outcrossed progeny. The proportion of heterozygous loci in selfed progeny wad 0.30 compared with 0.60 in the outcrossed progeny. The degree of heterozygosity within selfed progeny did not exhibit any significant deviation from that which would be expected from the parental genotype.

When the growth traits of saplings were tested for correlation against individual genetic parameters (multi-locus outcrossing rate (tm), proportion of heterozygous loci), several significant correlations were detected (Table 14), however significant correlations were much more frequent between growth traits and the degree of heterozygosity than between growth traits and the multi-locus outcrossing rate (Table 14).

The level of correlation between seed/growth traits and genotype (proportion of heterozygous loci, multi-locus outcrossing rate) within trees was inconsistent and predominately weak (Appendix 5). However, where they occurred the correlation was stronger between heterozygosity and growth traits rather than the multi-locus outcrossing rate. This was particularly evident in the DRT2 tree (Table 15 and Figure 25).

When the correlation between growth traits and degree of heterozygosity was calculated with seedlings grouped as either selfed or outcrossed there was no consistent correlations across traits. However highly significant correlations were detected between above and below ground biomass and heterozygosity in the selfed group ($p = 0.0025$ and $p = 0.0082$ respectively) (Appendix 4).

Pairwise t-tests that compared seed and seedling traits between selfed and outcrossed progeny detected significantly greater height measurements (p range 0.002–0.026) in the outcross progeny in the majority of comparisons (55%). A number of early leaf traits were also significantly greater in the outcrossed progeny. However, there was no significant difference in either the condition rating or the final measures of biomass. Correlations between the same growth traits over time diminished indicating that earlier measures of growth traits were not good indicators of later stage seedling condition (Figure 22).

7.4.2 Parent tree analyses

The method of moment's estimator of multi-locus outcrossing rate indicated variable outcrossing between trees (Table 16). The lowest estimate (indicative of high levels of selfing) was detected in the DEN3 tree ($tm = 0.313$) while almost total outcrossing was detected in CAB2 ($tm = 0.981$). Intermediate values were detected in other trees (Table 16). Heterozygosity was lowest in the trees sourced from the Denman populations (Table 16) and low levels of genetic variability were associated with high standard errors indicating considerable variation among progeny. The majority of inbreeding coefficients were negative indicating either allelic frequency close to Hardy-Weinberg equilibrium, or with an excess of heterozygosity.

The multi-locus outcrossing rate estimated for parent trees was correlated with mean below ground biomass and condition rating (Appendix 6) indicating that parent outcrossing rate influenced the quality of offspring. Correlation analyses between mean seed and seedling traits across trees indicated that there was a negative and significant correlation between the mean weight of seed per capsule and both the percentage of seed germinated, and the number of seed germinated (Figures 23 & 24). Variable significant correlations were detected between a number of mean growth traits but this was due to the tendency of tall trees to exhibit higher number of leaves etc (Appendix 6).

Two trees at Denman (DEN2 & DEN3) exhibited significantly lower outcrossing rates than all other trees sampled and this corresponded with a significantly lower heterozygosity in DEN3 compared with CAA and CAC ($p = <0.0001$ & $p = 0.0405$ respectively). The progeny from the DEN3 and DEN2 also exhibited the two lowest levels of genetic diversity as measured by the expected heterozygosity (Table 16). DEN2 and DEN3 trees also exhibited significantly lower seed weight; however, reduced seed weight was not a consistent indication of low outcrossing rates. Low weights were detected in progeny from outcrossing individuals from CA and DRT populations. Nonetheless significantly ($p<0.05$) lower values for various other sapling measurements were also detected in pairwise comparisons between DEN3 and other trees (Table 18). Intriguingly, significant differences commonly occurred between DEN1 and DEN3 with DEN1 exhibiting significantly higher values for a range of attributes. The DEN1 parent tree had a significantly higher outcrossing rate than DEN3, however the level of genetic diversity detected within progeny was the third lowest of all trees.

7.4.3 Population level analyses

Mating system estimates calculated within remnants indicated variable levels of outcrossing with moderately high levels detected in Dartbrook (DRT) and Camyr Allyn (CA) but a very low level in the population at Denman (DEN) (Table 17). However, the comparison between multi-locus and single-locus outcrossing rate indicated minimal levels of biparental inbreeding.

7.5 Discussion

The genotype of outcrossed progeny (tm ≥1) in this study exhibited a high level of heterozygosity. However despite the evidence of outbreeding reported in the *Eucalyptus* (Butcher & Williams 2002; Burczyk et al. 2002; Drake 1981) and the usual allelic patterns previously detected in my work (Chapters 5 and 6) the results presented here gave no indication that heterozygosity was associated with poor performance. Rather, positive correlations between various growth traits and heterozygosity suggested that high levels of homozygosity may result in inbreeding depression. While the performance of seedlings under controlled conditions can differ from performance in the wild these results are in agreement with previous

research reporting on inbreeding depression in *E. camaldulensis* and in other eucalypts (Griffin & Cotterill 1988; Hardner & Potts 1995; Potts et al. 1987).

7.5.1 Heterozygosity-fitness correlations

Heterozygosity-fitness correlations have been previously reported in *E. camaldulensis* (Aradhya & Phillips 1995) although the link between heterozygosity-fitness associations and inbreeding depression are difficult to establish. Dewoody and DeWoody (2005) demonstrated that the level of heterozygosity at a sample of loci is insufficient to describe heterozygosity of the genome, however, associations between heterozygosity and fitness attributes may arise under extreme circumstances (Balloux et al. 2004; Szulkin et al. 2010). Balloux et al. (2004) claimed that for heterozygosity-fitness correlations at neutral loci to develop positive associations because of inbreeding depression, inbreeding needs to be 'both frequent and severe'. Small plant populations are much more likely to conform to these conditions than the human populations discussed by Balloux et al. (2004) due to their ability to self pollinate and their sessile nature.

The degree of correlation between heterozygosity and the inbreeding coefficient calculated from pedigree data can be assessed to determine if heterozygosity reflects inbreeding (Balloux et al. 2004). However, such information is not available in natural populations. Although only a small number of loci were amplified in this study, genotyping of the parent tree enabled the outcrossing rate to be estimated in the progeny. Although it is possible that individuals exhibiting only alleles from the parent might still be outcrossed (i.e. alleles not identical by decent) those individuals with alleles different from their parents were much more likely to exhibit higher levels of heterozygosity. However, growth attributes were correlated with heterozygosity irrespective of paternity and increasing heterozygosity among outcrossed progenies was associated with increased fitness. This is illustrated effectively in Figure 25 which describes the heterozygosity and height correlation between the progeny of the parent tree DRT2. This is important as it indicates that the degree of heterozygosity that results from mating pairs can impact upon the fitness of progeny. Thus the type of mates available can have a substantial impact upon the growth traits of progeny. This effect was variable between trees indicating that the susceptibility to fitness-related-declines associated with homozygosity was not consistent. It was most pronounced in the DRT2 tree, which exhibited a high

multi-locus outcrossing rate. Variable inbreeding depression has been documented in other species (Armbruster & Reed 2005) and may be attributed to divergent mating histories and variable genetic load in individual lineages; this is also likely to be an important causal factor in *E. camaldulensis.*

7.5.2 Individual outcrossing rates

The outcrossing rate and number of seed set was highly variable between trees. Almost total outcrossing was detected in most trees, however, two trees (DEN2 & DEN3) exhibited very low outcrossing estimates; substantially lower than has previously been recorded in this species (0.313 versus 0.60 in Butcher et al. 2002). Another tree failed to set any viable seed. High variation between family outcrossing rates within species is not uncommon and substantial variation has been documented in a number of *Eucalyptus* species (Griffin et al. 1987; Butcher & Williams 2002; McDonald et al. 2003; Byrne et al. 2008). Lowered outcrossing rates have been detected in fragmented versus continuous habitat (Aguilar et al. 2008) and increased self pollination has been detected in isolated eucalypt species (Butcher et al. 2005), thus the variation reported here may in part be due to fragmentation effects.

While the low outcrossing rate detected in this study may be influenced by the low canopy position of the capsules sampled, the outcrossing rate may also be impacted by habitat fragmentation. Fragmentation may impact upon the behavior of pollinators subsequently impacting the level of outcrossing. Although many flowers are open on a single tree at the same time offering substantial opportunity for geitonogamous pollination, (Moncur et al. 1995; House 1997) pollination still requires pollen to be distributed onto the stigma by a pollinator. The stigma does not become receptive until several days after pollen has dehisced subsequently limiting within flower pollination (Penfold 1961). Thus self pollination is not automatic and the rate of both self and outcross pollination is likely to be affected by pollinator type and abundance. Various environmental factors can impact upon pollinators (Hardner & Potts 1995; Aizen & Feinsinger 1994a). In the Hunter Valley, the variable land use surrounding remnant populations may influence pollinators while the distance and degree of connectivity between remnants is also likely to be important. A detailed investigation into pollinator type efficiency and abundance across fragmented habitat would greatly increase our understanding of pollination dynamics. Although eucalypts are purported to be pollinated by generalist pollinators (Southerton et al.

2004b) a detailed examination of pollinators in *E. camaldulensis* is missing. Determining which generalist pollinators are effecting pollination and their abundance in the landscape could assist in assessing fragmentation effects (e.g. increased introduced honey bees in fragmented systems, Aizen & Feinsinger 1994a).

Variation in the outcrossing rate between individuals in eucalypts has also been attributed to variable levels of self fertility (Patterson et al. 2004). In this study, the DRT1 tree exhibited substantially lower seed set than any other tree (bar DRT3 which set no seed), and only a portion of seed germinated successfully. The fact that some seed was set in the DRT1 tree indicated that flowers were visited by a pollinator and it is likely that the pollinator carried at least a portion of self pollen. The failure to set adequate seed set could, therefore, indicate that fertilisation and growth of seed via self pollen was prevented because the genotype of selfed progeny was not viable. The attrition of selfed zygotes where resources are not limited (i.e. where inclusion of selfed progeny would bolster overall recruitment) suggests that the abortion of seed is not always selective. Exploring the genotype of aborted seed would enable a much more informative insight into the post zygotic dynamics in natural populations.

The degree of outcrossing detected may also be influenced by where in the canopy the samples are sourced. Patterson (2004) demonstrated higher and more consistent outcrossing in the upper compared with the lower canopy. It was not possible to sample the top of the canopy in this study because of the height of the trees. Samples were consistently sourced from the lower part of the canopy (2-6m) and hence estimates of outcrossing may be biased downward.

7.5.3 Population outcrossing rates

Although only a limited number of trees were sampled in each remnant, preliminary results suggested the reproductive strategies in remnant populations were variable. Very low levels of outcrossing were detected in two individuals from the Denman population while overall seed set was greatly reduced in two individuals within the Dartbrook population. In contrast, all individuals within the Camyr Allyn population produced substantial numbers of outcrossed progeny. These results are congruent with results of earlier work where Camyr Allyn (one of the largest populations within the catchment) exhibited high levels of genetic variability conducive to high

outcrossing, and the Denman population (DEN) exhibited the highest inbreeding coefficient (F = 0.21) (Chapter 4) consistent with the low levels of outcrossing detected here. This suggests that population level genetic attributes might be useful indicators of individual reproductive processes.

From a conservation perspective, if populations vary in their degree of self-compatibility, then the susceptibility to genetic decline is also likely to vary between populations. Those species most susceptible to genetic decline are those that tend toward obligate outcrossing (Honnay & Jacquemyn 2007; Aguilar et al. 2008); where mating patterns are variable between populations of the same species, it may be necessary to understand the variation to ascertain the risk of genetic decline.

7.5.4 Selective seed abortion

Under selective seed abortion, progeny are hypothesised to be developed preferentially, with high quality seeds favoured over poor quality seeds. James and Kennington (1993) found evidence to support this phenomenon with a higher level of heterozygous offspring within self-pollination treatments than would be expected if progeny were developed randomly. In the current study, where heterozygosity appeared to be associated with increased growth traits, preference for the more heterozygous selfed progeny would be expected. The proportion of heterozygous to homozygous loci in the self pollinated portion of seed did not indicate any significant deviation in the direction of increased heterozygosity from that which would be expected given the maternal genotype. This indicated selfed seeds were a random selection of selfed progeny. This was contrary to the results presented by James and Kennington (1993).

7.5.5 Growth and seed traits

The negative correlation between both the number of seeds germinated per capsule, the percentage of seed germinated per capsule, and the mean weight of seeds within capsules (Figure 23 &24) indicates that the production of a large number of small seeds results in a higher levels of germination. This could enhance the possibility of successful germination in suitable habitat in those trees who adopt this strategy.

The pattern of decreasing correlation of between seedling traits (both within trait and between) indicates that the early vegetative characteristics are not an accurate

indication of the performance of older saplings. This is consistent with other research that indicates that the performance of trees varies temporally (Charlesworth & Hughes 1996). However, the negative and significant correlation between the percentage of total seed germinated and the total reproductive success indicates the initial distribution of resources to seed may have some impact on the survivorship and vitality of offspring.

7.6 Conclusion

The result of this study indicated that the outcrossing rate was positively correlated with a number of growth measures (e.g. height). This indicated that selfed progeny may be sensitive to inbreeding depression. The correlations were stronger, however, between the degree of heterozygosity (proportion of heterozygous loci) and seedling traits. Correlations were also variable between trees possibly indicating variable genetic load. Heterozygosity x growth trait correlation was the strongest in the predominantly outcrossing DRT2 tree. This suggests that outcrossing will not prevent inbreeding if mating is sufficiently restricted. The low seed set per capsule in some individuals suggests that seed set is inhibited under certain circumstances, but it is unknown if self infertility, predation or a lack of adequate pollination services is responsible. Substantial variation of the outcrossing rate was detected with several trees exhibiting predominant selfing while the progeny in other trees were almost all outcrossed. The high variation detected indicates that the reproductive processes are difficult to predict. A greater understanding of the underlying selective processes and the factors that impact mating system are critical to our understanding of how the species will persist in small, fragmented communities in the future.

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Table 14. Correlation analysis (Pearson's r) between seedling traits (pooled from 8 *Eucalyptus camaldulensis* trees) and the proportion of heterozygous loci, and between seedling traits (pooled from 8 *Eucalyptus camaldulensis* trees) and the individual outcrossing rate (tm)

Seedling trait	Days	Heterozygosity		tm	
		r	p value	r	p value
Seed weight	na	0.1205	0.1197	0.0885	0.2542
Days to germination	na	-0.0.161	0.8361	0.0280	0.7119
Height	14	0.0766	0.4157	-0.0911	0.3329
N leaves	14	0.0274	0.7712	0.0127	0.8928
Longest leaf	14	0.2408	0.0095***	0.1613	0.0851
Height	32	0.1262	0.1788	0.1311	0.1626
N leaves	32	0.1002	0.2868	0.0659	0.4842
Longest leaf	32	0.2863	0.0019**	0.1866	0.0458*
Height	52	0.2229	0.0111*	0.1583	0.0731
N leaves	52	0.1629	0.0651	0.1155	0.1924
Longest leaf	52	0.1463	0.0980	-0.0448	0.6140
Height	66	0.2564	0.0074**	0.0727	0.4546
N leaves	66	0.0829	0.3938	-0.0402	0.6793
Longest leaf	66	0.2961	0.0019**	0.1335	0.1684
Height	79	0.3479	0.0002***	0.1721	0.0749
N leaves	79	0.1079	0.2665	0.0075	0.9388
Longest leaf	79	0.3127	0.0010**	0.2302	0.0165*
Height	101	0.3336	0.0010**	0.2041	0.0485*
N leaves	101	-0.0073	0.9443	-0.106	0.3091
Longest Leaf	101	0.2590	0.0117*	0.2137	0.0386*
Height	132	0.4072	<.0001***	0.2787	0.0094**
Height	306	0.1295	0.2109	0.1051	0.3109
Longest leaf	306	0.0826	0.4315	-0.0619	0.5553
Condition	306	0.2787	0.0068**	0.1725	0.0982
Height	448	0.2037	0.0477*	0.1815	0.0784
Length	448	-0.0133	0.8979	0.0561	0.5889
Biomass above	448	0.2726	0.0075**	-0.0426	0.6820
Biomass below	448	0.3430	0.0007***	0.1137	0.2727

Days = n days since sowing, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locus outcrossing rate (Ritland 2002) * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Table 15. Correlation (Pearson's r) between seedling traits and the proportion of heterozygous loci, and between seedling traits and the individual outcrossing rate (Ritland 2002) calculated from seedlings sourced from a single adult *Eucalyptus camaldulensis* tree in the Dartbrook remnant (DRT2)

Seedling trait	Days	Heterozygosity		tm	
		r	p value	r	p value
Seed weight		0.139	0.5590	-0.2222	0.3465
Days to germination		0.1239	0.6132	-0.0536	0.8276
Height	14	0.1799	0.5050	0.4051	0.1196
N leaves	14	0.0241	0.9293	0.2460	0.3583
Longest leaf	14	0.5867	0.0169*	0.5582	0.0246*
Height	32	0.3757	0.1373	0.4568	0.0653
N leaves	32	-0.0773	0.7682	0.0590	0.8221
Longest leaf	32	0.5087	0.0378*	0.5287	0.0291*
Height	52	0.4815	0.0431*	0.1802	0.4742
N leaves	52	0.0159	0.9500	0.0567	0.8233
Longest leaf	52	0.4169	0.0853	0.3292	0.1823
Height	66	0.6493	0.0048**	0.4085	0.1035
N leaves	66	0.0226	0.9314	0.0617	0.8141
Longest leaf	66	0.5146	0.0346*	0.4269	0.0875
Height	79	0.5960	0.0116*	0.3391	0.183
N leaves	79	0.2753	0.2849	0.3123	0.2223
Longest leaf	79	0.5129	0.0353*	0.3715	0.1421
Height	101	0.4792	0.0516	0.3195	0.2112
N leaves	101	0.2142	0.409	0.1120	0.6687
Longest Leaf	101	0.2852	0.2671	0.2742	0.2869
Height	132	0.8205	<.0001***	0.5418	0.0302
Height	306	0.3924	0.1479	-0.1507	0.5919
Longest leaf	306	-0.3253	0.2367	0.2161	0.4392
Condition	306	-0.0891	0.7523	0.3649	0.1811
Height	448	0.2932	0.2888	0.1323	0.6385
Length	448	0.0023	0.9934	-0.198	0.4793
Biomass above	448	0.4310	0.1087	-0.0616	0.8275
Biomass below	448	0.5037	0.0556	0.1477	0.5995

Days = n days since sowing, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locus outcrossing rate (Ritland 2002) * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Table 16. Genetic parameters calculated from progeny arrays sourced from adult *Eucalyptus camaldulensis* trees (CAA, CAB, CAC, DEN1, DEN2, DEN3 DRT1 and DRT2) from three populations: Camyr Allyn (CA), Denman (DEN) and Dartbrook (DRT). The number of individuals in each family is provided in brackets.

Tree	CAA (22)	CAB (24)	CAC (33)	DEN1 (16)	DEN2 (15)	DEN3 (25)	DRT1 (14)	DRT2 (19)
tm	0.960	0.981	0.850	0.838	0.358	0.313	0.963	0.949
He	0.633	0.519	0.543	0.475	0.447	0.324	0.480	0.545
SE	0.024	0.082	0.057	0.109	0.151	0.120	0.113	0.046
F	-0.119	-0.064	-0.027	-0.111	0.041	-0.004	-0.064	0.097
SE	0.052	0.116	0.045	0.052	0.128	0.044	0.062	0.139

tm = multi-locus outcrossing rate (Ritland 2002), He = expected heterozygosity, F = inbreeding coefficient, SE = standard error calculated from 1000 bootstraps

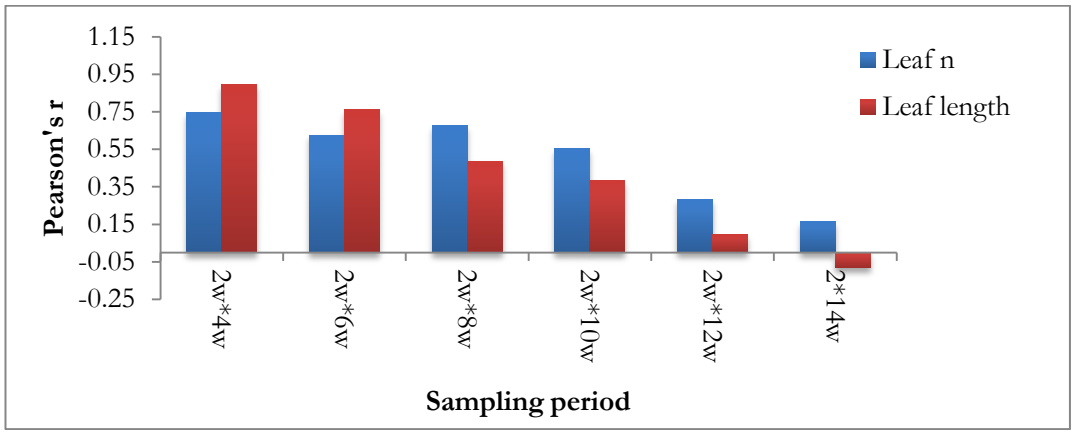

Figure 22. Decreasing correlation (Pearson's r) of re-sampled attributes in *Eucalyptus camaldulensis* seedlings (leaf number and leaf length) at 2, 4, 6, 8, 10, 12, and 14 weeks

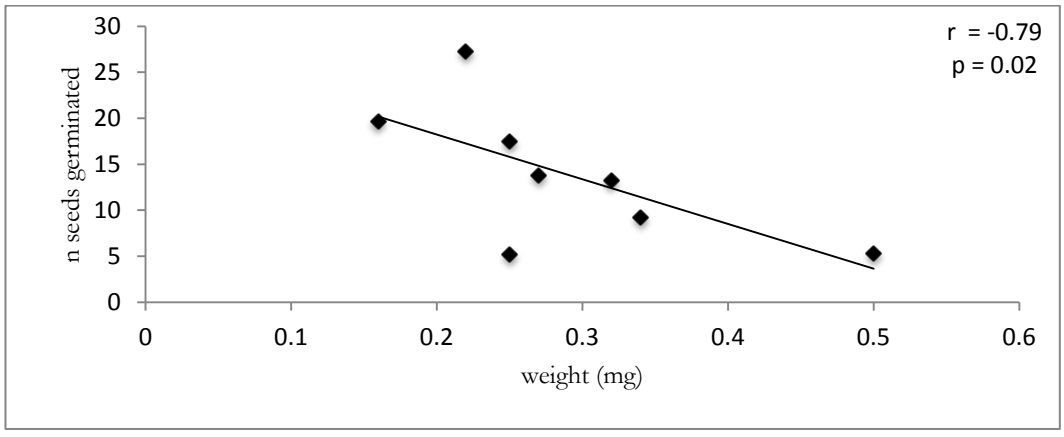

Figure 23. The mean weight of seeds (mg) per capsule collected from eight *Eucalyptus camaldulensis* trees plotted against the mean number of seeds germinated (n seeds) per capsule

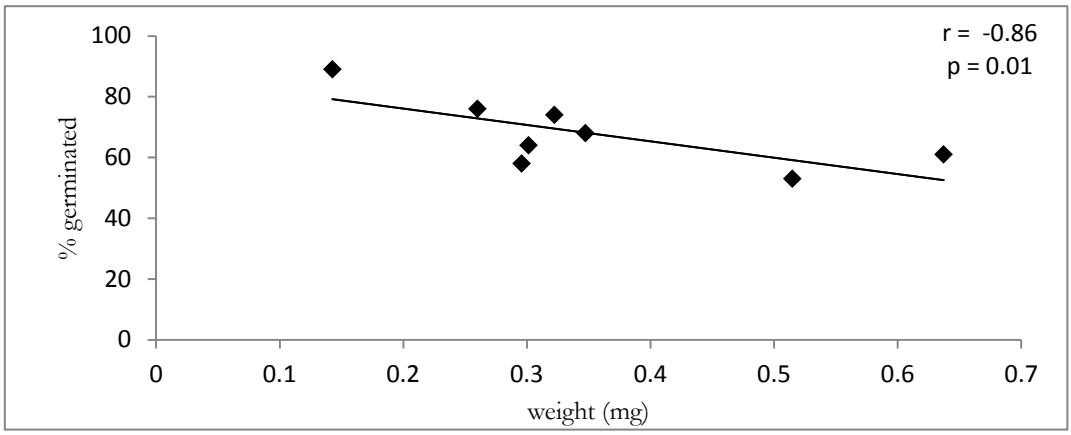

Figure 24. The mean weight of seeds (mg) per capsule collected from eight *Eucalyptus camaldulensis* trees plotted against percentage of seeds germinated (% germinated) per capsule

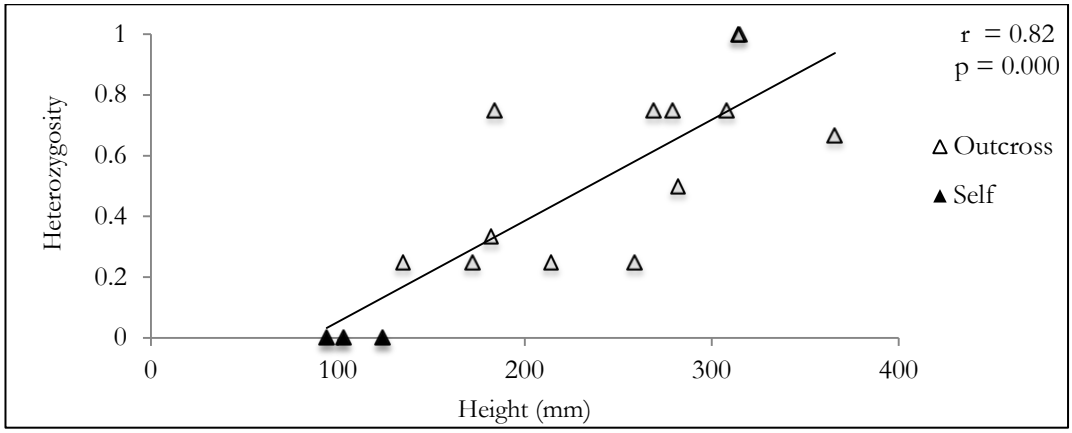

Figure 25. Correlation (Pearson's r) between the proportion of heterozygous loci and seedling height in progeny from an individual *Eucalyptus camaldulensis* tree (DRT2) with individuals designated as selfed (tm < 1) outcrossed (tm ≥ 1)

Table 17. Single and multi-locus mating system estimates for three populations of *Eucalyptus camaldulensis* populations based on seed sourced from individual trees from Dartbrook (2 trees), Denman (3 trees) and Camyr Allyn (3 trees)

Population	tm (SE)	ts (SE)	tm-ts (SE)	rpm (SE)	rps (SE)
Dartbrook	0.755 (0.279)	0.693 (0.259)	0.062 (0.044)	0.065 (0.221)	0.071 (0.052)
Denman	0.379 (0.091)	0.267 (0.096)	0.122 (0.025)	0.397 (0.213)	-0.249 (0.347)
Camyr Allyn	0.786 (0.158)	0.639 (0.183)	0.148 (0.059)	0.127 (0.075)	0.036 (0.053)

tm = multi-locus population outcrossing rate, ts = single locus outcrossing rate, tm-ts = measure of biparental inbreeding, rpm = multi-locus correlated paternity, rps = single locus correlated paternity, SE = standard error

Table 18. Significant differences between mean growth traits of progeny from parent trees (CAA, CAB, CAC, DEN1, DEN2, DEN3, DRT1, DRT2) of *Eucalyptus camaldulensis*

Attribute	Date	Higher	Lower	p value
Condition rating	(18/02/10)	CAA	DEN3	0.0001
Condition rating	(18/02/10)	CAB	DEN3	0.0086
Condition rating	(18/02/10)	CAC	DEN3	<.0001
Condition rating	(18/02/10)	DEN1	DEN3	0.0002
Condition rating	(18/02/10)	DRT2	DEN3	0.0222
Heterozygous/total loci	na	CAA	DEN3	<.0001
Heterozygous/total loci	na	CAC	DEN3	0.0206
Longest leaf	(11/06/09)	DEN1	CAA	0.0292
Longest leaf	(11/06/09)	DEN1	CAA	0.0055
Longest leaf	(11/06/09)	DEN1	DEN3	0.0229
Longest leaf	(11/06/09)	DEN1	DEN3	0.0255
Longest leaf	(11/06/09)	DEN1	DRT1	0.0222
Number leaves	(23/06/09)	DRT1	CAB	0.0486
Number of leaves	(11/07/09)	DEN1	DEN2	0.0459
Number of leaves	(11/07/09)	DEN1	DRT2	0.0354
Number of Leaves	(18/02/10)	CAA	DEN3	0.0440
Number of Leaves	(18/02/10)	DEN2	DEN3	0.0076
Number of leaves	(28/07/09)	DEN1	CAA	0.0020
Number of leaves	(28/07/09)	DEN1	CAC	0.0237
Number of leaves	(28/07/09)	DEN1	DRT1	0.0016
Number of leaves	(28/07/09)	DEN1	DRT2	0.0280
outcrossing rate (tm)	na	CAA	DEN2	0.0009
outcrossing rate (tm)	na	CAA	DEN3	<.0001
outcrossing rate (tm)	na	CAB	DEN2	0.0004
outcrossing rate (tm)	na	CAB	DEN3	<.0001
outcrossing rate (tm)	na	CAC	DEN2	0.0055
outcrossing rate (tm)	na	CAC	DEN3	0.0003
outcrossing rate (tm)	na	DEN1	DEN2	0.0339
outcrossing rate (tm)	na	DEN1	DEN3	0.0069
outcrossing rate (tm)	na	DRT1	DEN2	0.0039
outcrossing rate (tm)	na	DRT1	DEN3	0.0005
outcrossing rate (tm)	na	DRT2	DEN2	0.0016
outcrossing rate (tm)	na	DRT2	DEN3	<.0001
Seed weight (mg)	na	CAB	DEN3	0.0005
Seed weight (mg)	na	CAC	CAA	<.0001
Seed weight (mg)	na	CAC	CAB	<0.001
Seed weight (mg)	na	CAC	DEN2	0.0013
Seed weight (mg)	na	CAC	DEN3	<.0001
Seed weight (mg)	na	CAC	DRT1	0.0004
Seed weight (mg)	na	CAC	DRT2	<.0001
Seed weight (mg)	na	DEN1	CAA	<.0001
Seed weight (mg)	na	DEN1	CAB	<.0001
Seed weight (mg)	na	DEN1	DEN2	<.0001
Seed weight (mg)	na	DEN1	DEN3	<.0001
Seed weight (mg)	na	DEN1	DRT1	<.0001
Seed weight (mg)	na	DEN1	DRT2	<.0001
Seed weight (mg)	na	DEN2	DEN3	0.0193
Seed weight (mg)	na	DRT2	DEN3	0.0386
Seedling height (cm)	(18/02/10)	CAA	DEN3	0.0261
Seedling height (cm)	(18/02/10)	DEN1	DEN3	0.0343
Seedling height (cm)	(4/07/10)	DEN1	DEN3	0.0243
Seedling height (cm))	(4/07/10)	CAA	DENN3	0.0232

date = sampling date, p = significance value calculated via the Tukey-Kramer test

8 Discussion and Conclusions

8.1 Introduction

This research assessed genetic resources in remnant *E. camaldulensis* populations; however the research aimed to determine not only the levels of genetic diversity that were present, but also how the diversity was structured and maintained, what impact genotypic variability had on the immediate fitness of individuals, and how informative the information would be for conserving and regenerating remnant stands.

Knowledge of the importance of genetic variability stems from an understanding of evolutionary processes, but it is also an acknowledgement of the important role genotype plays in determining the fitness of progeny and the success of mating in the immediate future. A central aim of assessing genetic variation in fragmented habitat is to try to and ascertain if the genetic profiles of affected remnant populations are comparable to those taken from genetically viable populations. Of particular importance in sessile organisms from restricted habitats is the capacity to reproduce – and to reproduce viable, comparably fit offspring.

Processes that compromise the genetic variability of small populations increase their susceptibility to extinction (Aguilar et al. 2008). Principle among these is the threat of genetic drift, the threat of increased population differentiation and the possibility of decreased fitness due to increased self and close mating (Young et al. 1996). However, these processes need to be considered within the context of other threatening processes. This is particularly important when habitat modification threatens recruitment on a number of different grounds. In *E. camaldulensis* remnants, ongoing successful recruitment and persistence is threatened by a multitude of interacting factors and maintaining genetically variable populations in which regular recruitment and persistence is a challenge. Herein I discuss what this research has contributed to our understanding of how to maintain self-sustainable, genetically variable populations of *E. camaldulensis* within the highly modified region of the Hunter Valley.

The original perturbation of *E. camaldulensis* populations was caused by the initial clearing that all but removed riparian vegetation entirely. Although the historical

extent of *E. camaldulensis* within the Hunter Valley region is uncertain, it is clear that what we have left is most likely only a small portion of what we had. Remaining populations of *E. camaldulensis* are small and variously isolated. Because of their small size, they are threatened by a range of factors including environmental and genetic stochastisity (Lande 1988). This research revealed, however, that remnants varied not only in size, but also in the levels of genetic variability that they contained, the rate of population growth they exhibited, the habitat that they occupied and possibly the degree of outcrossing that was practiced. It was clearly established that the viability of remnant populations was not equal and that contextualizing conservation of genetic resources within the environment will be a pre-requisite for effective long term management of *E. camaldulensis*. Herein I have endeavored to begin this process. In this chapter I incorporate all the information gained throughout my research to address three questions:

- Are remnant populations of *E. camaldulensis* genetically impoverished?
- Are low levels of genetic variation likely to impact upon reproductive success?
- What impact does habitat and environmental factors have on remnants and how does this relate to remnant genetic diversity and structure?

Finally I will briefly touch on some analysis issues.

8.2 Are remnant populations of *Eucalyptus camaldulensis* genetically impoverished?

It is difficult to determine if a population of a species is genetic impoverished. Genetic impoverishment is a relative term that describes the level of genetic diversity within a population in relation to the ideal level of genetic diversity for the species, or the level of genetic diversity that is required to maintain sexual reproduction and evolutionary processes. More often than not, the pre-fragmentation levels of genetic variability are unknown. However, because species that exhibit similar life-history traits exhibit similar levels of genetic diversity (Hamrick & Godt 1996) comparisons with other species can be informative. The mean level of genetic variability found within *E. camaldulensis* remnants in the Hunter Valley (He =0.74) was similar to other widespread species (e.g. He = 0.75 in *E. globulus*, Steane et al. 2006), and similar to the levels detected in the same species over a wider range (He = 0.77 in *E.*

camaldulensis subsp. *camaldulensis*, Butcher et al. 2009). However the levels of genetic variability varied substantially ((He = 0.60-0.81) between remnant stands. This indicated that some remnants were more genetically impoverished than others.

Habitat fragmentation has been found to reduce genetic diversity (Aguilar et al. 2008) and the relationship between the level of genetic diversity and population size is well established (Leimu 2006). We could predict, therefore, that the levels of genetic impoverishment would be greater in smaller fragments than larger fragments. However there was no relationship between population size and genetic variability in this study. Some relatively large populations were genetically impoverished, while some very small populations exhibited high levels of genetic diversity. In the small population at Muswellbrook, high levels of genetic diversity were detected as a result of re-vegetation that included planting *E. camaldulensis* saplings from outside of the catchment. The high genetic diversity was anthropogenically initiated. However, the lack of correlation between population size and genetic diversity was also attributed to the low levels of genetic variability found in some large populations. Low levels of genetic variability in large populations is likely to have resulted from founder effects; either a large population was founded by a limited seed source, or a population that has suffered a substantial bottleneck is growing, but the adult seed sources are limited. The lack of correlation between genetic diversity and population size indicates that population size should be used cautiously to prioritise the conservation of populations when genetic conservation is being considered.

Comparing the levels of genetic diversity across different age classes (dbh cohorts) within remnants populations suggested that in most remnants, declines in genetic variability were occurring and these were not associated with inflated inbreeding coefficients indicating that genetic decline was independent of differential selection. Overall the younger cohort exhibited levels of genetic variability that were more akin to restricted species (e.g. He = 0.70 in *Eucalyptus bethamii*, Butcher et al. 2005). If the levels of genetic variability in younger cohorts are an accurate indication of the levels of genetic variability that will be maintained within remnants, remnant populations will suffer lower levels of genetic variability in the future. The higher levels of genetic variability in older cohorts indicated that some extant older trees may be failing to recruit successfully, and therefore failing to pass genetic variability onto the next generation (genetic drift).

There was substantial variation between the age-class structure of remnants suggesting that population growth was not equal. Successful reproduction is a pre-requisite for population viability. When reproductive processes are operating, genetic variation can be passed between generations, and the remediation of genetic decline can be achieved by successful reproduction following gene-flow (Aguilar et al. 2008). Recruitment is difficult in *E. camaldulensis* due to the stringent water requirements of seeds and seedlings (Di Stefano 2002). Remnant populations, therefore, may vary in their potential to maintain and increase genetic variability based on the rate of population growth. The presence of a diverse age-class structure is likely to be at least as important, perhaps even more important, than the current level of genetic diversity within a remnant. A good example is the Redbournberry remnant where a low level of genetic diversity was accompanied with a diverse age-class structure and a temporal increase in genetic diversity across age classes. The genetic parameters of this population signaled recovery, and the age-class structure indicated that the ecological requirements for germination and survival were met.

The potential for genetic recovery within isolated remnants of once widespread species is likely to be significantly influenced by gene-flow. The impact of landscape context on pollen and seed dispersal is therefore critical. Seed of *E. camaldulensis* is reported to float and has been found in flood water suggesting that water mediated seed dispersal may be important (but see Butcher et al. 2009). Both the connectivity between populations by water, as well as the geographical distance between remnants may, therefore, be important. Within the remnants studied, several were located on the river margins of major tributaries (Redbournberry, Hunterview, Sandy Hollow), while others were either disconnected (e.g. Denman) or on very small, sometimes dry tributaries (e.g. Bylong). Further, the distance between populations and their nearest neighbour was highly variable. This gave an opportunity to explore genetic differentiation and genetic isolation by distance. The results indicated that the degree of differentiation (Dest) between the majority of population pairs was weak and the overall genetic differentiation within the catchment was low. Further, the correlation between geographic and genetic distance was also very weak (although significant = $r^2 = 0.06$). These results suggested that substantial gene-flow had occurred, however because the degree of differentiation was determined by combining multiple aged trees within each population, it was not possible to determine whether a lack of

population differentiation had been established when populations were more connected, or whether contemporary gene-flow events are responsible, or whether both historic and contemporary processes contributed.

In two populations (Bylong and Hunterview) a large portion of sampled individuals exhibited relatively high levels of genetic similarity and consistent patterns of genetic admixture (Figure 14). This was particularly evident in the Bylong and Hunterview remnants. Bylong resides in an upper tributary of the Goulburn River in an isolated pocket and is separated from other populations by considerable terrain; hence genetic similarity between individuals in this remnant may represent historical reproductive isolation. However, the population remnant at Hunterview is centrally located on the Hunter River within close proximity (1.9km) to a neighbouring population (Redbournberry). The Hunterview population is dominated by young trees. Hence the genetic similarity between individuals in this population is likely to have arisen more recently. Although more in-depth study would be required to determine how genetic similarity has arisen, it is likely that it has resulted from local reproduction of the few reproductively mature trees remaining after clearing. If genetic similarity has established in this way, it indicates that the close proximity of Redbournberry has not prevented the growth of a population that is genetically more homogenous than is typical in other remnants. This could be an early indication that gene-flow may be restricted. While population genetic variability may decline because of genetic drift, species wide genetic variability may be enhanced in rare or less common alleles become fixed in reproductively isolated fragments. More in depth study including full genetic profiles of populations may be required to understand gene-flow within the catchment. It would also be beneficial to perform an assessment including chloroplast markers to differentiate between maternal and paternal contribution to gene-flow.

The levels of genetic variability detected within remnants populations were also affected by the regeneration of sites by outsourced *E. camaldulensis* trees. Redbournberry, Hunterview, Dartbrook, Denman and Muswellbrook remnants have all had *E. camaldulensis* individuals planted within close proximity to naturally recruited individuals. In Muswellbrook, outsourced individuals were sampled and genotyped and this substantially inflated the level of private alleles in the population.

If outsourced trees in any of the other sites (Redbournberry, Hunterview, Denman, Dartbrook) were reproductively mature (no evidence of flowering was detected in this study) they may have contributed genetic variability to the younger generation via seed or pollen (but see *E. regnans*, Burczyk et al. 2002). The long term ramifications of the altered genetic content within these remnant stands are uncertain Follow up investigation should be conducted. How outsourced and naturally recruited individuals behave reproductively will have a significant impact upon future levels of genetic variability and their impact on population sustainability within the catchment.

8.3 Is a lack of genetic variability a possible cause of recruitment failure?

The variable age-class structure between *E. camaldulensis* remnants indicated that recruitment between populations was not equal. However this did not appear to be associated with genetic attributes. In two sites (Hunterview and Redbourberry) a large number of young trees were observed, however, these sites exhibited relatively low levels of genetic variability. This suggested that low levels of genetic diversity detected at the population level with neutral markers were not significantly restricting recruitment, at least not at these locations. In another component of the current research, however, measurement of the association between genotype and various growth attributes indicated that inbred, more homogenous genotypes were, on average, likely to exhibit lower seedling growth traits. Thus, while populations that were genetically impoverished could recruit successfully, the impact of increased homozygosity was negative. Under extreme circumstance, low levels of genetic variability and restricted mate choice could therefore, impact negatively on recruitment.

The impact of genotype on growth traits, however, varied substantially between trees, with strong heterozygosity x growth trait correlations within the progeny of some trees, but not others. This suggested that the impact of genetic impoverishment is variable between individuals. Very preliminary evidence presented here also indicates that individuals within remnants may exhibit similar reproductive strategies – the knowledge of which would substantially increase the capacity to predict the impact of genetic decline. Variation of outcrossing levels is a characteristic of the

genus (e.g. Griffin et al. 1987; Butcher & Williams 2002; McDonald et al. 2003; Byrne et al. 2008). This variation is likely to be related to the reproductive options (hybridisation, seed abortion) open to the species, and how population history and environmental context has shaped them. While the low (and in one tree absent) seed set in some individuals indicates that seed set may be affected in a detectable way despite the number of potential seeds that can be produced, it was not possible to determine if inadequate genetic variability or pollinator scarcity caused reduced seed set. Detailed work on pollinator type and efficiency is urgently required to fill the gaps, particularly, additional research is required to determine if differences in the outcrossing rates between individuals are driven by pollination paucity, or if they are a genetically based, or possibly a combination of both.

Although the species is strictly self-compatible, self-incompatibility via early acting inbreeding depression may be present in some individuals if the selfed genotypes are not viable. Under these circumstances, predominantly outcrossed progeny are produced, as self progenies are aborted. This could explain the variable outcrossing rate detected. The latter would indicate that individuals (and possibly populations) may have different genetic thresholds. For example, if some populations are largely self incompatible, they may need to be larger and more genetically diverse to maintain reproductive processes in the short term.

The sheer number of seed produced in eucalypts requires only a small portion to succeed to achieve replacement. However, the number of good quality seeds to achieve reproductive viability heavily depends on the availability of safe recruitment sites. Drake (1981) suggested that the number of seed required is large if the availability of suitable recruitment sites is low since the chance distribution of a high quality seed finding a secure sight depends on the number of sites available. Further, the quality of safe sites is also likely to have a significant impact on the range of genotypes that are able to succeed. As Reed et al. (2002) demonstrated inbreeding depression is more prevalent under stressful conditions. Thus both the suitability of habitat and the quality of seed may interact to determine the susceptibility of a remnant to genetic decline. As such the threats presented by genetic decline are likely to be population and habitat specific.

8.4 What impact does habitat and environmental factors have on remnants and how does this relate to remnant genetic diversity and structure?

Eucalyptus camaldulensis is restricted to riparian and floodplain habitat because of the dependence of early stages (seed germination and seedling growth) on water availability (Di Stefano 2002). This strong dependence on water drives the patterns of recruitment. The age-class structure of remnant stands indicates that recruitment within floodplain habitat occurs episodically while recruitment along river margins is much more continuous. The results of this work suggested that recruitment patterns influenced by different water regimes impacted upon the genetic structure of remnant populations. In floodplain habitat, seedlings within recruitment groups exhibited low levels of genetic variability, similar to those detected in the progeny of individual trees. Neighbourhoods in riparian habitat exhibited very different genetic structure; genetic diversity was high and stretches of juvenile trees were often dissimilar despite close proximity indicating that they had been sourced from different trees and that seed had been distributed randomly.

However when the correlation between genetic diversity were assessed (Chapter 4) non-linear predominantly floodplain populations exhibited relatively high diversity while low levels of genetic diversity were detected in linear riparian habitat. Riparian habitats with low levels of genetic diversity had other commonalities (e.g. the low number of old trees) that may have influenced the level of genetic variability. While typical floodplain habitat incorporated a relative large number of old trees (in the two largest dbh cohorts) riparian populations exhibited fewer old trees and a relative higher number of young trees. Although correlations between the proportion of trees in dbh cohorts against genetic diversity were not high, the lack of correlation was largely due to a single population; a population dominated by large old trees with overall low levels of genetic variability. It may be that populations with a small proportion of old trees compared with young reliably exhibit low levels of genetic variability, but populations of large old trees may also exhibit low levels of genetic diversity, possibly due to founder effect.

Safe recruitment sites clearly vary between floodplain and riparian habitat and this is an obvious driver of recruitment. Recruitment in some floodplain populations is clearly limited and long term conservation of these populations *in situ* will most likely require active management. In *E. camaldulensis*, the spatially restricted genetic diversity

detected within floodplain habitat suggested that a large portion of seed is dispersed beneath the parent tree. This suggests that declines in suitable sites in floodplain habitat will result in recruitment of progeny only from parent trees whose seed rain overlaps with suitable recruitment habitat. As safe recruitment sites are reduced, genetic decline due to genetic drift can, therefore, proceed rapidly. Thus the ecological decline of floodplain habitat may contribute to amplified genetic drift. This is likely to be important in other niche specialists. While changes to the environment may prevent regeneration all together, genetic decline is likely if changes to the species habitat result in patchy and subsequently insufficient sites suitable for germination.

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Appendix 1.

Table 19. Embra (Brondani et al. 2006) and Eg (Benson 1991) primers: annealing temperatures, sequences, fragment size range (base pairs) and the chapter which primers were used

Primer	Temp.	Sequence (FW)	Sequence (RVSE)	bp range	Chapter
Embra 8	58°C	CACAACTAAAAATCAAAACCC	AAAGAGCAGATTATTACAGAAGC	129–167	4,5,6
Embra 20	53°C	GTGAGTGGGTATCCATCG	GCTGGAACTGGTCTTGAG	102–154	4,5,6
Embra 17a	53°C	AGGATACTCGTGAGAGAAGC	GTAGATCTGTTCTGCATGTTG	118–160	4,5,6,7
Embra 17b	53°C	AGGATACTCGTGAGAGAAGC	GTAGATCTGTTCTGCATGTTG	200–268	4,5,6
Embra 2	53°C	CGTGACACCAGGACATTAC	ACAAATGCAAATTCAAATGA	106–134	4,5
Eg1062	55°C	GGGTGCATTACTGATCCCAC	TTCATCTTCATTGGCACACG	184–222	4,5,6,7
Eg1096	55°C	CCAGGGAAAACAATTCAAGC	GAGCGACAAACCCAAGTTTC	263–283	4,5,6,7
Eg1067	55°C	TTTTCTGCACGTGTGTTTCC	GTGGAAGAGAAGTCGCCAAG	155–223	4,5,6
Eg1028	55°C	TTTTGAAGGTAGATGAGGGG	CCATGAAAACCAGCAAAATATG	174–228	4,5,6,7

bp = base pairs, Chapter indicates the chapters in which specific primers were used

Appendix 2.

Table 20. Polymerase Chain Reaction protocol (see Table 19 for annealing temperatures)

Temp	Time (sec)	n. repeats
94°C	600	1
94°C	45	
45°C	Anneal temp	
72°C	160	30
72°C	600	1

Appendix 3.

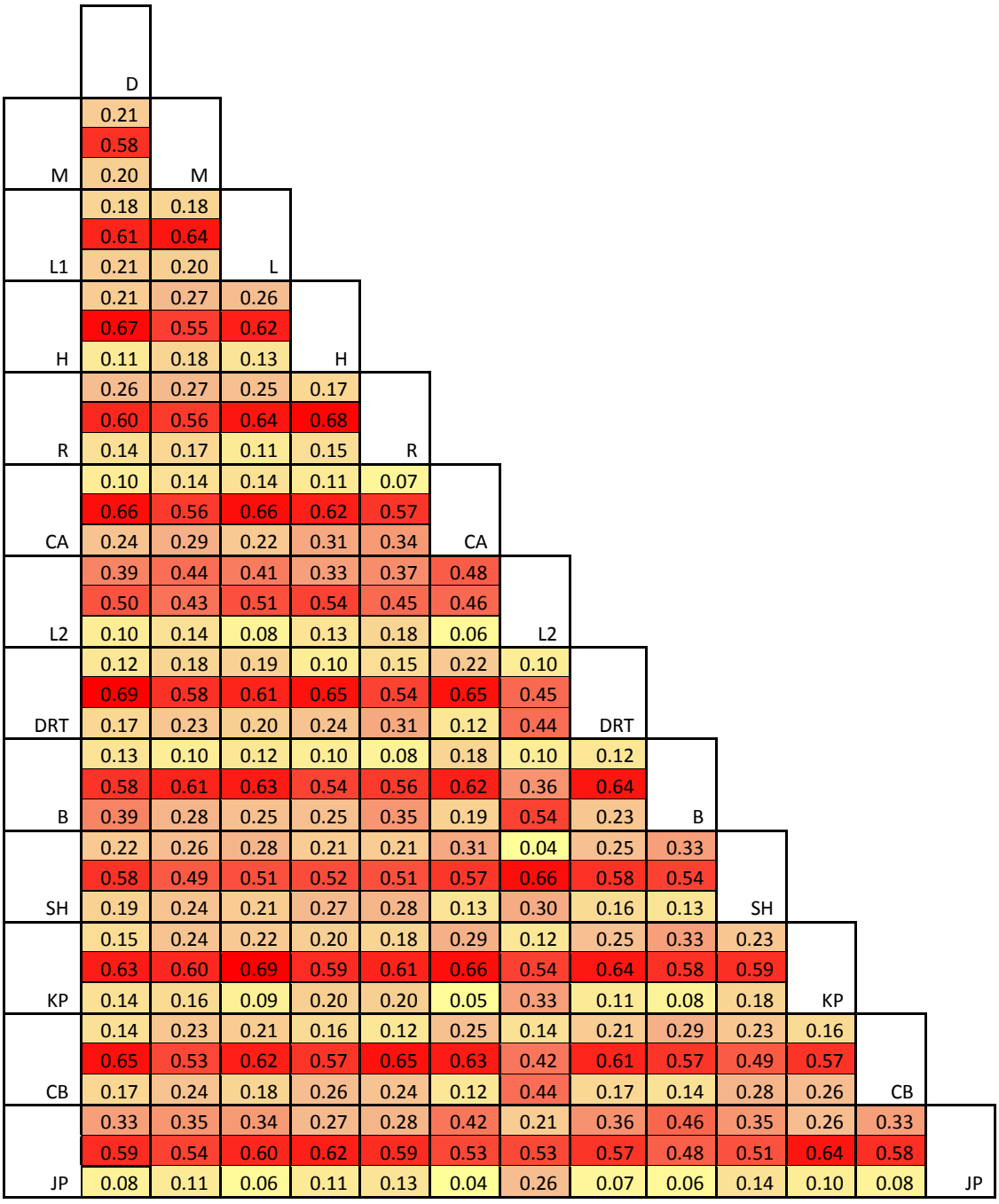

Figure 26. Pairwise assessment of the percentage of shared alleles (middle row) and those unique (above and below) to each *Eucalyptus camaldulensis* remnants (D = Denman, M = Muswellbrook, L = Lemington, H = Hunterview, R = Redbournberry, CA = Camyr Allyn, L2 = Lemington 2, DRT = Dartbrook, B = Bylong, SH = Sandy Hollow, KP = Kingdom Ponds, CB = Carrington Billabong and JP = Jefferson Park) in the Hunter Valley.

Appendix 4.

Table 21. Correlation (Pearson's r) between seedling traits and heterozygosity within outcrossed (tm ≥ 1) progeny of *E. camaldulensis* pooled from eight parent trees

Seedling trait	Date	Heterozygosity	
		r	p
Seed weight	na	0.0135	0.8892
Days to germination	na	0.0363	0.7077
N leaves	2/05/2009	-0.1079	0.3669
Longest leaf	2/05/2009	0.0288	0.8105
Height	2/05/2009	-0.0043	0.9713
N leaves	20/05/2009	0.0464	0.6949
Longest leaf	20/05/2009	0.1142	0.3326
Height	20/05/2009	0.0004	0.9972
N leaves	11/06/2009	-0.0841	0.4760
Longest leaf	11/06/2009	0.1489	0.2056
Height	11/06/2009	0.0185	0.8760
N leaves	23/06/09	-0.0128	0.9140
Longest leaf	23/06/2009	0.2181	0.0620
Height	23/06/2009	0.1620	0.1680
N leaves	6/07/2009	-0.0242	0.8410
Longest leaf	6/07/2009	0.1908	0.1109
Height	6/07/2009	0.1844	0.1237
N leaves	28/07/2009	-0.0349	0.7810
Longest leaf	28/07/2009	0.2102	0.0902
Height	28/07/2009	0.2118	0.0877
Height	28/08/2009	-0.0232	0.8525
Condition	18/02/2010	0.3058	0.0119*
Longest leaf	18/02/2010	0.1749	0.1568
Height	18/02/2010	-0.090	0.5962
Height	10/07/2010	0.2847	0.0318*
N leaves	10/07/2010	-0.2801	0.0931
Biomass below	10/17/10	0.0680	0.6894
Biomass above	10/17/10	0.2662	0.1113

Heterozygosity = the proportion of heterozygous loci, * $p < 0.05$

Table 22. Correlation (Pearson's r) between seedling traits and heterozygosity within selfed (tm < 1) progeny of *E. camaldulensis* pooled from eight parent trees

Seedling trait	Date	Heterozygosity	
		r	p
Seed weight	na	0.0978	0.4614
Days to germination	na	-0.161	0.2233
N leaves	2/05/2009	0.1897	0.2231
Longest leaf	2/05/2009	0.2644	0.0866
Height	2/05/2009	0.2113	0.1738
N leaves	20/05/2009	0.1926	0.2276
Longest leaf	20/05/2009	0.2755	0.0812
Height	20/05/2009	0.0598	0.7103
N leaves	11/06/2009	0.1228	0.4108
Longest leaf	11/06/2009	0.1978	0.1826
Height	11/06/2009	0.1464	0.326
N leaves	23/0/09	-0.002	0.9916
Longest leaf	23/06/2009	0.1464	0.4318
Height	23/06/2009	0.1181	0.5268
N leaves	6/07/2009	0.167	0.3231
Longest leaf	6/07/2009	0.1615	0.3396
Height	6/07/2009	0.3534	0.0319*
N leaves	28/07/2009	0.0925	0.6332
Longest leaf	28/07/2009	0.1309	0.4985
Height	28/07/2009	0.2922	0.1240
Height	28/08/2009	0.3410	0.0703
Condition	18/02/2010	0.1729	0.3789
Longest leaf	18/02/2010	0.1433	0.4671
Height	18/02/2010	0.1963	0.3168
Height	10/07/2010	0.2883	0.1294
N leaves	10/07/2010	0.0915	0.6369
Biomass below	10/17/10	0.4814	0.0082**
Biomass above	10/17/10	0.5393	0.0025**

Heterozygosity = the proportion of heterozygous loci, * $p < 0.05$, ** $p < 0.01$

Appendix 5.

Table 23. Correlation (Pearson's r) between *Eucalyptus camaldulensis* seedling traits and genotype (heterozygosity, outcrossing rate) from seedlings sourced from the same parent tree (CAA)

Trait	Date	Heterozygosity		tm	
		r	p	r	p
Seed weight	na	0.2944	0.1835	0.0318	0.8827
Days to germ.	na	-0.1140	0.6136	-0.2097	0.3368
N leaves	2/05/09	0.6441	0.0848	0.4284	0.0761
Longest leaf	2/05/09	0.5814	0.1306	0.3624	0.1394
Height	2/05/09	0.5186	0.1879	0.2559	0.3055
N leaves	20/05/09	0.4033	0.1717	0.4281	0.1267
Longest leaf	20/05/09	0.5178	0.0699	0.3897	0.1684
Height	20/05/09	0.5880	0.0346	0.2501	0.3884
N leaves	11/06/09	0.5067	0.0644	0.2399	0.3084
Longest leaf	11/06/09	0.5341	0.0491	0.2168	0.3585
Height	11/06/09	0.4897	0.0755	0.2816	0.2291
N leaves	23/0/09	0.4982	0.1188	0.0003	0.9990
Longest leaf	23/06/09	0.3861	0.2408	-0.1933	0.4423
Height	23/06/09	0.4724	0.1424	0.0062	0.9807
N leaves	6/07/09	0.6435	0.0852	0.3912	0.1084
Longest leaf	6/07/09	0.4272	0.2911	0.3492	0.1555
Height	6/07/09	0.4646	0.2461	0.3514	0.1528
N leaves	28/07/09	0.4009	0.3250	0.1333	0.6497
Longest leaf	28/07/09	0.4210	0.2989	0.3038	0.2910
Height	28/07/09	0.5293	0.1773	0.3465	0.2248
Height	28/08/09	-0.3610	0.3796	0.2758	0.3618
Condition	18/02/10	0.2857	0.4927	-0.237	0.4584
Longest leaf	18/02/10	0.2494	0.5514	0.3425	0.2758
Height	18/02/10	-0.5873	0.2204	-0.0852	0.7924
Height	10/07/10	0.6023	0.1524	-0.1564	0.5933
N leaves	10/07/10	0.0280	0.9580	-0.8093	0.0005***
Biomass below	10/17/10	0.2333	0.6564	0.215	0.4604
Biomass above	10/17/10	-0.3863	0.4493	-0.1761	0.5471

r = Pearson's correlation coefficient, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locu outcrossing rate (Ritland 2002) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 24. Correlation (Pearson's r) between *Eucalyptus camaldulensis* seedling traits and genotype (heterozygosity, outcrossing rate) from seedlings sourced from the same parent tree (CAB)

Trait	Date	Heterozygosity		tm	
		r	p	r	p
Seed weight	na	0.1195	0.5782	0.0318	0.8827
Days to germ.	na	-0.0183	0.9341	-0.2097	0.3368
N leaves	2/05/09	0.3004	0.2259	0.4284	0.0761
Longest leaf	2/05/09	0.3234	0.1905	0.3624	0.1394
Height	2/05/09	0.1488	0.5557	0.2559	0.3055
N leaves	20/05/09	0.4379	0.1173	0.4281	0.1267
Longest leaf	20/05/09	0.4733	0.0874	0.3897	0.1684
Height	20/05/09	0.3074	0.2851	0.2501	0.3884
N leaves	11/06/09	0.3192	0.1701	0.2399	0.3084
Longest leaf	11/06/09	0.4014	0.0794	0.2168	0.3585
Height	11/06/09	0.3204	0.1684	0.2816	0.2291
N leaves	23/0/09	0.0431	0.8652	0.0003	0.9990
Longest leaf	23/06/09	-0.0039	0.9877	-0.1933	0.4423
Height	23/06/09	0.0459	0.8565	0.0062	0.9807
N leaves	6/07/09	0.3101	0.2105	0.3912	0.1084
Longest leaf	6/07/09	0.3168	0.2002	0.3492	0.1555
Height	6/07/09	0.2436	0.3299	0.3514	0.1528
N leaves	28/07/09	0.1898	0.5158	0.1333	0.6497
Longest leaf	28/07/09	0.3863	0.1725	0.3038	0.2910
Height	28/07/09	0.2329	0.4229	0.3465	0.2248
Height	28/08/09	0.141	0.6459	0.2758	0.3618
Condition	18/02/10	0.2758	0.3856	-0.237	0.4584
Longest leaf	18/02/10	0.243	0.4467	0.3425	0.2758
Height	18/02/10	-0.368	0.2392	-0.0852	0.7924
Height	10/07/10	-0.3917	0.1661	-0.1564	0.5933
N leaves	10/07/10	-0.391	0.1669	-0.8093	0.0005***
Biomass below	10/17/10	0.3457	0.2260	0.215	0.4604
Biomass above	10/17/10	0.2366	0.4154	-0.1761	0.5471

r = Pearson's correlation coefficient, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locu outcrossing rate (Ritland 2002) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 25. Correlation (Pearson's r) between *Eucalyptus camaldulensis* seedling traits and genotype (heterozygosity, outcrossing rate) from seedlings sourced from the same parent tree (CAC)

Trait	Date	Heterozygosity		tm	
		r	p	r	p
Seed weight	na	-0.0844	0.6462	-0.0932	0.6118
Days to germ.	na	-0.2339	0.2135	-0.1017	0.5928
N leaves	2/05/09	0.0922	0.6911	0.2081	0.3653
Longest leaf	2/05/09	-0.1559	0.4997	0.0212	0.9273
Height	2/05/09	0.0801	0.7298	0.2509	0.2726
N leaves	20/05/09	0.076	0.7570	-0.004	0.9871
Longest leaf	20/05/09	-0.0489	0.8425	0.0798	0.7454
Height	20/05/09	-0.4031	0.0870	-0.1742	0.4756
N leaves	11/06/09	0.1839	0.3897	0.334	0.1107
Longest leaf	11/06/09	0.0849	0.6932	0.0915	0.6706
Height	11/06/09	-0.0066	0.9757	0.1083	0.6143
N leaves	23/0/09	0.2151	0.3243	0.0244	0.9119
Longest leaf	23/06/09	0.0878	0.6904	-0.0071	0.9744
Height	23/06/09	0.1455	0.5076	0.0454	0.8369
N leaves	6/07/09	0.0986	0.6626	0.0722	0.7494
Longest leaf	6/07/09	0.0815	0.7185	0.4317	0.0448
Height	6/07/09	0.2353	0.2917	0.351	0.1093
N leaves	28/07/09	-0.0406	0.8576	0.1655	0.4617
Longest leaf	28/07/09	0.1064	0.6374	0.3985	0.0662
Height	28/07/09	0.2479	0.2660	0.3607	0.0991
Height	28/08/09	0.2502	0.3882	0.4314	0.1236
Condition	18/02/10	0.1459	0.4964	-0.209	0.327
Longest leaf	18/02/10	0.1684	0.4316	-0.1387	0.5181
Height	18/02/10	-0.0012	0.9956	-0.1895	0.3752
Height	10/07/10	0.3505	0.0858	-0.0283	0.8933
N leaves	10/07/10	0.1239	0.5551	0.0874	0.6778
Biomass below	10/17/10	0.2935	0.1544	-0.0305	0.8849
Biomass above	10/17/10	0.6292	0.0008***	0.1354	0.5187

r = Pearson's correlation coefficient, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locus outcrossing rate (Ritland 2002) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 26. Correlation (Pearson's r) between *Eucalyptus camaldulensis* seedling traits and genotype (heterozygosity, outcrossing rate) from seedlings sourced from the same parent tree (DEN1)

Trait	Date	Heterozygosity		tm	
		r	p	r	p
Seed weight	na	-0.1061	0.6958	-0.3099	0.2427
Days to germ.	na	-0.0717	0.7918	0.0839	0.7573
N leaves	2/05/09	-0.3955	0.1811	-0.3	0.3193
Longest leaf	2/05/09	-0.291	0.3348	0.4669	0.1077
Height	2/05/09	0.0454	0.8829	-0.1102	0.72
N leaves	20/05/09	-0.2662	0.4030	-0.1549	0.6307
Longest leaf	20/05/09	0.0067	0.9835	-0.0821	0.7998
Height	20/05/09	-0.2079	0.5167	0.0009	0.9979
N leaves	11/06/09	-0.2434	0.4459	-0.1519	0.6374
Longest leaf	11/06/09	-0.0755	0.8156	-0.7231	0.0079**
Height	11/06/09	0	1	-0.2923	0.3565
N leaves	23/0/09	0.604	0.0491*	-0.1491	0.6618
Longest leaf	23/06/09	0.654	0.0291*	-0.4747	0.1401
Height	23/06/09	0.6189	0.0423*	-0.4736	0.1411
N leaves	6/07/09	-0.0021	0.9951	-0.3706	0.2618
Longest leaf	6/07/09	-0.4623	0.1303	-0.2764	0.3844
Height	6/07/09	-0.1467	0.6492	-0.553	0.0622
N leaves	28/07/09	-0.5177	0.0847	-0.249	0.4351
Longest leaf	28/07/09	0.3125	0.3494	-0.4399	0.1758
Height	28/07/09	0.4858	0.1298	-0.6003	0.0508
Height	28/08/09	0.1213	0.7223	-0.3299	0.3218
Condition	18/02/10	-0.2203	0.5151	0	1
Longest leaf	18/02/10	-0.431	0.1857	0.2451	0.4675
Height	18/02/10	-0.0327	0.9239	0.0108	0.9748
Height	10/07/10	-0.0319	0.9257	0.1242	0.716
N leaves	10/07/10	-0.3827	0.2453	0.2684	0.4248
Biomass below	10/17/10	-0.3377	0.3097	0.2968	0.3754
Biomass above	10/17/10	0.1181	0.7294	0.1722	0.6126

r = Pearson's correlation coefficient, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locus outcrossing rate (Ritland 2002) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 27. Correlation (Pearson's r) between *Eucalyptus camaldulensis* seedling traits and genotype (heterozygosity, outcrossing rate) from seedlings sourced from the same parent tree (DEN2)

Trait	Date	Heterozygosity		tm	
		r		r	p
Seed weight	na	-0.2028	0.4685	-0.3872	0.1539
Days to germ.	na	-0.3803	0.1620	-0.2105	0.4515
N leaves	2/05/09	0.0417	0.9152	-0.1285	0.7418
Longest leaf	2/05/09	0.4992	0.1712	-0.2859	0.4558
Height	2/05/09	0.4044	0.2804	-0.5535	0.1221
N leaves	20/05/09	0.5975	0.0893	-0.1818	0.6398
Longest leaf	20/05/09	0.6705	0.0481*	-0.2768	0.4708
Height	20/05/09	0.0128	0.9738	-0.2912	0.4470
N leaves	11/06/09	0.7467	0.0131*	0.1824	0.6139
Longest leaf	11/06/09	0.7323	0.0160*	0.1316	0.7171
Height	11/06/09	0.6534	0.0405*	-0.1503	0.6785
N leaves	23/0/09	0.7093	0.0743	0.1754	0.7067
Longest leaf	23/06/09	0.8461	0.0164*	0.4015	0.3720
Height	23/06/09	0.5857	0.1671	0.1339	0.7747
N leaves	6/07/09	0.553	0.1551	-0.0922	0.8281
Longest leaf	6/07/09	0.4588	0.2529	0.1108	0.7940
Height	6/07/09	0.2999	0.4705	0.0087	0.9838
N leaves	28/07/09	-0.2458	0.6902	-0.0792	0.8993
Longest leaf	28/07/09	0.1932	0.7555	0.8223	0.0875
Height	28/07/09	-0.223	0.7185	0.526	0.3625
Height	28/08/09	-0.6382	0.3618	-0.1407	0.8593
Condition	18/02/10	0.6814	0.2053	0.7469	0.1469
Longest leaf	18/02/10	0.1293	0.8358	-0.4421	0.4561
Height	18/02/10	0.4533	0.4432	-0.0215	0.9727
Height	10/07/10	0.6387	0.1722	0.0416	0.9377
N leaves	10/07/10	0.3183	0.5387	0.621	0.1882
Biomass below	10/17/10	0.4692	0.3478	-0.2932	0.5728
Biomass above	10/17/10	0.5626	0.2452	-0.3155	0.5425

r = Pearson's correlation coefficient, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locus outcrossing rate (Ritland 2002) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 28. Correlation (Pearson's r) between seedling *Eucalyptus camaldulensis* traits and genotype (heterozygosity, outcrossing rate) from seedlings sourced from the same parent tree (DEN3)

Trait	Date	Heterozygosity		tm	
		r	p	r	p
Seed weight	na	0.052	0.805	0.2135	0.3055
Days to germ.	na	0.0656	0.7555	-0.0632	0.7643
N leaves	2/05/09	0.2093	0.3377	0.366	0.0859
Longest leaf	2/05/09	0.3165	0.1412	0.258	0.2346
Height	2/05/09	0.0857	0.6975	0.014	0.9493
N leaves	20/05/09	0.2986	0.1664	0.19	0.3852
Longest leaf	20/05/09	0.0625	0.7771	0.2614	0.2282
Height	20/05/09	0.1764	0.4208	0.2132	0.3286
N leaves	11/06/09	0.1868	0.3820	0.1857	0.3849
Longest leaf	11/06/09	0.3315	0.1136	0.2018	0.3443
Height	11/06/09	0.1645	0.4423	0.1604	0.4539
N leaves	23/0/09	-0.0658	0.8086	-0.1266	0.6404
Longest leaf	23/06/09	0.6341	0.0083**	0.2682	0.3151
Height	23/06/09	0.51	0.0435*	0.1603	0.5532
N leaves	6/07/09	-0.1313	0.6037	-0.0437	0.8634
Longest leaf	6/07/09	0.2745	0.2702	0.0671	0.7912
Height	6/07/09	0.3377	0.1705	0.1772	0.4818
N leaves	28/07/09	-0.0411	0.8798	-0.2901	0.2758
Longest leaf	28/07/09	0.2508	0.3488	0.0131	0.9616
Height	28/07/09	0.4361	0.0913	0.2438	0.3628
Height	28/08/09	0.6427	0.0098**	0.4112	0.1278
Condition	18/02/10	0.5707	0.0331*	0.3429	0.2300
Longest leaf	18/02/10	0.0478	0.8712	0.1016	0.7296
Height	18/02/10	0.1987	0.4958	0.6389	0.0139**
Height	10/07/10	-0.0436	0.8773	0.3688	0.1761
N leaves	10/07/10	0.1732	0.537	-0.1209	0.6679
Biomass below	10/17/10	0.2411	0.3867	-0.1665	0.5531
Biomass above	10/17/10	0.2705	0.3296	0.444	0.0973

r = Pearson's correlation coefficient, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locus outcrossing rate (Ritland 2002) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 29. Correlation (Pearson's r) between *Eucalyptus camaldulensis* seedling traits and genotype (heterozygosity, outcrossing rate) from seedlings sourced from the same parent tree (DRT1)

Trait	Date	Heterozygosity		tm	
		r	p	r	P
Seed weight	na	-0.1815	0.5345	-0.0187	0.9494
Days to germ.	na	-0.0352	0.909	0.038	0.9019
N leaves	2/05/09	-0.1936	0.6774	-0.0913	0.8457
Longest leaf	2/05/09	-0.1914	0.681	-0.1767	0.7048
Height	2/05/09	0.1641	0.7251	-0.0506	0.9141
N leaves	20/05/09	-0.1708	0.7143	-0.1936	0.6774
Longest leaf	20/05/09	0.304	0.5074	0.4309	0.3345
Height	20/05/09	0.2102	0.651	0.2588	0.5753
N leaves	11/06/09	-0.2415	0.6018	-0.3347	0.4631
Longest leaf	11/06/09	-0.6202	0.1373	-0.8751	0.0099**
Height	11/06/09	-0.1206	0.7967	-0.0955	0.8385
N leaves	23/0/09	-0.4275	0.4728	-0.1873	0.7629
Longest leaf	23/06/09	-0.6271	0.2575	-0.7231	0.1675
Height	23/06/09	-0.4726	0.4215	-0.1253	0.8408
N leaves	6/07/09	-0.8992	0.1008	-0.8704	0.1296
Longest leaf	6/07/09	-0.233	0.767	-0.152	0.848
Height	6/07/09	0.6229	0.3771	0.8423	0.1577
N leaves	28/07/09	-0.9583	0.0102*	-0.904	0.0352*
Longest leaf	28/07/09	-0.9129	0.0305*	-1	<.0001 ***
Height	28/07/09	-0.9129	0.0305*	-1	<.0001***
Height	28/08/09	-0.6909	0.1964	-0.5256	0.363
Condition	18/02/10	-0.1566	0.8015	-0.343	0.572
Longest leaf	18/02/10	-0.679	0.2075	-0.8462	0.0707
Height	18/02/10	0.0357	0.9545	0.1729	0.7809
Height	10/07/10	0.0381	0.9516	0.1841	0.7669
N leaves	10/07/10	-0.0627	0.9202	-0.206	0.7396
Biomass below	10/17/10	0.1479	0.8124	0.203	0.7433
Biomass above	10/17/10	0.0276	0.9649	-0.1496	0.8102

r = Pearson's correlation coefficient, Heterozygosity = the proportion of heterozygous loci, tm = the multi-locus outcrossing rate (Ritland 2002) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Appendix 6.

Table 30. Correlation (Pearson's r) between mean seed and seedling traits of progeny sourced from eight parent trees (n = 8). Presented values are signficant (p >0.05). Correlations including mean seed weight are highlighted yellow; those with correlations including the multi-locus outcrossing rate (tm) are highlighted orange

Variable	Variable	r
(length x n) + height 11/06/09	mean length (cm) 11/06/09	0.99167
(length x n) + height 18/02/10	mean height (cm) 18/02/10	0.98993
((lxn) + height) x n individuals 6/07/09	((lxn) + height) x n individuals 23/06/09	0.9795
((lxn) + height) x n individuals 28/08/09	((lxn) + height) x n individuals 23/06/09	0.96903
SE mean height (cm) 28/08/09	SE mean height (cm) 20/05/09	-0.9684
mean length (cm) 28/07/09	SE mean length (cm) 11/06/09	0.96366
SE mean height (cm) 20.05.09	mean height (cm) 20/05/09	0.95706
(length x n) + height 23/06/09	(length x n) + height 11/06/09	0.9531
((lxn) + height) x n individuals 28/08/09	((lxn) + height) x n individuals 6/07/09	0.95184
(length x n) + height 28/08/09	mean length (cm) 28/07/09	0.95039
(length x n) + height 28/08/09	mean height (cm) 28/07/09	0.9448
SE mean number of seeds/cap	mean number of seeds/cap	0.944
mean n germ seeds/cap	mean number of seeds/cap	0.94222
(length x n) + height 28/08/09	SE mean length (cm) 11/06/09	0.94073
(length x n) + height 23/06/09	mean length (cm) 11/06/09	0.93355
mean height (cm) 23/06/09	SE mean height (cm) 11/06/09	0.93218
mean height (cm) 11/06/09	mean n true leaves 11/06/09	0.93005
((lxn) + height) x n individuals 18/02/10	((lxn) + height) x n individuals 23/06/09	0.92994
((lxn) + height) x n individuals 28/08/09	((lxn) + height) x n individuals 20/05/09	0.92619
SE mean n germ seeds/cap	mean number of seeds/cap	0.92281
mean length (cm) 23/06/09	SE mean length (cm) 11/06/09	0.92217
SE mean height (cm) 28/08/09	SE mean height (cm) 28/07/09	0.91858
total biomass	Biomass above	0.91691
SE mean height (cm) 11/06/09	mean height (cm) 11/06/09	0.91471
SE mean length (cm) 6/07/09	mean n true leaves 23/06/09	-0.91051
((lxn) + height) x n individuals 28/08/09	SE mean length (cm) 11/06/09	0.91023
((lxn) + height) x n individuals 6/07/09	((lxn) + height) x n individuals 11/06/09	0.90885
mean height (cm) 6/07/09	mean height (cm) 11/06/09	0.90834
height (cm) 10/07/10	mean % seed germinated	-0.90822
SE mean n germ seeds/cap	SE mean number of seeds/cap	0.90692
(length x n) + height 23/06/09	mean n true leaves 11/06/09	0.90454
SE mean length (cm) 11/06/09	SE mean n true leaves 11/06/09	-0.90374
mean n true leaves 11/06/09	mean n true leaves 20/05/09	0.9037
DELELTE #65	mean height (cm) 23/06/09	0.90311
height (cm) 10/07/10	mean height (cm) 28/08/09	0.90282
SE condition (cm) 18/02/10	mean n true leaves 28/07/09	-0.90162
(length x n) + height 28/08/09	mean length (cm) 23/06/09	0.90154
SE mean height (cm) 28/07/09	SE mean height (cm) 20/05/09	-0.89964
mean length (cm) 20/05/09	mean length (cm) 2/05/09	0.89658
height (cm) 10/07/10	mean height (cm) 18/02/10	0.89408
biomass below	SE height (cm) 10/07/10	0.89239
height (cm) 10/07/10	(length x n) + height 18/02/10	0.89203
SE mean height (cm) 28/08/09	mean height (cm) 20/05/09	-0.89046
SE mean length (cm) 6/07/09	mean n true leaves 20/05/09	-0.88684
mean n true leaves 6.07.09	mean n true leaves 23/06/09	0.8857
((lxn) + height) x n individuals 18/02/10	((lxn) + height) x n individuals 28/08/09	0.88527
SE n leaves 10/07/10	n leaves 10/07/10	0.88399

Table 30 cont.		
((lxn) + height) x n individuals 18/02/10	((lxn) + height) x n individuals 6/07/09	0.88377
SE mean length (cm) 18/02/10	((lxn) + height) x n individuals 20/05/09	-0.88352
(length x n) + height 23/06/09	mean height (cm) 11/06/09	0.88285
SE height (cm) 10/07/10	mean height (cm) 6/07/09	-0.88175
((lxn) + height) x n individuals 6/07/09	((lxn) + height) x n individuals 20/05/09	0.88136
SE mean length (cm) 6/07/09	total seed (g)	0.88027
mean height (cm) 28/07/09	mean length (cm) 23/06/09	0.87978
mean length (cm) 23/06/09	SE mean n true leaves 11/06/09	-0.87963
mean length (cm) 6/07/09	SE mean n true leaves 20/05/09	-0.87905
mean length (cm) 23/06/09	((lxn) + height) x n individuals 11/06/09	0.8761
mean height (cm) 28/07/09	mean length (cm) 28/07/09	0.87316
SE mean length (cm) 6/07/09	SE mean length (cm) 23/06/09	0.87271
(length x n) + height 6/07/09	mean height (cm) 23/06/09	0.87102
mean height (cm) 23/06/09	mean n true leaves 11/06/09	0.8707
mean height (cm) 28/07/09	SE mean length (cm) 11/06/09	0.87033
((lxn) + height) x n individuals 23/06/09	((lxn) + height) x n individuals 11/06/09	0.86933
mean n germ seeds/cap	SE mean number of seeds/cap	0.86912
height (cm) 10/07/10	condition rating	0.86851
SE mean length (cm) 6/07/09	mean n true leaves 6.07.09	-0.86691
(length x n) + height 6/07/09	mean n true leaves 11/06/09	0.86686
mean length (cm) 28/07/09	SE mean n true leaves 11/06/09	-0.8667
(length x n) + height 28/08/09	SE mean height (cm) 20/05/09	-0.86667
mean n true leaves 23/06/09	total seed (g)	-0.86578
SE mean n true leaves 20/05/09	SE mean % seed germinated	-0.86515
(length x n) + height 20/05/09	mean length (cm) 2/05/09	0.86507
SE mean length (cm) 11/06/09	((lxn) + height) x n individuals 20/05/09	0.86275
(length x n) + height 6/07/09	(length x n) + height 20/05/09	0.86229
SE height (cm) 10/07/10	(length x n) + height 6/07/09	-0.86143
Biomass above	(length x n) + height 18/02/10	0.86143
(length x n) + height 6/07/09	mean length (cm) 6/07/09	0.8612
mean length (cm) 28/07/09	SE mean height (cm) 20/05/09	-0.86075
SE mean length (cm) 20/05/09	mean number of seeds/cap	-0.85971
mean height (cm) 6/07/09	mean n true leaves 11/06/09	0.8597
Biomass above	mean height (cm) 18/02/10	0.85923
SE mean height (cm) 28/07/09	mean height (cm) 20/05/09	-0.85878
(length x n) + height 23/06/09	((lxn) + height) x n individuals 11/06/09	0.85834
mean n true leaves 6.07.09	mean n true leaves 20/05/09	0.85798
mean % seed germinated	mean seed weight (mg)	-0.85728
SE mean length (cm) 23/06/09	mean n true leaves 23/06/09	-0.85714
biomass below	SE mean height (cm) 18/02/10	0.85638
mean length (cm) 18/02/10	((lxn) + height) x n individuals 20/05/09	-0.85611
biomass below	SE mean % seed germinated	-0.85585
(length x n) + height 20/05/09	(length x n) + height 2/05/09	0.85374
mean height (cm) 11/06/09	(length x n) + height 20/05/09	0.85358
SE mean height (cm) 28/07/09	SE mean length (cm) 20/05/09	-0.85321
(length x n) + height 20/05/09	mean length (cm) 20/05/09	0.853
((lxn) + height) x n individuals 23/06/09	% survival	0.85298
mean height (cm) 23/06/09	mean height (cm) 11/06/09	0.85003
(length x n) + height 2/05/09	mean length (cm) 2/05/09	0.8493
SE mean length (cm) 18/02/10	mean length (cm) 18/02/10	0.84919
((lxn) + height) x n individuals 23/06/09	((lxn) + height) x n individuals 20/05/09	0.84726
SE height (cm) 10/07/10	mean length (cm) 6/07/09	-0.84654
mean height (cm) 6/07/09	(length x n) + height 23/06/09	0.84627
((lxn) + height) x n individuals 28/08/09	((lxn) + height) x n individuals 11/06/09	0.84535
mean n true leaves	total seed (g)	-0.84481
mean length (cm) 28/07/09	mean length (cm) 23/06/09	0.84382
total biomass	(length x n) + height 18/02/10	0.8426

Table 30. cont.		
mean length (cm) 23/06/09	SE mean n true leaves 23/06/09	-0.8414
SE mean height (cm) 28/08/09	(length x n) + height 28/08/09	0.84047
SE Cap/mean	mean days to germination	0.83943
SE mean height (cm) 28/08/09	mean n true leaves 28/07/09	0.83878
SE mean length (cm) 18/02/10	((lxn) + height) x n individuals 11/06/09	-0.83826
SE mean length (cm) 6/07/09	mean n true leaves	-0.83684
mean length (cm) 28/07/09	((lxn) + height) x n individuals 20/05/09	0.8368
(length x n) + height 6/07/09	mean height (cm) 11/06/09	0.83533
mean height (cm) 20/05/09	SE mean length (cm) 20/05/09	0.83532
(length x n) + height 18/02/10	mean % seed germinated	-0.83479
SE mean height (cm) 11/06/09	mean n true leaves 11/06/09	0.83439
((lxn) + height) x n individuals 6/07/09	% survival	0.83136
(length x n) + height 6/07/09	SE mean days to germination	0.83134
(length x n) + height 23/06/09	mean length (cm) 23/06/09	0.83047
mean height (cm) 23/06/09	(length x n) + height 20/05/09	0.82858
((lxn) + height) x n individuals 28/08/09	mean length (cm) 23/06/09	0.82844
SE mean length (cm) 28/07/09	mean length (cm) 2/05/09	-0.82763
(length x n) + height 6/07/09	mean height (cm) 6/07/09	0.8272
SE mean n true leaves 28/07/09	SE mean number of seeds/cap	-0.82716
mean height (cm) 18/02/10	mean % seed germinated	-0.82712
((lxn) + height) x n individuals 28/08/09	mean length (cm) 28/07/09	0.82698
((lxn) + height) x n individuals 23/06/09	SE mean length (cm) 11/06/09	0.82685
(length x n) + height 28/08/09	SE mean n true leaves 23/06/09	-0.82505
(length x n) + height 23/06/09	mean n true leaves 20/05/09	0.82505
SE mean n true leaves	mean % seed germinated	0.82331
height (cm) 10/07/10	SE mean n true leaves	-0.82331
n leaves 10/07/10	mean length (cm) 18/02/10	0.82239
SE mean length (cm) 28/07/09	mean length (cm) 20/05/09	-0.82222
(length x n) + height 11/06/09	mean seed weight (mg)	0.82188
SE mean n true leaves 23/06/09	SE mean length (cm) 11/06/09	-0.82159
(length x n) + height 11/06/09	mean height (cm) 11/06/09	0.8215
mean n true leaves	SE mean n germ seeds/cap	-0.82149
SE mean n true leaves 11/06/09	((lxn) + height) x n individuals 20/05/09	-0.82133
(length x n) + height 28/08/09	mean n true leaves 28/07/09	0.8213
total biomass	mean height (cm) 18/02/10	0.82021
SE mean length (cm) 20/05/09	mean n germ seeds/cap	-0.8199
mean length (cm) 23/06/09	mean length (cm) 11/06/09	0.81913
mean length (cm) 20/05/09	SE mean % seed germinated	0.8187
SE mean n true leaves 20/05/09	SE mean days to germination	-0.81863
mean height (cm) 18/02/10	mean height (cm) 28/08/09	0.81668
mean n true leaves 20/05/09	(length x n) + height 2/05/09	0.81642
SE mean height (cm) 11/06/09	mean number of seeds/cap	-0.81588
mean height (cm) 11/06/09	mean length (cm) 20/05/09	0.81501
(length x n) + height 28/08/09	((lxn) + height) x n individuals 20/05/09	0.81315
(length x n) + height 6/07/09	(length x n) + height 23/06/09	0.81311
mean length (cm) 23/06/09	((lxn) + height) x n individuals 20/05/09	0.81234
(length x n) + height 2/05/09	mean n true leaves	0.81232
SE mean length (cm) 6/07/09	(length x n) + height 2/05/09	-0.81215
mean height (cm) 6/07/09	(length x n) + height 11/06/09	0.81188
SE mean length (cm) 20/05/09	SE mean n germ seeds/cap	-0.81128
mean length (cm) 11/06/09	mean seed weight (mg)	0.81096
SE mean height (cm) 20/05/09	SE mean height (cm) 2/05/09	0.8106
SE mean height (cm) 18/02/10	mean height (cm) 28/07/09	-0.81046
((lxn) + height) x n individuals 28/08/09	SE mean n true leaves 11/06/09	-0.80965
mean length (cm) 28/07/09	mean height (cm) 20/05/09	-0.80822
condition rating	SE Cap/mean	0.8068
SE mean % germ.	(tm) outcrossing rate	0.80226

Table 30. cont.		
SE mean length (cm) 18/02/10	((lxn) + height) x n individuals 6/07/09	-0.80594
mean length (cm) 6/07/09	SE mean % seed germinated	0.80498
(length x n) + height 18/02/10	mean height (cm) 28/08/09	0.80481
(length x n) + height 28/08/09	SE mean n true leaves 11/06/09	-0.8047
((lxn) + height) x n individuals 11/06/09	((lxn) + height) x n individuals 20/05/09	0.80442
SE mean height (cm) 18/02/10	mean length (cm) 23/06/09	-0.80411
SE mean length (cm) 18/02/10	((lxn) + height) x n individuals 2/05/09	-0.80362
SE mean n germ seeds/cap	mean n germ seeds/cap	0.80346
mean n true leaves 11/06/09	(length x n) + height 20/05/09	0.80314
SE condition (cm) 18/02/10	((lxn) + height) x n individuals 2/05/09	-0.803
(length x n) + height 11/06/09	mean n true leaves 11/06/09	0.80233
SE mean height (cm) 28/07/09	Cap/mean	-0.80205
mean n true leaves 23/06/09	mean height (cm) 20/05/09	0.80133
(length x n) + height 11/06/09	mean length (cm) 20/05/09	0.80063
(length x n) + height 11/06/09	mean length (cm) 2/05/09	0.79761
condition rating	mean % seed germinated	-0.79758
mean height (cm) 11/06/09	mean length (cm) 2/05/09	0.79734
(length x n) + height 6/07/09	PASTE #65	0.79568
mean length (cm) 28/07/09	SE mean n true leaves 23/06/09	-0.79562
SE mean length (cm) 20/05/09	SE mean height (cm) 2/05/09	0.79512
((lxn) + height) x n individuals 18/02/10	SE mean height (cm) 18/02/10	-0.79503
SE mean length (cm) 11/06/09	SE mean height (cm) 20/05/09	-0.79465
SE mean length (cm) 20/05/09	SE mean number of seeds/cap	-0.79391
((lxn) + height) x n individuals 18/02/10	% survival	0.79388
mean n germ seeds/cap	mean seed weight (mg)	-0.79321
SE mean height (cm) 18/02/10	SE mean height (cm) 2/05/09	0.7929
mean height (cm) 28/07/09	SE mean height (cm) 20/05/09	-0.79283
((lxn) + height) x n individuals 20/05/09	number of trees	0.79245
SE mean n true leaves 20/05/09	SE total seed (g)	-0.79199
mean length (cm) 11/06/09	mean length (cm) 20/05/09	0.7915
SE mean height (cm) 20/05/09	SE mean length (cm) 20/05/09	0.78994
mean n true leaves 20/05/09	total seed (g)	-0.78957
SE mean n true leaves 28/07/09	SE mean height (cm) 23/06/09	0.78731
((lxn) + height) x n individuals 6/07/09	SE mean length (cm) 11/06/09	0.78687
(length x n) + height 11/06/09	mean n true leaves 20/05/09	0.786
biomass below	mean height (cm) 6/07/09	-0.78559
((lxn) + height) x n individuals 18/02/10	SE mean length (cm) 11/06/09	0.78472
mean n true leaves 6.07.09	(length x n) + height 20/05/09	0.78471
SE mean % seed germinated	mean % seed germinated	-0.78445
SE mean height (cm) 11/06/09	(length x n) + height 20/05/09	0.78353
((lxn) + height) x n individuals 6/07/09	mean length (cm) 23/06/09	0.78329
mean height (cm) 6/07/09	SE mean height (cm) 11/06/09	0.7812
mean height (cm) 28/08/09	mean % seed germinated	-0.78047
SE mean n true leaves 23/06/09	SE mean height (cm) 2/05/09	0.78005
condition rating	SE mean % seed germinated	0.7797
mean height (cm) 28/07/09	((lxn) + height) x n individuals 11/06/09	0.77937
mean height (cm) 11/06/09	(length x n) + height 2/05/09	0.7787
biomass below	mean length (cm) 6/07/09	-0.77853
mean height (cm) 6/07/09	((lxn) + height) x n individuals 11/06/09	0.7785
((lxn) + height) x n individuals 28/08/09	(length x n) + height 28/08/09	0.77821
mean height (cm) 23/06/09	mean n true leaves 20/05/09	0.7781
SE mean height (cm) 28/08/09	SE mean n true leaves 23/06/09	-0.77763
(length x n) + height 20/05/09	mean n true leaves 20/05/09	0.77748
SE mean height (cm) 28/08/09	mean length (cm) 28/07/09	0.77731
SE mean length (cm) 6/07/09	(length x n) + height 20/05/09	-0.77713
SE height (cm) 10/07/10	SE mean n true leaves 20/05/09	0.77678
SE height (cm) 10/07/10	SE mean % seed germinated	-0.77673

Table 30. cont.		
SE mean height (cm) 18/02/10	SE mean n true leaves 23/06/09	0.77588
mean n true leaves 6.07.09	total seed (g)	-0.77489
(length x n) + height 6/07/09	(length x n) + height 11/06/09	0.77473
mean length (cm) 23/06/09	(length x n) + height 11/06/09	0.77462
((lxn) + height) x n individuals 28/08/09	% survival	0.77459
mean height (cm) 28/07/09	mean n true leaves 28/07/09	0.77431
(length x n) + height 6/07/09	mean n true leaves 20/05/09	0.77372
mean height (cm) 6/07/09	mean length (cm) 11/06/09	0.77369
((lxn) + height) x n individuals 11/06/09	mean length (cm) 11/06/09	0.77316
SE mean length (cm) 18/02/10	(length x n) + height 23/06/09	-0.77256
((lxn) + height) x n individuals 11/06/09	number of trees	0.77256
mean length (cm) 20/05/09	mean seed weight (mg)	0.77251
mean length (cm) 23/06/09	SE mean % seed germinated	0.7714
mean n true leaves 11/06/09	(length x n) + height 2/05/09	0.77126
((lxn) + height) x n individuals 23/06/09	mean length (cm) 23/06/09	0.77115
mean length (cm) 6.07.09	(tm) outcrossing rate	0.77079
SE mean length (cm) 6/07/09	mean height (cm) 23/06/09	-0.77057
SE mean n true leaves 23/06/09	SE mean n true leaves 11/06/09	0.77009
(length x n) + height 23/06/09	mean length (cm) 20/05/09	0.77001
SE mean height (cm) 11/06/09	SE mean number of seeds/cap	-0.76979
mean length (cm) 6/07/09	SE mean days to germination	0.76844
((lxn) + height) x n individuals 2/05/09	mean days to germination	-0.76842
SE mean length (cm) 18/02/10	((lxn) + height) x n individuals 28/08/09	-0.76835
mean n true leaves 6.07.09	mean n true leaves 11/06/09	0.76818
((lxn) + height) x n individuals 11/06/09	SE mean length (cm) 11/06/09	0.76692
(length x n) + height 11/06/09	(length x n) + height 20/05/09	0.76688
SE height (cm) 10/07/10	SE mean days to germination	-0.76654
mean height (cm) 23/06/09	mean n true leaves 23/06/09	0.76629
mean height (cm) 6/07/09	mean height (cm) 23/06/09	0.76623
(length x n) + height 28/08/09	mean height (cm) 20/05/09	-0.76575
SE mean length (cm) 20/05/09	Cap/mean	0.76525
SE mean % seed germinated	mean seed weight (mg)	0.76462
((lxn) + height) x n individuals 18/02/10	((lxn) + height) x n individuals 11/06/09	0.76452
biomass below	mean length (cm) 23/06/09	-0.76445
((lxn) + height) x n individuals 11/06/09	(length x n) + height 11/06/09	0.76428
Biomass above	mean length (cm) 18/02/10	0.7641
SE height (cm) 10/07/10	SE mean height (cm) 18/02/10	0.76352
mean height (cm) 23/06/09	total seed (g)	-0.76318
mean n germ seeds/cap	Cap/mean	-0.76249
mean length (cm) 11/06/09	mean length (cm) 2/05/09	0.76231
SE mean n true leaves 23/06/09	mean length (cm) 11/06/09	-0.76189
SE height (cm) 10/07/10	mean height (cm) 11/06/09	-0.7617
DELETE #104	mean length (cm) 18/02/10	0.76129
((lxn) + height) x n individuals 11/06/09	% survival	0.76092
SE mean height (cm) 28/08/09	mean height (cm) 28/07/09	0.7605
mean height (cm) 11/06/09	mean length (cm) 11/06/09	0.76014
biomass below	condition rating	-0.75992
SE mean height (cm) 18/02/10	((lxn) + height) x n individuals 11/06/09	-0.75934
SE mean length (cm) 18/02/10	mean n true leaves 11/06/09	-0.75932
mean n true leaves 28/07/09	SE mean height (cm) 20/05/09	-0.7589
mean n true leaves 23/06/09	SE mean number of seeds/cap	-0.75778
mean height (cm) 28/08/09	mean length (cm) 2/05/09	0.75773
mean length (cm) 2/05/09	mean seed weight (mg)	0.75743
SE mean n true leaves 28/07/09	SE mean n germ seeds/cap	-0.75661
SE mean length (cm) 23/06/09	SE mean n germ seeds/cap	0.75653
(length x n) + height 18/02/10	SE mean n true leaves	-0.75643
SE mean length (cm) 6/07/09	SE mean n germ seeds/cap	0.75625

Table 30. cont.		
condition rating	mean height (cm) 28/08/09	0.75489
condition rating	SE mean n true leaves	-0.75487
biomass below	(tm) outcrossing rate	-0.75443
SE mean length (cm) 6/07/09	mean number of seeds/cap	0.75426
mean height (cm) 11/06/09	mean n true leaves 20/05/09	0.75425
SE mean n germ seeds/cap	total seed (g)	0.75382
SE mean height (cm) 28/08/09	SE mean length (cm) 11/06/09	0.75284
SE mean height (cm) 18/02/10	SE mean length (cm) 11/06/09	-0.75198
(length x n) + height 23/06/09	(length x n) + height 20/05/09	0.75071
biomass below	SE mean n true leaves 20/05/09	0.75041
mean height (cm) 28/08/09	SE mean n true leaves	-0.75027
Biomass above	SE n leaves 10/07/10	0.75016
SE mean length (cm) 18/02/10	mean length (cm) 23/06/09	-0.74999
(length x n) + height 6/07/09	SE mean height (cm) 11/06/09	0.7499
mean height (cm) 6/07/09	mean length (cm) 20/05/09	0.74965
SE mean length (cm) 11/06/09	number of trees	0.74903
SE mean n true leaves 23/06/09	SE mean height (cm) 20/05/09	0.74834
mean height (cm) 18/02/10	SE mean n true leaves	-0.74798
mean height (cm) 2/05/09	SE total seed (g)	0.74718
(length x n) + height 23/06/09	mean seed weight (mg)	0.7465
mean length (cm) 28/07/09	SE mean height (cm) 2/05/09	-0.74603
SE mean height (cm) 28/08/09	SE mean height (cm) 2/05/09	-0.7454
SE mean height (cm) 18/02/10	SE mean % seed germinated	-0.74498
mean height (cm) 20/05/09	SE mean height (cm) 2/05/09	0.74441
SE condition (cm) 18/02/10	mean days to germination	0.7444
mean n true leaves 23/06/09	SE mean n germ seeds/cap	-0.74428
mean n true leaves 20/05/09	mean n true leaves	0.74416
mean height (cm) 28/07/09	((lxn) + height) x n individuals 20/05/09	0.7435
SE mean length (cm) 28/07/09	(length x n) + height 20/05/09	-0.74264
(length x n) + height 28/08/09	((lxn) + height) x n individuals 11/06/09	0.74228
mean n true leaves 6.07.09	SE mean height (cm) 11/06/09	0.74216
SE mean length (cm) 28/07/09	(length x n) + height 2/05/09	-0.74161
SE mean height (cm) 18/02/10	SE mean n true leaves	0.74056
SE height (cm) 10/07/10	(length x n) + height 23/06/09	-0.74039
mean height (cm) 11/06/09	mean seed weight (mg)	0.73851
SE mean n true leaves 23/06/09	((lxn) + height) x n individuals 11/06/09	-0.73823
(length x n) + height 18/02/10	SE Cap/mean	0.73813
SE mean n true leaves 28/07/09	total seed (g)	-0.73719
SE mean length (cm) 23/06/09	total seed (g)	0.73635
SE mean length (cm) 23/06/09	SE mean number of seeds/cap	0.73414
Biomass above	n leaves 10/07/10	0.734
SE mean length (cm) 6/07/09	mean n true leaves 11/06/09	-0.73358
mean length (cm) 11/06/09	mean n true leaves 11/06/09	0.73357
SE mean height (cm) 18/02/10	(length x n) + height 28/08/09	-0.73293
SE mean length (cm) 11/06/09	mean height (cm) 20/05/09	-0.7327
SE mean length (cm) 6/07/09	SE mean length (cm) 20/05/09	-0.73222
(length x n) + height 2/05/09	mean number of seeds/cap	-0.73183
SE mean height (cm) 28/08/09	Cap/mean	-0.73174
height (cm) 10/07/10	SE mean % seed germinated	0.73111
SE mean height (cm) 11/06/09	mean n germ seeds/cap	-0.73031
((lxn) + height) x n individuals 28/08/09	SE mean n true leaves 23/06/09	-0.73006
(length x n) + height 20/05/09	mean seed weight (mg)	0.72881
SE height (cm) 10/07/10	(length x n) + height 11/06/09	-0.72862
((lxn) + height) x n individuals 11/06/09	mean n true leaves 11/06/09	0.72811
SE mean height (cm) 18/02/10	mean length (cm) 28/07/09	-0.7276
biomass below	((lxn) + height) x n individuals 18/02/10	-0.72754
mean height (cm) 6/07/09	mean length (cm) 2/05/09	0.72746

Table 30. cont.		
mean n true leaves 23/06/09	mean n true leaves 20/05/09	0.72692
(length x n) + height 20/05/09	mean number of seeds/cap	-0.72674
mean length (cm) 11/06/09	SE mean % seed germinated	0.7267
SE mean length (cm) 18/02/10	((lxn) + height) x n individuals 23/06/09	-0.72647
mean % seed germinated	SE Cap/mean	-0.72554
mean n true leaves 11/06/09	total seed (g)	-0.72541
SE mean height (cm) 18/02/10	condition rating	-0.72531
SE mean height (cm) 28/07/09	SE mean height (cm) 2/05/09	-0.72305
SE mean height (cm) 6/07/09	SE mean seed weight (mg)	-0.723
((lxn) + height) x n individuals 28/08/09	mean height (cm) 28/07/09	0.72196
mean length (cm) 18/02/10	((lxn) + height) x n individuals 6/07/09	-0.7215
(length x n) + height 6/07/09	mean length (cm) 11/06/09	0.72149
SE mean n true leaves 11/06/09	SE mean % seed germinated	-0.72092
mean height (cm) 11/06/09	mean number of seeds/cap	-0.72076
SE mean height (cm) 18/02/10	mean length (cm) 11/06/09	-0.71887
mean length (cm) 11/06/09	mean n true leaves 20/05/09	0.7188
SE mean height (cm) 20/05/09	Cap/mean	0.71393
((lxn) + height) x n individuals 18/02/10	mean length (cm) 23/06/09	0.7136
((lxn) + height) x n individuals 18/02/10	SE mean height (cm) 2/05/09	-0.71348
SE height (cm) 10/07/10	mean length (cm) 11/06/09	-0.71318
SE mean height (cm) 11/06/09	(length x n) + height 2/05/09	0.71282
(length x n) + height 6/07/09	mean length (cm) 20/05/09	0.71263
total biomass	mean length (cm) 18/02/10	0.71196
mean length (cm) 28/07/09	mean n true leaves 23/06/09	-0.71085
mean length (cm) 28/07/09	((lxn) + height) x n individuals 23/06/09	0.71054
mean height (cm) 28/07/09	mean height (cm) 20/05/09	-0.70802
mean height (cm) 23/06/09	mean number of seeds/cap	-0.70738
biomass below	mean length (cm) 20/05/09	-0.70704
condition rating	(tm) outcrossing rate	0.70645
mean length (cm) 23/06/09	mean length (cm) 20/05/09	0.70602
SE mean height (cm) 10.07.10	(tm) outcrossing rate	-0.70574
n leaves 10/07/10	mean length (cm) 6/07/09	-0.70488
mean height (cm) 2/05/09	% survival	-0.70482
mean n true leaves 6.07.09	(length x n) + height 2/05/09	0.70466
mean height (cm) 28/07/09	SE mean n true leaves	-0.70453
(length x n) + height 18/02/10	condition rating	0.70403
mean seed weight (mg)	mean number of seeds/cap	-0.70396
mean height (cm) 28/08/09	mean length (cm) 20/05/09	0.70389
mean length (cm) 11/06/09	(length x n) + height 20/05/09	0.70366
mean length (cm) 28/07/09	SE mean height (cm) 23/06/09	-0.70333
SE mean length (cm) 6/07/09	SE mean number of seeds/cap	0.70313
mean length (cm) 20/05/09	(length x n) + height 2/05/09	0.70199
mean n true leaves 28/07/09	mean length (cm) 11/06/09	0.70195
mean height (cm) 6/07/09	(length x n) + height 20/05/09	0.70174
SE mean length (cm) 23/06/09	mean n true leaves	-0.70087
SE mean days to germination	mean days to germination	0.70041
mean n true leaves 28/07/09	((lxn) + height) x n individuals 2/05/09	0.70024
SE mean n true leaves 23/06/09	((lxn) + height) x n individuals 20/05/09	-0.70021
SE mean height (cm) 28/08/09	SE mean length (cm) 20/05/09	-0.7001

length = length of the longest leaf, height = height of seedling (cm), SE = standard error