III.7 Molecular Markers in Tree Improvement: Characterisation and Use in *Eucalyptus*

M. Shepherd and M.E. Jones[1]

1 Introduction

Eucalypts are the most widely planted hardwood trees in the world, occupying a global estate of around 12 million ha (Turnbull 1999). The genus comprises over 700 species, most of which are endemic to Australia, and its diverse membership offers species with adaptability to a range of exotic tropical and temperate conditions with high growth rates on productive sites (Eldridge et al. 1994). They are a major source of wood for paper pulp and construction timber, as well as fuelwood for industrial and domestic purposes in many developing countries (Eldridge et al. 1994).

Domestication of eucalypts is still at an early stage, with most breeding populations only several generations removed from wild populations (Eldridge et al. 1994). The challenge for eucalypt breeders, as with most tree crops, is to make genetic gains in the face of long generations and delays in selecting mature traits, which can be as long as 20–30 years for wood properties. Eucalypts, having mixed mating systems, are predominantly outcrossing (rates between 0.7 and 0.92), and are thought to possess high levels of genetic load and exhibit deleterious effects when inbred (Eldridge 1970; Potts and Reid 1990; Myburg et al. 2000). Consequently, breeders tend to avoid inbreeding, instead they manage broadly based breeding as well as specialty populations to select for genetic gains (Eldridge et al. 1994). Eucalypts, as a group, are recognised as being promiscuous, with weak reproductive barriers amongst taxa (Pryor 1976), and a frequency of natural and artificial hybridisation that declines as taxonomic distance between parents increases (Griffin et al. 1988; Potts et al. 2001). Interspecific F_1 hybrids feature in a number of breeding programs, often because they combine desirable characteristics from the parental taxa, but also for hybrid superiority imparted through heterosis, epistasis or trait complementarity (Nikles and Griffin 1992). Many tropical eucalypts are amenable to vegetative propagation, hence, one strategy for improvement is intensive within family selection followed by mass clonal reproduction of elite hybrid trees (Eldridge et al. 1994).

[1] Centre for Plant Conservation Genetics, Southern Cross University, P.O. Box 157, Lismore, NSW 2480 Australia

Biotechnology in Agriculture and Forestry, Vol. 55
Molecular Marker Systems (ed. by H. Lörz and G. Wenzel)

Molecular markers have been embraced enthusiastically in the face of these complex and varied challenges for eucalypt breeders, as they offer hope of circumventing some restrictions to or accelerating improvement. In this review, we provide representative or unique case studies where applications of molecular markers are benefiting eucalypt breeders. Initially, we consider how gene resource (base) populations can be managed and best exploited with the aid of molecular markers. Then, a study to determine whether anthropological factors influence the genetic diversity of base populations of a eucalypt is reviewed. Next, we consider how markers are helping to define the gene pool available for breeding eucalypts and how they are revealing new understanding about the genetic mechanisms of hybrid inviability. We review two recent accounts of gene flow and paternity analysis that are providing data to optimise seed orchard design and finally, perhaps the area where markers will ultimately have the greatest impact on breeding, molecular breeding. In recent years, research into the molecular breeding of eucalypts has entered a new era with an emphasis on multi-allelic markers and candidate gene mapping. This second generation of experiments addresses short falls and builds on the discoveries of a first generation of genetic mapping and quantitative trait loci (QTL) experiments in the earlier years of the last decade. On the subject of molecular breeding, our review updates an excellent recent review in this area (Grattapaglia 2000). Grattapaglia (2000) also provides a review of applications of DNA fingerprinting and germplasm management not considered in this review. For a comprehensive review of eucalypt genetics and genecology, see Potts and Wiltshire (1997), and for tree improvement of eucalypts in general, Eldridge et al. (1994).

2 Base Population Characterisation – *Eucalyptus globulus* is Geographically Structured with Chloroplast Haplotypes Coincident with Quantitative Genetic Variation

Our understanding of the natural population structure of *E. globulus* is perhaps the most detailed of any eucalypt. It is a major plantation species for temperate regions in Australia, Chile, Portugal, Spain and China (Eldridge et al. 1994) and has been subject to a concerted research effort over the past decade that has revealed a detailed picture of the relationships and evolutionary forces shaping the quantitative and molecular variation in this species.

Eucalyptus globulus has a broad natural distribution in south-eastern Australia with populations in Victoria, Tasmania and on the islands of Bass Strait. It is currently recognised as having four geographical subspecies, spp. *globulus*, spp. *biocostata*, spp. *pseudoglobulus* and spp. *maidenii* (Chippendale 1988). A major latitudinal cline was evident in random amplified polymorphic DNA (RAPD) markers from the northern mainland Australian localities to the southern Tasmanian localities (Nesbitt et al. 1995). Like

RAPD, restriction fragment length polymorphism (RFLP) variation of *E. globulus* chloroplast revealed lineages that showed strong geographic structuring rather than alignment with taxonomic subspecies boundaries (Jackson et al. 1999). This study also found that chloroplast haplotypes transcended species boundaries, as haplotypes were shared with co-occurring endemic eucalypts. Hybridisation and introgression of chloroplasts is believed to be the most likely explanation for this, emphasising the importance of reticulate evolution in the eucalypt group.

Chloroplast lineages within Tasmania were also found to be coincident with patterns of quantitative genetic variation that had been used to establish a racial classification for *E. globulus* (Dutkowski and Potts 1999; Jackson et al. 1999). Traits including bark thickness, wood basic density and flowering precocity exhibited strong spatial patterns. Racial groups are of interest to breeders as they summarise complex patterns of variation and may improve prediction of breeding values (Dutkowski and Potts 1999).

The development of microsatellite markers in *E. globulus* (Steane et al. 2001) has enabled further molecular characterisation of this species. These markers were found to be highly polymorphic across the geographic range of *E. globulus*. Microsatellite analysis found significant differentiation across the geographic range of ssp. *globulus* and was consistent with the previous RAPD and quantitative genetic studies, providing a further tool for race identification and exploring relationships within and between races.

3 Effect of Utilisation on the Base Population Resource – Influence of Silvicultural and Harvesting on *Eucalyptus sieberi* Genetic Diversity

The impact of native forest management, including logging, upon genetic diversity of *E. sieberi,* a eucalypt from lowland mixed-species forests of south-eastern Australia, was recently examined (Glaubitz et al. 1999; Moran et al. 2000). Three silvicultural treatments that were commonly practiced in this region were investigated; clearfelling with aerial resowing, regeneration from seed trees following burning, and regeneration from seed trees following mechanical disturbance. Several measures of genetic diversity at RFLP and microsatellite loci indicated there were no significant differences amongst treatments or within unharvested controls. As *E. sieberi* is abundant in the region, it was thought that the diversity in regenerated areas was maintained in this system by a high number of pollen parents contributing to offspring in harvested coupes. Although the silvicultural practices apparently do not significantly affect diversity for common widespread species over one generation, it remains to be seen whether these practices are suitable for more localised species or for longer time frames (Moran et al. 2000).

4 Defining the Gene Pool for Breeding – Hybridisation

Eucalypt hybrids have been an important part of plantation forestry, particularly in the tropics (Potts and Dungey 2001). DNA markers are helping breeders to characterise the gene pool available to create hybrid eucalypts and the genetic causes of hybrid inviability.

4.1 Resolving Anomalies in the Success of Wide Hybrids

Hybridisation between subgenera for a long time was thought to delimit the extent of hybridisation in eucalypts (Pryor and Johnson 1981). Reports of an occurrence of a putative natural hybrid between *E. cloeziana* and *E. acmenodies*, members of the *Idiogenes* and *Monocalyptus* subgenera, respectively, were thought to be the single exception (Brooker and Kleinig 1994). Analysis of morphological characters as well as the chloroplast J_{LA} region verified hybridisation and established the direction of crossing between these two species (Stokoe et al. 2001). Genealogical analysis of hybrid offspring based on microsatellite markers indicated that the extent of hybridisation was likely to be restricted to an F_1 generation (Stokoe 2002). Phylogenetic analysis of the J_{LA} region *E. cloeziana* and 20 subgenus *Monocalyptus* species, however, found no supporting evidence for a division between the single monotypic subgenus *Idiogenes* and *Monocalyptus* (Stokoe et al. 2002). Molecular evidence, therefore, suggests there is no basis for a transgression of the subgenera rule.

4.2 Genetic Causes of Hybrid Inviability

A glimpse of the importance and complexity of genic mechanisms maintaining species and defining patterns of hybridisation was obtained from comparisons of transmission ratio distortion (TRD) in genetic maps from interspecific crosses of *E. globulus* and *E. grandis* (Myburg et al. 2003, 2004). In this study, AFLP markers were used to generate maps of an interspecific F_1 individual and two backcross parents. The F_1 *E. globulus* × *E. grandis* cross exhibits high levels of hybrid inviability and breakdown (Griffin et al. 2000). As with many wide crosses, a high proportion (27 %) of markers were distorted, but these loci mapped to a few regions and marker alleles were biased to either of the two parents, supporting biological rather than methodological causes of distortion. Multiple putative TRD loci (TRDL) were found in each map, but there was surprisingly little evidence of epistasis between these loci. Those interactions that were detected tended to be between donor and recurrent parent alleles and they tended to be positive, increasing hybrid fitness. Remarkably, the donor genes were not predominantly selected against

in the recurrent background, as might be expected with dysfunctional homologous recombination. Preferential survival of donor alleles suggest either the presence of "selfish" genes that enhance the success of gametes or that there was alleviation of genetic load, which is a major factor influencing fitness in forest trees (Williams and Savolainen 1996). Comparison of TRDL between maps revealed some loci were fixed, whereas other loci segregated between the two species.

5 Direct Measures of Gene Flow – Implications for Orchard Design

Gene flow analysis in eucalypts is increasingly relying on direct measures derived from molecular markers because they provide absolute measures, high resolution paternity analysis and enable the separate contributions from pollen and seed to be distinguished (reviewed in Potts and Wiltshire 1997). Isozyme analysis of gene flow in a 10-year-old seed orchard of the insect-pollinated *E. regnans* indicated the likely importance of floral phenology, tree spacing, provenance, and within-orchard position in determining complex mating patterns (Burczyk et al. 2002). Asynchronicity in floral phenology between provenances, for example, was thought to largely explain why intra-provenance crosses were three times more likely to occur than inter-provenance crosses. Despite a preference for within-provenance mating, pollen dispersal was found to be extensive throughout the orchard with 50 % of all effective pollen travelling a distance of at least 40 m and bypassing large numbers of nearer neighbours.

Microsatellite markers and paternity assignment were used to study pollen flow in a seed orchard of *E. grandis* (Jones et al., in prep.). Nearly half of the progeny analysed (46 %) was found to be the result of pollen originating from trees located outside the seed orchard, probably from nearby surrounding *E. grandis* plantations (Fig. 1). The high level of pollen contamination from outside the *E. grandis* orchard suggested that longer-range pollen dispersal vectors such as flying fox bats may be important for pollen dispersal as they are in some south-eastern, coastal eucalypts (House 1997). Pollination distance within the seed orchard was also extensive with 48 % of identified parent pairs located more than 50 m apart, and pollen travelling distances up to the maximum detectable distance of ~192 m. Of the pollen parents identified within the orchard, the majority were from a provenance that was different to the mother tree (inter-provenance) and low intra-provenance and within-family crossing was observed.

The low level of intra-provenance provenance pollination in the *E. grandis* study contrasted with the preferential intra-provenance mating exhibited in the *E. regnans* orchard (Burczyk et al. 2002). This difference between the two studies may be accounted for by the greater breadth of material studied in the

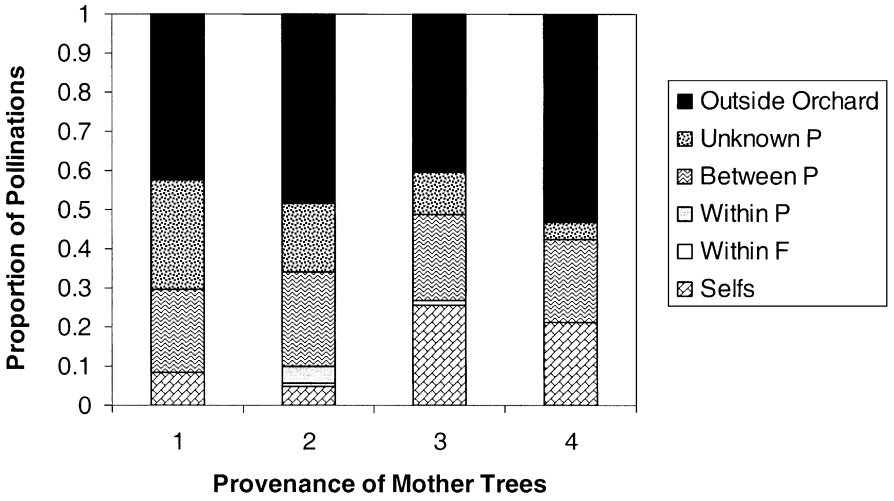

Fig. 1. Pollination events categorised by the degree of relationship between the pollen donor and the mother tree for six *E. grandis* trees. The mother trees are grouped into four provenances: *1* Boambee SF, *2* Pine Creek plantation 3, *3* Newry plantation, *4* Pine Creek plantation 17. Pollination events were classified as; self fertilisation (*Selfs*), within-family (*Within F*), within-provenance (*Within P*), between provenance (*Between P*), unknown provenance (*Unknown P*; orchard trees for which no provenance data is available) and contamination from outside the orchard (*Outside Orchard*)

E. regnans orchard compared to the *E. grandis* orchard. The *E. regnans* orchard was derived from two distinct provenances that differed in flowering time, whereas the *E. grandis* orchard was based on material from the Coffs Harbour region of New South Wales. Although the Coffs Harbour region has been recognised as encompassing multi-provenance sources (Burgess et al. 1996), it is unlikely this material would exhibit the marked differences in flowering phenology observed in the *E. regnans* study, as *E. grandis* is known for high flowering synchronicity across provenances compared to other species (Law et al. 2000).

Among the six mother trees studied in the Coffs Harbour seed orchard, the proportion of progeny derived from self-fertilisation ranged between zero and 36% with an overall average of 13%, consistent with the high degree of between-tree and within-canopy variability in self fertilisation rates of eucalypts (Potts and Wiltshire 1997). This observed outcrossing rate compared closely with an 'effective' multi-locus outcrossing rate (t) of 0.89 based on eight microsatellite markers generated using MLTR v2.4 (Ritland 2002) and was similar to the rate typical for natural populations of this species (0.84; J.C. Bell in Eldridge et al. 1994 p. 194). The level of selfing was apparently higher, however, than that detected in an *E. grandis* orchard in Uruguay, which had low levels of selfing for this species (5%; Russell et al. 2001). At present, we may only speculate on possible causes for apparent differences in

breeding system parameters between these studies because of the many sources of variation influencing fecundity that prevent critical comparison. Nonetheless, it seems that the planting of eucalypts as exotics does not necessarily lead to large increases in selfing rates as might be expected with some "offsite" plantings due to poor or sporadic flowering.

6 Genetic Architecture of Commercial Traits – Quantitative Trait Loci and Candidate Gene Mapping

6.1 First Generation Genetic Mapping and Quantitative Trait Loci Studies – Detection of Major Effect Genes and Quantitative Trait Loci Stability Across Physiological Age Classes

Genetic mapping and QTL detection studies in eucalypts over the early part of the last decade were characterised by the use of unplanned crosses, typically F_1 families from interspecific matings and the use of the pseudo-testcross mapping strategy (e.g., Grattapaglia and Sederoff 1994; Grattapaglia et al. 1996; Verhaegen and Plomion 1996; Shepherd et al. 1999). Hybrid families were targeted for these studies because of the favourable prospects for gains from early within-family selection using marker-aided selection (MAS) directly in populations used for deployment (Bradshaw and Grattapaglia 1994). Gains can be realised even earlier in many tropical eucalypts because of their amenability to vegetative propagation (Bradshaw and Foster 1992; Eldridge et al. 1994). There were important exceptions to this approach, however that were based on multi-generation intraspecific crosses or that used family arrays or factorial designs in attempts to identify QTL of average effect (O'Malley and McKeand 1994; Byrne et al. 1995; Grattapaglia et al. 1996; Verhaegen and Plomion 1996; Squilassi and Grattapaglia 1998). A thorough review of earlier work has been recently published (Grattapaglia 2000). Here, we report only the major outcomes, as a prelude to a review of more recent reports.

A major theme that emerged from early genetic mapping and QTL detection experiments was the high proportion of variation in many traits that were apparently controlled by a few major genes (Grattapaglia 2000). The detection of major gene effects for traits believed to have simple underlying genetic control was expected, and recently it has been possible to validate some of these putative QTL for vegetative propagation characteristics (Marques et al. 2002; also see below).

The detection of major gene effects for traits considered as quantitative, however, such as diameter or volume stem growth, was less expected. This outcome may have been a consequence of the predominance of studies based on wide hybrids where atypical large effects segregated as a consequence of hybrid incompatibility. Alternatively, there are confounding issues of limited

experimental power and potential problems with sampling effects due to small population sizes, which may have led to detection of false QTL or inflated QTL parameters (Beavis 1998). This issue needs to be resolved by large-scale QTL detection and validation experiments before too much emphasis can be placed on these early estimates of QTL effects (Grattapaglia 2000).

Another outcome from early QTL detection studies was the discovery that the stability of QTL across different physiological ages may be higher than initially thought (Campinhos et al. 1997; Verhaegen et al. 1997; Grattapaglia 2000; P. Bundock, pers. comm.). This was important, as one difficulty in tree improvement is the need to establish reliable correlations between a tree's performance at a young age and that at harvest, as different genes may be influencing a trait at different ages in a tree's life. These studies suggested, however, that some genes (or at least their effect), are important for growth for long periods in a tree's life time.

It was also clear from early QTL studies that there was a need to investigate QTL variability across populations to identify the most useful QTL for breeding (Grattapaglia 2000). Several studies had indicated that genetic background significantly affects QTL detection (Grattapaglia et al. 1996; Verhaegen et al. 1998). Furthermore, little was known about QTL × site interaction (Bradshaw and Grattapaglia 1994; Grattapaglia 2000). These early studies also highlighted the need for more informative multi-allelic marker types, such as microsatellites to facilitate exchange of genetic information within and across species in the genus (Grattapaglia 2000).

6.2 Second Generation Experiments – Multi-Allelic Markers, Quantitative Trait Loci × Site Effects, Candidate Gene Mapping

6.2.1 Microsatellites and Genus-Wide Maps

A genus-wide map for *Eucalyptus* is now feasible with the availability of an abundance of highly variable, multi-allelic microsatellite markers and their ready transfer amongst related species (Brondani et al. 1998, 2002; Byrne et al. 1996; Glaubitz et al. 2001; Jones et al. 2001; Steane et al. 2001). A genetic map with 240 microsatellite loci was developed from a pool of over 500 microsatellite markers (Brondani et al. 2001). Many of these loci exhibited high levels of polymorphism, with average expected heterozygosities in the range of 0.82–0.87 (Brondani et al. 2002). Transferability of microsatellites was high amongst eucalypts with around 80–90% of loci transferring amongst the key commercial species belonging to the *Symphyomyrtus* group, *E. globulus*, *E. grandis*, *E. urophylla* and *E. tereticornis* (Brondani et al. 2001). Similarly high levels of transfer were found for microsatellite markers developed from *Corymbia variegata* (spotted gum; formerly *Eucalyptus*; Jones et al. 2001). All 14 loci tested, transferred to another species within the same

Corymbia section (*Politaria*). Transfer to species belonging to the *Eucalyptus* subgenus *Symphyomyrtus* was also high (40–50%), with lower transfer to more distal species in the subgenus *Monocalyptus* (21%) and *E. cloeziana* (29%; subgenus *Idiogenes*).

6.2.2 *Transfer of Genetic Information Across Populations and Species – Quantitative Trait Loci Stability Across Genetic Backgrounds and Environments*

The development of highly informative, transferable microsatellite markers has been a major step towards studies of QTL diversity at a breeding population level in eucalypts, and the exchange of genetic information amongst pedigrees and species. The advantage in exchanging genetic information across species was demonstrated in the validation of QTL for vegetative propagation characteristics (Marques et al. 2002). Comparative mapping of microsatellite loci and QTL influencing sprouting and adventitious rooting ability was possible in four species, *E. grandis*, *E. urophylla*, *E. tereticornis* and *E. globulus*. Using a set of 40 microsatellites, many homeologous linkage groups were identified amongst these species and in most cases, locus order was conserved. Putative QTL for adventitious root formation were located on homeologous linkage groups of two species, providing independent validation that genes influencing rooting were located in this region of the eucalypt genome. Putative QTL for sprouting were located on a homeologous linkage group of a third species, indicating that a cluster of genes influencing different aspects of vegetative propagation could be located in this region, or that there was pleiotropy of the same major gene.

Highly variable co-dominant markers in mapping experiments will also assist to transfer genetic information amongst pedigrees in efforts to introgress early flowering genes into elite clones in eucalypts (Missiaggia et al. 2002). Genes for early flowering could be important in a eucalypt breeding program to allow accelerated breeding cycles and the potential to develop inbred lines (Missiaggia et al. 2002). Linkage between a mutant early flowering phenotype and a microsatellite marker was established by selective genotyping in one family and it is anticipated that once other markers are found to bracket the QTL, it will be transferred into elite clones.

A recent QTL detection study in *E. globulus* was encouraging in that at least some QTL for wood density were stable across environments (P. Bundock, pers. comm.). In this experiment, a single family was grown across seven sites in Australia. There was no evidence of QTL × site interaction for pilodyn penetration, an indirect measure of wood density, yet volume growth exhibited large QTL × site interaction.

6.2.3 Gene Discovery and Candidate Gene Mapping

There has been an intensive effort over the past few decades to understand the cellular processes and more recently the molecular aspects of wood formation (Jain and Minocha 2000; Savidge et al. 2000). As a result, many of the key features of the development and function of the vascular cambium, the differentiation and control of xylem (wood) cell formation, and the biosynthesis of the major components of their cell walls, cellulose and lignin, have been elucidated. Some of the genes involved in these processes are known and cloned as a result of functional or mutation analysis (Bossinger and Leitch 2000; Hertzberg et al. 2001; Whetten et al. 2001). Other genes with unknown function, but believed to be involved in the processes of cell wall formation, developmental regulation, signal transduction and hormone biosynthesis have also been cloned (Sterky et al. 1998). Currently, there are over 273 eucalypt sequences in the genetic database GenBank (March 2002), with about 50 % of these associated or likely to be associated with wood formation. Recently, a major genomics initiative in eucalypts, the GENOLYPTUS project has commenced in Brazil (Grattapaglia 2002; D. Grattapaglia, pers. comm.). The objective of this project is to discover, map, validate and characterise genes of economic importance in eucalypts. A key aspect of this work will be the establishment of segregating reference populations for genetic mapping experiments similar to those available in humans.

In the first step toward understanding the relationship between variation in gene sequence and its phenotypic effect, candidate genes for wood quality and other traits were mapped in eucalypts. In one example, six genes of known function involved in lignin biosynthesis or the common phenylpropanoid pathway, and which may be related to QTL controlling wood quality, as well as two genes implicated in morphological formation in roots, were mapped using polymorphism detected by single strand conformation polymorphism (SSCP) in a *E. grandis* × *E. urophylla* family (Gion et al. 1999). In a second study, genes of known function involved in monolignol biosynthesis and floral expression in addition to 31 cambium-specific expressed sequence tags (EST), were mapped by RFLP on to a genetic map for *E. globulus* (Thamarus et al. 2002). The next crucial step in this area will be the linking of candidate genes with phenotypic values of characteristics of economic importance by QTL or association studies (Brown et al. 2001; Thamarus et al. 2002).

Acknowledgements. The authors thank B. Potts, R. Vaillancourt, P. Bundock, R. Griffin and Z. Myburg for helpful comments on the manuscript.

References

Beavis WD (1998) QTL analysis: power, precision, and accuracy. In: Paterson AH (ed) Molecular dissection of complex traits. CRC Press, Boca Raton, pp 145–162

Bossinger G, Leitch M (2000) Isolation of cambium-specific genes from *Eucalyptus globulus* Labill. In: Savidge R, Barnett J Napier (eds) Cell and molecular biology of wood formation. BIOS Scientific, Oxford, pp 203–207

Bradshaw HD, Foster GS (1992) Marker aided selection and propagation systems in trees. Advantages of cloning for studying quantitative inheritance. Can J For Res 22:1044–1049

Bradshaw HD, Grattapaglia D (1994) QTL mapping in interspecific hybrids of forest trees. For Genet 1:191–196

Brondani RPV, Brondani C, Tarchini R, Grattapaglia D (1998) Development, characterisation and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. Theor Appl Genet 97:816–827

Brondani R, Kirst M, Ribeiro V, Gaiotto F, Marques C, Nichols D, Williams E, Grattapaglia D (2001) Fingerprinting and mapping *Eucalyptus* with large batteries of microsatellite loci. Paper presented at the plant and animal genome IX conference, 13–17 Jan 2001, San Diego

Brondani RPV, Brondani C, Grattapaglia D (2002) Towards a genus-wide reference linkage map for *Eucalyptus* based exclusively on highly informative microsatellite markers. Mol Genet Genom 267:338–347

Brooker MIH, Kleinig DA (1994) Field guide to eucalypts, vol. 3. Northern Australia. Inkata Press, Sydney

Brown GR, Gill GP, Sewell MM, Wheeler NC, Megraw RA, Neale DB (2001) Towards association studies in forest trees: wood property QTL verification, candidate genes, and SNPs in Loblolly pine (*Pinus taeda* L.). Paper presented at the international conference on wood, breeding, biotechnology and industrial expectations, 11–14 June 2001, Bordeaux, France

Burczyk J, Adam W, Moran G, Griffin A (2002) Complex patterns of mating revealed in a *Eucalyptus regnans* seed orchard using allozyme markers and the neighbourhood model. Mol Ecol 11:2379–2391

Burgess IP, Williams ER, Bell JC, Harwood CE, Owen JV (1996) The effect of outcrossing rate on the growth of selected families of *Eucalyptus grandis*. Silvae Genet 45(2–3):97–101

Byrne M, Murrell JC, Allen B, Moran G (1995) An integrated genetic linkage map for eucalypts using RFLP, RAPD and isozyme markers. Theor Appl Genet 91:869–875

Byrne M, Marquez-Garcia MI, Uren T, Smith DS, Moran GF (1996) Conservation and genetic diversity of microsatellite loci in the genus *Eucalyptus*. Aust J Bot 44:331–341

Campinhos EN, Grattapaglia D, Alfenas AC, Bertolucci FL (1997) Stability of expression of QTL alleles controlling growth across variable genetic backgrounds in *Eucalyptus*. Paper presented at the proceedings of the IUFRO conference on silviculture and improvement of eucalypts, 24–29 Aug 1997, Salvador, Brazil

Chippendale GM (1988) *Eucalyptus*, *Angophora* (Myrtaceae). Flora of Australia, vol 19. Australian Govt. Publishing Service, Canberra

Dutkowski GW, Potts BM (1999) Geographic patterns of genetic variation in *Eucalyptus globulus* spp. *globulus* and a revised racial classification. Aust J Bot 47:237–263

Eldridge K (1970) Breeding system of *Eucalyptus regnans*. Paper presented at the proceedings of IUFRO section 22 working group on sexual reproduction of forest trees, Varparanta, Finland

Eldridge K, Davidson J, Harwood C, van Wyk G (1994) Eucalypt domestication and breeding, 1st edn. Oxford University Press, Oxford

Gion J-M, Rech P, Grima-Pettenati J, Verhaegen D, Plomion C (1999) Mapping candidate genes in *Eucalyptus* with emphasis on lignification genes. Mol Breed 6:441–449

Glaubitz J, Strk J, Moran G (1999) Genetic impact of different silvicultural practices in native eucalypt forests. Paper presented at the forest genetics and sustainability; Proceeding of IUFRO Conference, Beijing, China

Glaubitz J, Emebiri, Moran G (2001) Dinucleotide microsatellites in *Eucalyptus sieberi*: inheritance, diversity and improved scoring of single base differences. Genome 44:1041–1045

Grattapaglia D (2000) In: Jain SM, Minocha SC (Eds.) Molecular biology of wood plants, vol. 1. Kluwer Academic, Dordrecht, pp 451–474

Grattapaglia D (2002) Integrating genomic biotechnologies into genetic improvement of *Eucalyptus*: the GENOYPTUS project in Brazil. Paper presented at the Simposio Internacional sobre socioeconomia, tecnologia, patologia y sostenibilidad del eucalipto, Pontevedra, Spain, 29–31 May 2002, 16 pp

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137:1121–1137

Grattapaglia D, Bertolucci FLG, Penchel R, Sederoff R (1996) Genetic mapping of quantitative trait loci controlling growth and wood quality traits in *Eucalyptus grandis* using a maternal half-sib family and RAPD markers. Genetics 144:1205–1214

Griffin AR, Burgess IP, Wolf L (1988) Patterns of natural and manipulated hybridisation in the genus *Eucalyptus* L'Herit – a review. Aust J Bot 36:41–66

Griffin A, Harbard J, Centurion C, Santini P (2000) Breeding *Eucalyptus grandis* × *globulus* and other inter-specific hybrids with high inviability – problem analysis and experience with Shell forestry projects in Uruguay and Chile. Hybrid Breeding and Genetics Symposium, 9–14 April 2000, Noosa, Queensland, Australia, pp 1–13

Hertzberg M, Aspeborg H, Schrader J, Andersson A, Erlandsson R, Blomqvist K, Bhalerao R, Uhlen M, Teeri TT, Lundeberg J, Sundberg B, Nilsson P, Sandberg G (2001) A transcriptional roadmap to wood formation. Proc Natl Acad Sci USA 98:14732–14737

House SM (1997) Reproductive biology of eucalypts. In: Williams J, Woinarski J (eds) Eucalypt ecology. Cambridge Univ Press, Cambridge, pp 30–55

Jackson HD, Steane DA, Potts BM, Vaillancourt RE (1999) Chloroplast DNA evidence for reticulate evolution in *Eucalyptus* (Myrtaceae). Mol Ecol 8:739–751

Jain SM, Minocha SC (2000) Molecular biology of woody plants, vol 1. Kluwer Academic, Dordrecht

Jones M, Stokoe R, Cross M, Scott L, Maguire T, Shepherd M (2001) Isolation of microsatellite loci from spotted gum (*Corymbia variegata*), and cross-species amplification in *Corymbia* and *Eucalyptus*. Mol Ecol Notes 1:276–278

Law B, Mackowski C, Schoer L, Tweedie T (2000) Flowering phenology of myrtaceous trees and their relation to climatic, environmental and disturbance variables in northern New South Wales. Aust Ecol 25:160–178

Marques C, Brondani R, Grattapaglia D, Sederoff R (2002) Conservation and synteny of SSR loci and QTLs for vegetative propagation in four *Eucalyptus* species. Theor Appl Genet 105:474–478

Missiaggia M, Piacezzi A, Grattapaglia D (2002) A major effect QTL for early flowering in *Eucalyptus* mapped by selective genotyping of microsatellite markers detected in fluorescent multiplexes. Paper presented at the Proceedings of the 48th Brazilian Congress of Genetics, 17–20 Sept 2002, Aguas de Lindoir

Moran GF, Butcher PA, Glaubitz JC (2000) Application of genetic markers in the domestication, conservation and utilisation of genetic resources of Australasian tree species. Aust J Bot 48:313–320

Myburg AA, Griffin R, Sederoff RR, Whetten R (2000) Genetic analysis of interspecific backcross families of a hybrid of *Eucalyptus grandis* and *Eucalyptus globulus*. Paper presented at the Hybrid Breeding and Genetics of Forest Trees Proceedings of QFRI/CRC-SPF Symposium, 9–14 April 2000, Noosa, Queensland, Australia, pp 462–467

Myburg AA, Griffin RA, Sederoff RR, Whetten RW (2003) Comparative genetic linkage maps of *Eucalyptus grandis*, *Eucalyptus globulus* and their F-1 hybrid based on a double pseudo-backcross mapping approach Theor Appl Genet 107:1028–1042

Myburg AA, Vogl C, Griffin RA, Sederoff RR, Whetten RW (2004) Genetics of postzygotic isolation in *Eucalyptus* II. Whole-genome analysis of barriers to introgression in a wide interspecific cross of *E. grandis* and *E. globulus*. Genetics 166:1405–1418

Nesbitt KA, Potts BM, Vaillancourt RE, West AK, Reid JB (1995) Partitioning and distribution of RAPD variation in a forest tree species, *Eucalyptus globulus* (Myrtaceae). Heredity 74:628–637

Nikles DG, Griffin AR (1992) Breeding hybrids of forest trees: definitions, theory, some practical examples and guidelines on strategy with tropical acacias. ACIAR Proc 37:101–109

O'Malley DM, McKeand SE (1994) Marker-assisted selection for breeding value in forest trees. For Genet 1:207–218

Potts BM, Reid JB (1990) The evolutionary significance of hybridisation in *Eucalyptus*. Evolution 44:2151–2152

Potts BM, Wiltshire RJE (1997) Eucalypt genetics and genecology. In: Williams J, Woinarski J (eds) Eucalypt ecology: individuals to ecosystems. Cambridge Univ Press, Cambridge, pp 56–91

Potts BM, Dungey HS (2001) Hybridisation of *Eucalyptus*: key issues for breeders and geneticists. Paper presented at the IUFRO Conference: Developing the eucalypt of the future, Valdivia, Chile.

Potts BM, Barbour RC, Hingston AB (2001) Genetic pollution from farm forestry using eucalypt species and hybrids. A report for RIRDC/L&WA/FWPRDC joint venture agroforestry program, Sept 2001, RIRDC publ no 01/114

Pryor LD (1976) The biology of eucalypts. Arnold, London

Pryor LD, Johnson LAS (1981) *Eucalyptus*, the universal Australian. In: Keast A (ed) Ecological biogeography of Australia. Junk, The Hague, pp 499–536

Ritland K (2002) Extensions of models for the estimation of mating systems using *n* independent loci. Heredity 88:221–228

Russell J, Marshall D, Griffin R, Harbard J, Powell W (2001) Gene flow in South American *Eucalyptus grandis* and *Eucalyptus globulus* seed orchards. In: Barros S (ed) "Developing the eucalypt of the future." Proceedings of IUFRO international symposium, Valdivia, Chile

Savidge R (2000) Biochemistry of seasonal cambial growth and wood formation – an overview of the challenges. In: Savidge R, Barnett J, Napier R (eds) Cell and molecular biology of wood formation. BIOS Scientific Ltd, Oxford, pp 1–28

Shepherd M, Chaparro JX, Teasdale R (1999) Genetic mapping of monoterpene composition in an interspecific eucalypt hybrid. Theor Appl Genet 99: 1207–1215

Squilassi M, Grattapaglia D (1998) Mapping QTL using linkage disequilibrium and efficiency of early marker-assisted selection in *Eucalyptus.* Paper presented at the plant and animal genome VI, Jan 1998, San Diego

Steane DA, Vaillancourt RE, Russell J, Powell W, Marshall D, Potts BM (2001) Development and characterisation of microsatellite loci in *Eucalyptus globulus* (Myrtaceae). Silvae Genet 50:89–91

Sterky F, Regan S, Karlsson J, Hertzberg M, Rohde A, Holmberg A, Amini B, Bhalerao R, Larsson M, Villarroel R, Vanmontagu M, Sandberg G, Olsson O, Teeri TT, Boerjan W, Gustafsson P, Uhlen M, Sundberg B, Lundeberg J (1998) Gene discovery in the wood-forming tissues of poplar – analysis of 5,692 expressed sequence tags. Proc Natl Acad Sci USA 95:13330–13335

Stokoe RL (2002) Pattern of genetic diversity and hybridisation of *Eucalyptus cloeziana* F. Muell (Myrtaceae), PhD Thesis, Southern Cross University, Lismore

Stokoe RL, Shepherd M, Lee D, Nikles DG, Henry RJ (2001) Natural interspecific hybridisation between *Eucalyptus acmenoides* Schauer and *E. cloeziana* F. Muell (Myrtaceae). Ann Bot 88:563–570

Thamarus K, Groom K, Murrell J, Byrne M, Moran G (2002) A genetic linkage map for *Eucalyptus globulus* with candidate loci for wood, fibre and floral traits. Theor Appl Genet 104:379–387

Turnbull J (1999) Eucalypt plantations. New For 17:37–52

Verhaegen D, Plomion C (1996) Genetic mapping in *Eucalyptus urophylla* and *E. grandis* using RAPD markers. Genome 39:1051–1061

Verhaegen D, Plomion C, Gion JM, Poitel M, Costa P, Kremer A (1997) Quantitative trait dissection analysis in *Eucalyptus* using RAPD markers 1. Detection of QTL in interspecific hybrid progeny, stability of QTL expression across different ages. Theor Appl Genet 95:597–608

Verhaegen D, Plomion C, Poitel M, Costa P, Kremer A (1998) Quantitative trait dissection analysis in *Eucalyptus* using RAPD markers 2. Linkage disequilibrium in a factorial design between *E. urophylla* and *E. grandis*. For Genet 5:61–69

Whetten R, Sun Y, Zhang Y, Sederoff R (2001) Functional genomics and cell wall biosynthesis in loblolly pine. Plant Mol Bio 47:275–291

Williams CG, Savolainen O (1996) Inbreeding depression in conifers. For Sci 41:1–20