Genetic Improvement of Eucalypts

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Introduction

Eucalypts are virtually endemic to Australia; they are the tallest flowering plants on earth and the most widely grown hardwood plantation species. No sector of world forestry has expanded as rapidly as the industrial use of eucalypts and, while they are still at the early stages of domestication compared with crop species, they are fast becoming amongst the most advanced genetic material in forestry. This article overviews (1) the unique biological features of the genus, including its distribution and taxonomy, breeding systems, and natural regeneration mechanisms; (2) the history of its domestication, from its first discovery in the late eighteenth century, through its rapid dispersal around the world in the nineteenth century to its prominent role in the industrial plantations of the late twentieth century for the pulp and paper markets; (3) the genetic improvement of species, from provenance selection to advanced generation breeding strategies, including definition of breeding objectives and large-scale assessment of key biological traits affecting profitability; (4) the important role played by eucalypt hybrids, particularly in tropical and subtropical zones; (5) deployment options through seed and clonal propagation systems; and (6) progress towards molecular breeding and genetic engineering.

The Genus

Eucalypts are generally long-lived, evergreen hardwood species belonging to the predominantly southern hemisphere, angiosperm family Myrtaceae. They range in habit from shrubs and multistemmed mallees to enormous trees some which include the tallest flowering plants on earth (*Eucalyptus regnans*, up to 96 m). Most species are endemic to Australia but five tropical species are confined to islands north of Australia (e.g., *E. urophylla* and *E. deglupta*). A small group of species also extends outside of Australia into Papua New Guinea (e.g., *E. alba* and *E. tereticornis*). Eucalypts are the dominant species in open forests and woodlands throughout Australia but extend into a great diversity of habitats. They occur naturally from sea level to the alpine treeline, from high rainfall to semi-arid zones, and from the tropics to latitudes as high as 44° S, but they are absent from true arid and rainforest environments in Australia.

In the broad sense, eucalypts include the genera *Eucalyptus, Corymbia,* and *Angophora*. A key feature of the majority of eucalypts is the fusion of either the petals and/or sepals to form an operculum from which the eucalypts derive their name (from a Greek root *eu* – well and *calyptos* – covered). The operculum appears to have evolved independently in different eucalypt lineages and has not evolved in *Angophora*. There is some debate as to whether the *Corymbia* and *Angophora* genera (bloodwood taxa) warrant separation from the genus *Eucalyptus* in the strict sense (non-bloodwood taxa), but this split is supported by several independent DNA studies and is adopted herein. This dichotomy appears to be associated with differences in the structure of raised oil glands (termed bristle glands), wood properties (the bloodwood lineage for example only has solitary vessels in the xylem), leaf venation, and ovule arrangement. The latest taxonomic revision of the eucalypts recognizes just over 700 species that belong to 13 main evolutionary lineages (**Table 1**) but still treats the bloodwood eucalypts as subgenera of *Eucalyptus*. Most species belong to the subgenus *Symphyomyrtus*, and it is mainly species from three sections of this subgenus that are used in plantation forestry.

Eucalypts have several noteworthy biological features. Many species are heteroblastic, with leaves changing from a sessile, horizontally orientated juvenile form to a petiolate, vertically orientated adult form. This is also accompanied by changes in leaf anatomy, physiology, and chemistry. The timing of this heteroblastic transition is under strong genetic control and susceptibility to many pests is dependent upon the leaf type present in the canopy. Eucalypts also have well-developed mechanisms for vegetative recovery from defoliation arising from factors such as fire, drought, frost, or herbivory. The bark often protects numerous dormant vegetative buds that sprout to form epicormic shoots after defoliation. If the whole stem is killed by, for example fire, many species also have the possibility of resprouting from lignotubers. Lignotubers are organs that develop as swellings in the axils of the cotyledonary and early seedling nodes and comprise a mass of vegetative buds, vascular tissue, and food reserves. They usually become buried and allow the plant to regenerate after the death of the main stem. Epicormic or coppice shoots exhibit varying degrees of reversion to the juvenile leaf form. Other vegetative regeneration mechanisms such as rhizomes or root suckering have been reported in *Corymbia*. Regeneration usually occurs by seed that is stored in woody capsules, 5–30 mm in diameter, and often held on the

Table 1 Major evolutionary lineages[a] within the eucalypts

Pryor and Johnson's subgenera/genera	*Brooker's subgenera*	*Number of species*	*Examples of well-known forestry species*
Angophora (genus)	*Angophora*	7	
Blakella	*Blakella*	15	
Corymbia	*Corymbia*	67	*C. torelliana*, *C. citridora*, *C. maculata*
Eudesmia	*Eudesmia*	19	
Gaubaea	*Acerosa*	1	
Gaubaea	*Cuboidea*	1	
Idiogenes	*Idiogenes*	1	*E. cloeziana*
Monocalyptus	*Primitiva*	1	
Monocalyptus	*Eucalyptus*	110	*E. regnans*, *E. delegatensis*, *E. obliqua*, *E. marginata*, *E. fastigata*
Symphyomyrtus	*Cruciformes*	1	*E. guilfoylei*
Symphyomyrtus	*Alveolata*	1	*E. microcorys*
Symphyomyrtus	*Symphyomyrtus*	474	*E. camaldulensis*, *E. exserta*, *E. globulus*, *E. grandis*, *E. nitens*, *E. paniculata*, *E. robusta*, *E. saligna*, *E. tereticornis*, *E. urophylla*, *E. viminalis*
Telocalyptus	*Minutifructus*	4	*E. deglupta*

[a] The alignment of Pryor and Johnson's (1971) genera and subgenera with Brooker's (2000) subgenera. Pryor and Johnson's classification was informal, but widely used for 30 years. The number of species in each of Brooker's subgenera is indicated and examples of well-known forestry species are given. The subgenera *Blakella* and *Corymbia* had previously been treated as a separate genus *Corymbia* Hill and Johnson (Hill and Johnson 1995) and the subgenus *Angophora* treated as a genus and this treatment has been adopted in the text.

Sources: Pryor LD and Johnson LAS (1971) *A Classification of the Eucalypts*. Canberra: Australian National University Press; Brooker MIH (2000) A new classification of the genus *Eucalyptus* L'Her. (Myrtaceae). *Australian Systematic Botany* 13: 79–148; Hill KD and Johnson LAS (1995) Systematic studies in the eucalypts 7. A revision of the bloodwoods, genus *Corymbia* (Myrtaceae). *Telopea* 6: 185–504.

tree for several years. In good years, large numbers of seed are shed, particularly following wildfire. The seeds generally have no special adaptation for dispersal and, with the exception of a few cases of water dispersal (e.g., *E. camaldulensis*), seed dispersal is mainly by wind and normally occurs over short distances. Eucalypt seed is short-lived in the soil seed bank.

Eucalypt flowers are occasionally solitary (e.g., *E. globulus*), but often occur in clusters of three or more in umbels (**Figure 1**) or terminal inflorescences. The eucalypt flower is normally bisexual, with numerous stamens that expand outwards after the operculum is shed to form the conspicuous floral display. Eucalypts are predominantly animal-pollinated, with vectors encompassing a wide variety of insects, birds, and marsupials, and a few bat species. They have a mixed mating system, but are generally preferential outcrossers, with high levels of outcrossing maintained by protandry and various incomplete pre- and postzygotic barriers to self-fertilization. The postzygotic barriers include intense selection against the products of inbreeding. For example, inbreeding depression for growth in selfed *E. globulus* is nearly 50%, and this is quite typical. Consistent with most myrtaceous genera, eucalypts are diploids with virtually all having a chromosome

Figure 1 Flowers and flower buds of *Eucalyptus nitens. Eucalyptus nitens* bears its flowers in umbels of up to seven flowers. The figure shows buds just about to shed their inner operculum and those from which the operculum has been shed. In this group of eucalypts, the inner operculum is derived from fused petals and shed just before the anthers expand and shed pollen. The outer operculum is derived from the fused sepals and is shed early in bud development. The stigma of this species becomes receptive 5–7 days after operculum shed, at a stage when most pollen has been released from the anthers. Photograph courtesy of Dean Williams.

number of $2n = 22$. While the major eucalypt subgenera do not hybridize, reproductive barriers between species within subgenera are often weak. Hybridization and intergradation between recognized taxa are common in nature, often making delineation of species difficult. Many artificial hybrid combinations have been produced. In general, hybrid inviability tends to increase with increasing taxonomic distance between the parents, but there are exceptions.

History of Domestication

Eucalypts are the most planted hardwood trees in the world. Following their discovery in the late eighteenth century, they were spread rapidly around the world and were early introduced into countries such as India (*c.* 1790), France (*c.* 1804), Chile (1823), Brazil (1825), South Africa (1828), and Portugal (1829). Initially they were introduced as botanical curiosities but, as the potential for some species to grow fast was quickly recognized, they were grown for windbreaks, land reclamation, and leaf-oil production, but mainly for fuel wood and timber production. Plantations were established, for example, to provide railway crossties and fuel for wood-burning locomotives in Brazil and South Africa, mine props in Chile and South Africa, and charcoal in Brazil for iron and steel production. One factor causing their rapid early spread appears to be the belief that growing eucalypts could banish diseases such as malaria, and they became known as 'fever gums' in the latter half of the nineteenth century.

Eucalypts became renowned for species with fast growth, straight form, valuable wood properties, wide adaptability to soils and climates, and ease of management through coppicing. They are now found in more than 90 countries where the various species are grown for products as diverse as sawn timber, mine props, poles, firewood, pulp, charcoal, essential oils, honey, and tannin as well as for shade, shelter, and soil reclamation. The exotic eucalypts became an important source of fuel and building material in rural communities in countries such as India, China, Ethiopia, Peru, and Vietnam. However, it was the great global demand for short-fiber pulp that drove the massive expansion of eucalypt plantations throughout the world during the twentieth century. Their high fiber count relative to other wood components, coupled with the uniformity of fibers relative to other angiosperm species, has caused high demand for eucalypt pulp for coated and uncoated free-sheet paper, bleach board, sanitary products (fluff pulp), and secondarily for top liner on cardboard boxes, corrugating medium, and as a filler in long-fiber conifer products such as newsprint and containerboard. New technologies are also increasing interest in the use of plantation eucalypts for sawnwood, veneer, medium density fiberboard, and as extenders in plastic and molded timber.

No sector of world forestry has expanded as rapidly as the industrial use of eucalypts. While precise global figures are difficult to obtain, it is estimated there were 9.5 million ha of industrial eucalypt plantations in the world in 1999 (**Table 2**), with the vast majority of these established since the 1950s. This area is predicted to reach 11.6 million ha in 2010 (**Table 2**). Other less conservative global estimates suggest that there were nearly 16 million ha of general eucalypt plantations by the 1990s which would reach 20 million ha by 2010. These figures compare with the estimated 30 million ha of tall (>30 m tall) and 240 million ha of open (10–30 m tall) native eucalypt forest in Australia in 2001. The majority of plantations consist of only a few eucalypt species and hybrids. The most important plantation eucalypts around the world are *E. grandis, E. globulus,* and *E. camaldulensis*, which together with their hybrids account for about 80% of the plantation area; these are followed by *E. nitens, E. saligna, E. deglupta, E. urophylla, E. pilularis, Corymbia citriodora*, and *E. tereticornis*. In the case of pulpwood, the market favorites are *E. grandis, E. urophylla*, and their hybrids in tropical and subtropical regions and *E. globulus* in temperate regions. However, eucalypt plantations of the traditional pulpwood species as well as other species (e.g., *C. citriodora, C. maculata, E. cloeziana*, and *E. nitens*) are increasingly being managed for solidwood production. While there are reports of eucalypt plantations achieving growth rates of over $60\,\mathrm{m^3\,ha^{-1}\,year^{-1}}$, with intensive management, typical growth rates reported for the *E. grandis* plantations in Brazil and South Africa are $40\,\mathrm{m^3\,ha^{-1}\,year^{-1}}$ with a harvest age of 6–8 years,

Table 2 Area of industrial plantations of eucalypts (millions of hectares)

Region	*1999*	*2010*	*Change*
Africa	1.08	1.00	−7%
Asia	2.43	3.20	32%
Europe	1.32	1.40	6%
North and Central America	0.08	0.10	30%
South America	4.09	5.00	22%
Oceania	0.48	0.90	89%
Total	9.48	11.60	22%

Source: Wood resources international cited in Raga FR (2001) Perspectiva para el eucalipto chileno. In *Developing the Eucalypt of the Future*, IUFRO International Symposium, 10–15 September 2001, pp. 13. Valdivia, Chile: INFOR.

and 20–22 $m^3 ha^{-1} year^{-1}$ for *E. globulus* plantations in Portugal and Chile with a harvest age of 8–10 years. The world average is suggested to be more like 20 $m^3 ha^{-1} year^{-1}$, but it is anticipated that this could reach 25–30 $m^3 ha^{-1} year^{-1}$ in the future with better silviculture and breeding.

Initially, the botanical gardens of southern Europe played a major role in the introduction of eucalypts to other parts of the world, including Africa and South America. Later in the nineteenth century eucalypts were introduced directly from Australia with, for example, large quantities of seed being sent out of Australia by Ferdinand von Mueller, a government botanist and eucalypt specialist. These early introductions were often used as the base to establish large-scale plantings, resulting in narrow, potentially inbred, genetic bases in some cases (e.g., *E. globulus* in South Africa and Ecuador). In other cases, seed for plantation establishment was collected from ornamentals or multispecies plantings, and appears to have contained high frequencies of interspecific hybrids. In the latter case, F_1 generation hybrids may have performed well but subsequent seed collection from these hybrid plantations resulted in F_2 and subsequent generations with poor growth and form as well as extreme variation (e.g., those derived from the Rio Claro *E. urophylla* hybrids in Brazil 'Brazil alba,' or Mysore *E. tereticornis* hybrid in India). In many countries where eucalypts have been introduced for a long time and continually reproduced from local seed sources, they have formed landraces that are adapted to the specific environment of the country. However, there are many examples where the initial use of a limited sample of the genetic diversity in the native gene pools, the use of suboptimal provenances, inbreeding, or hybrid breakdown have led to landraces being outperformed in field trials by seedlots from some native-stand provenance collections (e.g., *E. globulus* in Argentina, *E. grandis* in South Africa). The planting of suboptimal germplasm is particularly problematic in rural communities where seed obtained for new plantings has been collected with no or only little phenotypic selection from local plantings, generation after generation. In a few cases, where more active breeding has occurred the local landrace has outperformed newly imported native stand seedlots (e.g., *E. grandis* in Florida).

Some of the earliest breeding of eucalypts was undertaken by French foresters in Morocco in 1954–1955. Coincident with the increasing interest in industrial plantations of eucalypts, the 1960s saw a more formal approach to genetic improvement with, for example, the commencement of the Florida *E. grandis* breeding program in 1961, *E. globulus* breeding in Portugal in 1965, and establishment of large provenance tests of *E. camaldulensis* in many countries. Major advances in domestication of the genus occurred in the 1970s with, for example, the first commercial plantings of selected clones derived from hardwood cuttings at Pointe Noir in the Congo followed by Aracruz in Brazil (many of which were spontaneous hybrids), and the establishment in many countries of the first large base-population trials of species such as *E. urophylla* and *E. globulus*. These trials were established from open-pollinated seedlots collected from range-wide provenance collections and formed the bases for deployment and breeding populations in many countries. Many other major international base-population trials were established through the 1980s for species such as *E. grandis, E. tereticornis*, and *E. viminalis*, and using more intensive collections of elite provenances identified in earlier collections.

While eucalypts are still at the early stages of domestication compared to crop species, they are fast becoming amongst the most advanced genetic material in forestry, with stock originating from the *E. grandis* program in Florida already in its sixth generation. In Brazil, around 500 000 ha of plantation were apparently established with 'Brazil alba' seed between 1940 and 1970 before it was realized that the quality of the new plantations was much inferior to earlier plantations and *E. grandis* due to hybrid breakdown. The company Aracruz Celulose S.A. has subsequently doubled yields from its Brazilian plantations through species and provenance selection, breeding, and the use of proven clones. Domestication of eucalypts has proceeded faster in countries like Brazil that rely on plantations for their eucalypt wood than in Australia, where up until the 1990s wood products of eucalypts were derived almost entirely from native forests. However, major provenance trials of species such as *E. regnans, E. delegatensis, E. globulus*, and *E. nitens* were established in Australia in the late 1970s, and major breeding programs for *E. globulus* and *E. nitens* were started in the 1980s.

Species Improvement

A key feature of eucalypt species is the great diversity of the native gene pools. Large, genetic differences between provenances are the rule rather than the exception. In some cases, provenances of a single species may vary from tall forest forms to small trees and even shrubs when grown in common environment trials (e.g., *E. globulus*). This diversity may occur for all traits of interest to breeders such as growth and survival, pest resistance, and wood properties, as well as flowering season and precocity. Such provenance variation makes it important that, firstly, species-elimination trials are based on adequate provenance representation and, secondly, when establishing base populations for breeding the full range of genetic diversity is assessed. This is further complicated by the large provenance × environment interactions that have been revealed in multisite field trials of species such as *E. camaldulensis, E. deglupta, E. delegatensis, E. nitens, E. urophylla*, and *E. viminalis*. Increasing information is available for environmental matching of species and provenances to sites through comparison of local environmental profiles with native ranges in Australia as well as exotic environments where they have been successfully grown. However, a traditional approach for formation of base populations for breeding has been the establishment of large range-wide provenance trials, supplemented with more intensive collection from elite provenances in a second stage. While early provenance trials pooled individual-tree seedlots, later trials have tended to maintain family identity to allow better pedigree control and conversion of trials to seed orchards. Increasing international exchange of eucalypt germplasm amongst breeding programs now means that base populations are comprising not only seedlots from native stand collections in Australia but also material from landraces and more advanced breeding programs.

A focus of eucalypt breeding in recent years has been the clear definition of breeding objectives and identification of relevant selection traits. Major developments were made in the 1990s in clarifying breeding objectives for kraft and mechanical pulpwood production using eucalypt wood. Wood density, pulp yield, and volume per hectare were identified as the key biological traits influencing the economics of pulpwood production. Economic weights have been determined to allow estimation of total breeding value in terms of monetary value to the industry sector. Wood density and pulp yield were rarely considered in earlier selection programs, yet they can account for over 70% of the benefits from breeding for pulp production. Approaches have now been developed for the quick, cheap, and nondestructive measurement of many key wood properties (e.g., pilodyn, mechanized coring (**Figure 2**) and near-infrared reflectance analysis (NIRA)), allowing their widespread application in breeding programs. Extending such work beyond pulp to paper is the next step. There is increasing work being undertaken on identifying breeding objectives and selection traits for solidwood products; however, this is complicated by the greater range of products involved from sawn timber, composites, and veneer, and changing technologies, as well as the fact that plantations may be required for multiple products through thinning or changes in product pricing.

Figure 2 Coring *Eucalyptus globulus* for wood density assessment using a mechanized coring machine. Photograph courtesy of Carolyn Raymond, Cooperative Research Centre for Sustainable Production Forestry.

The amount of genetic variation and intercorrelation among selection traits in the breeding population has a major effect on genetic progress. The genetic correlation between growth and wood density, for example, is probably slightly adverse in species such as *E. globulus* and *E. nitens*, but estimates are variable ranging from zero to significantly adverse. Most genetic parameters such as heritabilities and genetic correlations published for eucalypts to date refer to the additive genetic variation within provenances and have been calculated based on open-pollinated progeny trials. As eucalypts have a mixed mating system and the male parent is unknown, the accuracy of these genetic parameter estimates is questionable, particularly from native-stand seed collections where outcrossing rates may vary markedly. In *E. globulus*, for example, no correlation has been observed between open-pollinated and control-pollinated breeding value estimates for growth traits, but the correlation is significant for traits of higher heritability such as wood density and disease-resistance traits. The other types of heritability often reported include additive and nonadditive components of genetic variation and are the clonal and family heritability (or repeatabilities of their means). This is a measure of the relative differentiation between genotypes or families and is a measure of the repeatability of performance for clonal or family deployment respectively.

Traditionally, eucalypt breeding has involved open-pollinated breeding strategies using single populations or sublines, possibly coupled with open- or controlled-pollinated nucleus populations of the most elite selections or specialized breeds (**Figure 3**). In programs where clones are deployed, the standard approach has involved progeny testing, and phenotypic or genetic selection of elite genotypes, followed by various stages of selection on cloning potential and clonal elimination trials. Selected clones are frequently used as parents in the subsequent generations. Traditionally, breeding programs have involved discrete generations. However, programs in Australia and Portugal are now implementing a 'rolling front' breeding strategy that has overlapping generations with selection, crossing, and trial planting done each year. This strategy is believed to be more flexible in the face of changing breeding objectives, technologies, resource allocation, and industry reorganization, which are becoming increasingly common. In this scheme, decisions are defined in terms of dynamic rules, where a general objective function guides the

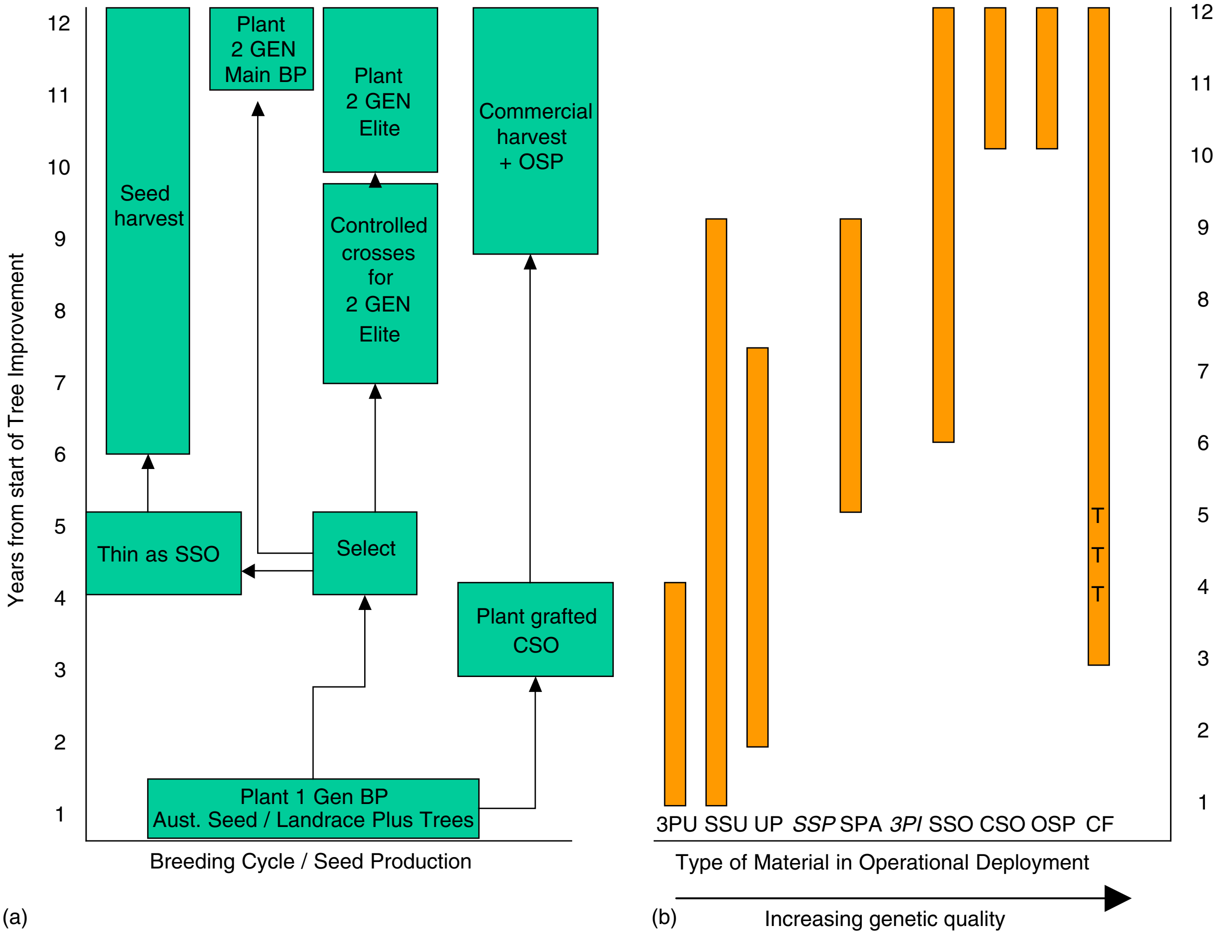

Figure 3 Elapsed time for first generation breeding and take-up of deployment options for *Eucalyptus globulus* in the company Forestal y Agricola Monteaguila S.A. in Chile (year 1 = 1990). (a) The type of genetic material deployed. The breeding population contained two sublines, a large Australian native stand collection and the other the Chilean landrace. The breeding populations (BP) were planted on multiple sites, the majority of the landrace selections from which seed was collected were also grafted and used to develop a clonal seed orchard (CSO) and a mass controlled pollination system based on 'one-stop pollination' (OSP). One site of the Australian subline was converted to a seedling seed orchard (SSO). (b) Planting started with importation of Australian seed (3PU), supplemented by harvests from the best trees in the plantations of the Chilean landrace (SSU). Within 3 years the superiority of the Jeeralang provenance in Australia (UP) was demonstrated and imported native stand seed formed a main component of the plantations established for the next 5 years until SSO and CSO seed came on stream. In 2000, backward and forward selected families from OSP contributed 30% of plantings and was expected to yield 30% volume improvement relative to Jeeralangs (UP). A clonal program (CF; T, test scale) was commenced in 1991, with cloning high rooting, selected families from the landrace and cold tolerant selection and later selections from the first generation breeding population (1GEN BP) trails. Commercial planting started in year 6 and by year 11, 20% of the total planting used clonal stock. Seed stands derived from plantations of selected provenance (SSP) or third party improved seed (3PI) were not utilized. From Griffin AR (2001) Deployment decisions: capturing the benefits of tree improvement with clones and seedlings. In *Developing the Eucalypt of the Future*, IUFRO International Symposium, 10–15 September 2001, pp. 16. Valdivia, Chile: INFOR.

selection and crossing done each year. Such a strategy exploits advances in genetic evaluation through best linear unbiased prediction (BLUP) methodology using individual-tree models that allow for overlapping generations, and complex pedigree- and field-trial structures. The costs of controlled pollination of many eucalypts have now been reduced substantially, through the development of techniques requiring only a single visit as opposed to three visits to the flower. Such reductions in costs have now made controlled crossing of the breeding population viable in species such as the large-flowered *E. globulus*, allowing improved accuracy of genetic evaluation over open-pollination where the male parent is unknown and breeding values could be biased by selfing and nonadditive genetic effects. Other major advances in genetic evaluation have come through improved trial designs such as incomplete-block and row–column designs, as well as including clonal information into evaluation models.

Interspecific Hybridization

Interspecific eucalypt hybrids have been used in forestry for decades and are a significant component of eucalypt plantation forestry, particularly in the tropics and subtropics. While some multiclonal seed orchards are used for F_1 hybrid production, cost-efficient clonal propagation is the key to their successful exploitation. Failures to develop such systems has limited deployment and hence reduced use of many desirable species combinations, particularly in temperate regions.

Clones of *E. urophylla* × *E. grandis* are easy to propagate and are widely planted in Brazil and Congo (**Figure 4**). Most eucalypt hybrids tested or deployed are either F_1s or composites derived from spontaneous hybridization (e.g., 'Brazil alba,' or Mysore hybrid in India) or more recently manipulated F_1 hybrids. The main hybrids used in industrial plantations are *E. grandis* × *E. urophylla, E. grandis* × *E. camaldulensis* (**Figure 5**), and cultivars including at least one of *E. saligna, E. pellita, E. exserta*, and *E. tereticornis*. Such hybrids are planted on a relatively large scale in Brazil and Congo, although sizeable plantations also occur in China, Indonesia, and South Africa. While hybrids are less utilized in more temperate zones, eucalypt hybridization programs involving controlled crossing were undertaken early in countries such as Russia and France, but hybrid development was curtailed by extreme frosts. Such artificial hybridization was undertaken early in temperate Australia, mainly aimed at understanding trait inheritance and the reproductive barriers between species. Hybrid development has focused on F_1 hybrids and has aimed to combine species with complementary attributes. However, as most traits are inherited in a more or less intermediate manner in the F_1s there is increasing interest in backcross and other advanced generation eucalypt hybrids to provide desirable combinations of traits. Some desirable species combinations produce high proportions of inviable/uncompetitive hybrids (e.g., *E. grandis* × *E. globulus, E. camaldulensis* × *E. globulus, E. urophylla* × *E. dunnii, E. dunnii* × *E. grandis*) and the key to hybrid selection appears to be rapid production and testing of large populations and application of high selection intensities. There are also advantages in selecting seed parents for both sexual and vegetative propagation traits.

Figure 4 Clonal plantation of an elite *Eucalyptus urophylla* × *E. grandis* hybrid at Pointe Noire, Congo. Photograph courtesy of Rod Griffin, Shell Forestry.

Deployment

Seedlings

In rural communities outside Australia, eucalypts have historically been propagated from the most accessible seed that is often derived from ornamentals or local plantations, with little attention paid to its

Figure 5 A phenotypically outstanding *Eucalyptus grandis* × *E. camaldulensis* F_1 hybrid in a family trial in Guangxi Dongmen Forest Farm, China. At 10 years the diameter at breast height of this tree was 32 cm compared with the adjacent tree in the foreground that measured only 11.3 cm. Such individuals are damaged at the base to cause coppice shoots that are multiplied by tissue culture to provide sufficient stock plants for production of hardwood cuttings for clonal tests.

Figure 6 Seed production stand of landrace *Eucalyptus globulus* near Lota, Chile after seed-bearing branches were harvested by climbers.

genetic quality. However, seed with varying levels of improvement has been obtained for deployment in industrial plantings from the best native-stand provenances in Australia, seed production areas (**Figure 6**), seed orchards, and more recently, mass controlled or supplementary pollination. The main problem with collection of seed from native stands is that genetic gain, from even the best provenances, may be limited due to varying degrees of inbreeding from selfing or crossing between related individuals which often grow in close proximity in the forest owing to limited seed dispersal. Self-fertilization is particularly a problem when seed is collected from isolated trees. Nevertheless such an approach has been a means of rapidly obtaining genetic gain in plantations during the early stages of domestication (**Figure 3**). Similarly, seed-production areas were often established early by visually thinning even-aged exotic plantations to leave large trees of good form for seed collection in subsequent years (**Figure 6**). While this will avoid the problem of the neighborhood inbreeding that may occur in native forests, it does not avoid problems of inbreeding due to selfing or a narrow genetic base, and genetic gain may also be limited by the low heritability of growth and some tree-form traits.

A large component of improved seed for the main plantation species is now available from open-pollinated seedling or clonal seed orchards established in many countries (**Figure 3**). Seedling seed orchards can be rapidly obtained by thinning pre-established progeny tests based on phenotypic selection, or preferably breeding-value estimates. In species such as *E. globulus*, large genetically based differences in the season and age of flowering may limit outcrossing and the number of effective pollen parents. To improve flowering synchrony, specialized thinning or planting designs are often employed. Higher genetic gains are expected from the more expensive, clonal seed orchards established by either forwards or backwards selection of elite genotypes. Mature scion wood of most commercial eucalypt species can be grafted onto seedling rootstocks using a variety of techniques including bottle-, top-cleft-, patch-, and micrografting, but the success rate is variable at the species and genotype level. Loss of trees due to late-acting graft incompatibility can be a significant cost with such clonal orchards and can be overcome by the use of cuttings or micropropagated clones.

Two major advances have occurred in the production of improved eucalypt seed in the last decade. One was through the discovery that the gibberellin inhibitor paclobutrazol could be used not only to reduce tree growth and allow easier canopy management, but also to enhance flowering. The other advance has been the discovery that the stigma is not necessary for successful pollination and that the pollen will germinate on the surface of the style when it has been cut either just after or even just prior to operculum shed, which often occurs about a week before stigma receptivity. This development has enabled pollination to be undertaken at the same time as emasculation and, coupled with single-flower or style-isolation procedures, has allowed controlled pollination to be undertaken in a single visit to the flower (termed 'single-visit pollination' (SVP) or 'one-stop pollination' (OSP)) (**Figure** 7). The traditional approach involved three visits – emasculation and isolation at operculum shed, pollination at stigma receptivity, and then removal of isolation bags. In the large-flowered species, *E. globulus*, orchards are now established for the manual production of elite full-sib families for deployment using SVP, with or without style isolation and emasculation (**Figure** 8). This approach is also being widely adopted with small-flowered species such as *E. grandis*, for the large-scale production of interspecific hybrids for clonal testing.

Figure 7 One-stop pollination of *Eucalyptus globulus*. (a) Emasculation of the flower just prior to operculum shed before pollen is released; (b) isolation of the style after it has been cut transversely just below the stigma and pollen applied. Photograph courtesy of Dean Williams.

Vegetative Propagation

Industrial-scale clonal propagation of eucalypts is widespread, particularly in the tropics and subtropics. Selected eucalypt clones are now used routinely in countries such as Brazil, Congo (**Figure 4**), Morocco, and South Africa. Most clonal systems use hardwood (ripened-shoot) cuttings. Micropropagation is mainly used to rejuvenate adult material or rapidly bulking up mother plants for hardwood cutting production. Embryogenesis is still in the research stage with eucalypts. Cuttings can be obtained relatively easily from seedlings or from basal coppice of most eucalypts, but the ability of shoots to produce roots rapidly declines with tree age and with a few exceptions (e.g., *E. deglupta*), adult shoots will not root. Maturation usually occurs rapidly and appears to be due to the production of a rooting inhibitor in mature apical or epicormic leaves. However, rejuvenation of shoots from the crown of mature trees is possible through rapid, 'cascade' grafting (including micrografting) on juvenile rootstocks or micropropagation (five to six transfers are usually required). Felling mature

Figure 8 Grafted seed orchard of *Eucalyptus globulus* of Bosques Arauco S.A., Chile. The orchard is being used for the production of controlled cross seed using one-stop pollination procedures similar to those shown in **Figure 7**.

trees will usually result in stumps producing juvenile coppice shoots from which cuttings can be obtained and used to establish mother plants for subsequent harvesting. The rooting potential of cuttings is generally increased in the next phase when shoots are harvested directly from well-maintained mother plants. Rapid multiplication is often achieved by using sequential generations of cuttings for mother plants. Clone banks of mother plants from which basal shoots are regularly harvested are either maintained in containers or in field plots.

Cuttings are usually obtained by dipping stem cuttings of one or two nodes into 1–3% indole butyric acid (IBA) rooting hormone. Rooting is usually obtained within 6–12 days and aided by high humidy (e.g., misting) and bottom heating. However, species vary in their propensity to form well-rooted cuttings and the conditions to achieve their maximum rooting potential, particularly mother-plant environment and handling. Species such as *E. globulus, E. nitens*, and *E. regnans* have a reputation for being difficult to root, whereas *E. camaldulensis, E. deglupta, E. grandis*, and *E. robusta* are easy. Even within species, there is considerable variation between families and genotypes. Genotypes of a species may vary in the proportion of cuttings that root from 0% to 90%. For example, in *E. globulus* only 25% of selections have been reported to root at rates of over 75%. In *E. deglupta*, the mean success is between 85–90%. However, good rooting does not ensure high growth rates and there are many examples of good rooting clones which have below-average growth rates. High rooting ability is essential for the successful exploitation of eucalypt hybrids, and most indications to date suggest that it will be inherited in a predominantly additive manner in most interspecific combinations. For economic production of clones, rooting success greater than 70–80% is usually required, which often results in a large number of individuals initially selected on breeding-objective traits being discarded.

Recent advances in technology for industrial-scale clonal propagation of eucalypts have occurred with the development of intensive micro- and minicutting systems in Brazil. Microcuttings use apices obtained from micropropagated plantlets, while the minicutting is based on the rooting of axillary shoots derived from rooted stem-cuttings. In both systems, field clonal hedges are replaced by intensively managed minihedges grown indoors using hydroponic systems (**Figure 9**). This reduces costs and can also make the propagation cycle less dependent on weather conditions.

Figure 9 Indoor hydroponic, minicutting systems developed at Klabin Riocell, Brazil. Mother plants (left bottom) are grown indoors in hydroponic beds (left top) from where shoots are harvested for minicuttings. Cuttings are set in indoor rooting facilities (right top) and well-rooted cuttings obtained by 30 days (right bottom). Photograph courtesy of Teotônio Francisco de Assis.

Genetic Modification and Molecular Breeding

Development of genetically modified (GM) eucalypts has been slow compared with *Populus* species. Traits being considered for modification are no different from those being examined in other forest tree genera. Most transformation has involved marker genes, although genes of commercial significance including herbicide and insect resistance have been stably inserted. Genetic engineering of sexual sterility has been a major focus of research in Australia where eucalypts are native. Transgenic plantlets have been recovered from species such as *E. grandis, E. camaldulensis, E. globulus, E. saligna, E. urophylla, E. dunnii*, and various hybrids of these species. Field trials were established in the UK in 1995 and in Spain, Portugal, and South Africa in 1997. However, the development of fully tested GM clones to the stage of large-scale planting is likely to be a slow process, taking up to 12 years, and owing to regulatory problems research has, for the moment, shifted more towards molecular breeding through marker- or gene-assisted selection.

The first eucalypt gene sequenced was the important lignin gene CAD of *E. gunnii*, published in 1993 by French researchers working at the University of Toulouse. The first genomic maps of *Eucalyptus* appeared in the early 1990s and were based on random amplified polymorphic DNA (RAPDs) which are dominant polymerase chain reaction (PCR) markers or codominant restriction fragment length polymorphisms (RFLPs), and were used to study the genetic control of quantitative traits in species such as *E. grandis, E. urophylla, E. nitens*, and *E. globulus*. There are now hundreds of codominant (more informative) microsatellite loci developed for eucalypts that are transferable across species and have allowed alignment of genome maps from different studies and species. High consistency in marker order (synteny) is being revealed, and generic maps are emerging with candidate genes (e.g., for flowering and wood properties) positioned. Considerable progress has been made toward identifying genomic regions and markers associated with variation in quantitative traits (quantitative trait loci, QTL). QTL have been detected for numerous traits of economic significance including growth, propagation and wood properties, and in several cases these have been shown to colocate with candidate genes (e.g., *cinnamoyl* CoA reductase (CCR) gene with pulp yield, cellulose yield, and lignin quality (S/G ratio)). Research is now focusing on identifying genes and alleles responsible for the variation in traits of economic significance, particularly the highly heritable and expensive-to-measure wood property traits, through QTL and association studies. The next decade will see major advances in our understanding of the eucalypt genome and molecular breeding. There are now several privately owned databases containing partial sequences of many of the genes expressed in various tissues (e.g., cambium) of *Eucalyptus*, microchips have recently been produced to study eucalypt gene expression, and there is growing interest in large-scale sequencing of the eucalypt genome.

See also: **Genetics and Genetic Resources**: Genetic Systems of Forest Trees; Propagation Technology for Forest Trees. **Tree Breeding, Practices**: Genetics and Improvement of Wood Properties. **Tree Breeding, Principles**: Breeding Theory and Genetic Testing; Forest Genetics and Tree Breeding; Current and Future Signposts. **Tropical Ecosystems**: Eucalypts.

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Nitrogen-fixing Tree Improvement and Culture

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Introduction

This review focuses on the genetic improvement and culture of important tree species that fix nitrogen. About 700 tree species are known to fix nitrogen, among approximately 3000 suspected to do so. They represent 11 plant families. Most N-fixing trees (NFTs) are multipurpose and tropical in origin. They are often as valuable as fuelwood, green manure, or forage as they are for lumber or craftwood, and they are cultivated in a great diversity of agroforestry