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Active genetic conservation and utilisation of native radiata pine germplasm



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Active genetic conservation and utilisation of native radiata pine germplasm

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Forest & Wood Products Australia

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Executive Summary

This report details a large body of applied research and other activities aimed at genetically characterising the base population of radiata pine (*Pinus radiata* D. Don) for the purposes of effective *ex situ* gene conservation of the genetic resource in Australia and New Zealand through (i) specific genetic resource plantings and (ii) infusion of new germplasm into the current breeding program(s). The research was based on the 1978 ‘Eldridge collection’ of radiata pine seed that was used to establish provenance trials and large block plantings of gene-resource plantings between 1979 and 1982 at many locations in Australia and New Zealand. A gradual loss of these plantings due to the near-rotation ages of most sites and bush fire damage highlighted the need to implement a new strategy for re-conservation of radiata pine genetic resources.

The case for allocating resources and effort for *ex situ* gene conservation of this important forest genetic resource is strong from both an international and national perspective, including documented threats to the native populations Año Nuevo, Monterey and Cambria, on the Californian mainland coast and Guadalupe and Cedros Islands, off the coast of Baja California. From a commercial forest industry perspective, this importance is due to the fact that: (i) the radiata pine resource underpins a significant industry; (ii) the breeding programs in Australia and New Zealand appear to have a relatively narrow genetic base; and (iii) opportunities to expand the genetic base through direct importation of native germplasm is now limited due to the risk of introducing pitch canker disease which is impacting native stands in California. The need for expanding the genetic base of the breeding populations is mainly argued on the grounds of: (i) a need to maintain genetic diversity for climate change (including growth on drier sites) and pest/disease adaptation; (ii) reduced genetic variance for growth in current population; and (iii) enhance opportunities of adaptation to changing market demands.

We initiated and completed this radiata pine conservation and utilization project under a joint funding scheme by Forest and Wood Products Australia (FWPA), Southern Tree Breeding Association (STBA), Radiata Pine Breeding Company (RPBC), SCION (New Zealand Forest Research Institute Ltd) and Forests New South Wales (FNSW)). The objectives of the project were to:

- provide for radiata pine gene conservation for the next 50–60 years and beyond
- infuse new genotypes into breeding populations for increased genetic gain (especially increased growth and wood stiffness).

The project made significant progress in practical conservation, and generated scientific information for conservation strategy and utilisation of the conserved germplasm. Significant achievements included the following:

1. Catalogued the existing radiata pine conservation stands of 50 provenance trials and block plantings, 16 of which were established between 2004 and 2007. The updated catalogue provides forest managers with a one-stop-shop that lists details of trial establishment, location, owners and collaborators of the trials, and proposed schedule of activities recommended for each trial. The catalogue provides updated trials

information for ensuring gene conservation of radiata pine germplasm for the next 50 to 60 years.

2. Collected open-pollinated cones, seeds, and pollen from existing conservation plantings for implementing practical *ex situ* gene conservation. Large amounts of seeds from 992 genotypes from all five provenances are being kept in cold storage for long-term future use while some genetic material is being used to establish second-generation gene conservation trials at different sites in Australia and New Zealand to ensure long-term genetic security. Pollen collected from at least 72 genotypes spread across all provenances will be used for controlled crosses in order to increase genetic diversity. The strategy would conserve not only existing genetic diversity, but increase the genetic base necessary to adapt to changing environment and market demands.
3. Completed a comprehensive and large-scale genetic analysis of growth and stem quality traits in 11 trials and wood quality traits in five trials derived from the 1978 'Eldridge collection'. Eight of the trials in Australia were from effectively unselected germplasm collected from the wild and the results best reflect the genetic variation in the native population and the fact that females can be assumed unrelated probably provide some of the best estimates of genetic parameters due to the large number of founder parents (506). The other three trials were based on a population which is a generation removed from the base and has been subject to both natural and artificial (phenotypic on growth and form) selection in New Zealand. Analysis revealed there was abundant genetic variation in these populations. The significant differences in growth, form and wood properties among provenances, families and/or individual trees provide an opportunity for breeding programs to select superior trees for solid-wood production that will combine superior growth with desirable wood traits. Valuable ring-by-ring wood traits data provides an opportunity to investigate the genetic control of the actual age trajectory and how much of the genetic variation in the final harvest age properties between provenances and families can be explained by genetic differences in these trajectories.
4. Selected 210 elite trees representing all five provenances of radiata pine for possible infusion into existing breeding populations. These selections were based on an integrated index using DATAPLAN® and TREEPLAN® and were grafted into an archive for controlled crossings and subsequent infusion into breeding populations. The infusion would extend the future responses to selection by contributing favourable alleles not present in the existing breeding populations. Unique alleles in the Guadalupe, Cedros and Cambria populations which are currently not in the breeding population will now be included in the breeding populations and enable genetic adaptation to projected future environments (e.g. adaptation to climate change, pests and disease resistance).

This work has provided a valuable framework for future breeding, genetic resource conservation and research on radiata pine.

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Introduction

Genetic diversity is widely recognised as the key component for long-term survival of species. It is the foundation of sustainability because it provides raw material for adaptation, evolution, and survival of species and individuals, especially under changed environmental and disease conditions (e.g. Eriksson et al. 1993; FAO 2001; Frankham et al. 2002). Genetic uniformity may leave a species vulnerable to new environmental and biotic challenges (Ledig 1988). Genetic adaptation and rate of evolutionary response to selective forces, such as climate change, may depend on inherent levels of genetic diversity at the time the species faces a threat to its survival. Reductions in genetic diversity in trees can predispose forests to environment-related decline in health and productivity (Raddi et al. 1994). Therefore, maintaining genetic diversity and conserving forest genetic resources are critical to forest sustainability, ecosystem stability and species' continued adaptation and survival.

Gene conservation planning for a species begins with assessments of the genetic diversity and reproductive status of populations and species. For example, knowledge of population genetic structure can be crucial for formulating conservation strategies and sustainable management of forest genetic resources. Since different pictures of population genetic structure are often provided by molecular markers versus adaptive quantitative traits (e.g. Karhu et al. 1996; Merilä and Crnokrak 2001), integration of both kinds of information should be used to develop strategies for the genetic conservation of tree species (e.g. Frankham 2010). For example, quantitative traits (e.g. survival, resistance to biotic or abiotic stress and growth) are of economic significance and associated with population adaptation to different environments. Successful gene conservation efforts should not be directed at maintaining a forest population in a given state forever, but rather at ensuring the long-term enhancement of the genetic diversity presently available to meet future human needs. Moreover, gene conservation efforts should not concentrate only on those tree species and populations that are commonly used today, but also to those that may contain variation that will be useful in the future.

Because of anticipated adverse climatic change and resulting increases in disease and insect attacks in forest plantations in the future, forest industries must maintain broad genetic bases for adaptability and pest resistance. For the last three decades, researchers and scientists have urged that the forest community be more pro-active in the development of sound gene conservation programs to promote sustainable forestry, which includes maintaining and protecting genetic base populations (e.g. Dvorak 2012). Everyone agrees that "conservation is good", but the dialogue often stagnates on issues such as strategy and approaches (*ex situ* vs. *in situ*), the cost/benefits of long-term programs to maintain gene pools, and the justification for protecting commercially important tree species represented by large areas of exotic plantations (Eldridge 1978).

An important requirement in conserving quantitative variation is to maintain its dynamics (Namkoong 1998). Maintenance of continuing evolution should be primarily emphasised in developing conservation strategy. Conservation *ex situ* in the forms of seed bank, and tissue bank, called static conservation, decouples from dynamic environments and implies a risk of accumulation of genetic load (e.g. Hu and Li 2002). For example, seed from the 1978 'Eldridge collection' had viability levels down to 3% after 25 years in cold storage (Eldridge

– personal communication, 2004). Adaptation of materials in static conservation would be affected by the condition in storage and the time lasted. Poorly adaptive alleles are not eliminated during the period of storage, and will likely present maladaptation in the future. Thus, a large genetic differentiation between populations maintained *ex situ* and *in situ* is expected, especially for loci with large or adequate effects.

The natural populations of radiata pine

Radiata pine (*Pinus radiata* D. Don) is one of the Pacific coastal coniferous forest species and occurs naturally in five discrete populations over a small range (latitudes 28°N to 37°N) in North America (Fig 1). Año Nuevo, Monterey and Cambria are on the Californian mainland coast and Guadalupe and Cedros Islands are off the coast of Baja California. The spatial separation of the populations ranges from 60 km between Año Nuevo and Monterey, to 1,200km separating the Año Nuevo and Cedros provenances (Burns and Honkala, 1990). The five locations differ substantially from each other with respect to soil, elevation, temperature, rainfall and ecosystem associates (Libby 1997) and therefore selective pressures would also be expected to differ.

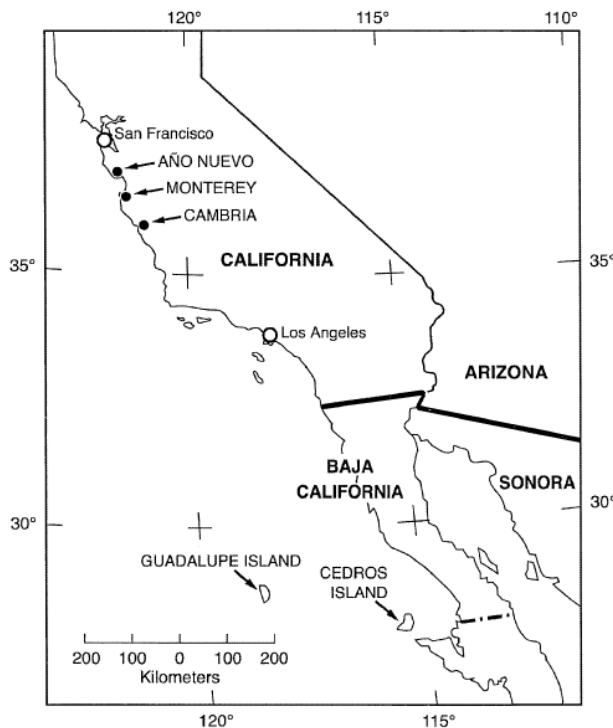


Fig 1. Location of natural radiata pine populations: Año Nuevo, Monterey and Cambria native to California and Guadalupe and Cedros Islands in Mexico. Adapted from Ledig et al. (1998)

The population history of the species has been well documented highlighting alternate scenarios (Axelrod 1981; Burdon 1992; Millar 2000; Karhu et al. 2006). Axelrod (1981) hypothesised that mainland populations are relicts of an earlier much wider distribution, reduced some 8 000 years ago. An alternate scenario put forward by Millar (2000) proposed that the species maintained a metapopulation structure throughout its history in the Californian region, growing in distinct coastal populations that were subjected to repeated events of colonisation, coalescence, and local extirpation in response to fluctuating climates.

Population differences are likely to reflect frequent founder and bottleneck effects, and complex introgression in addition to differences stemming from adaptations to the respective habitats (Millar 2000). Karhu et al. (2006) used microsatellite data to distinguish between the alternative scenarios and observed little evidence for the effects of long-term contraction, but some signature of population expansion.

The native stands of radiata pine are almost all threatened to some degree (Rogers et al. 2006; IUCN 2009). These native stands are infected with pitch canker, a fungal disease native to the southeast United States and found (in 1986) to have been introduced to California (Rogers et al. 2006; IUCN 2009). Habitat loss and fragmentation is often severe in the native stands. Details of the current status of radiata pine in its native habitat are given in Table 1. The two island populations have limited distribution with only approximately 220 mature radiata pine trees on Guadalupe Island occurring along the top of a ridge and no regeneration occurring due to grazing by goats (Eldridge 1978). Two disjunct subpopulations occur on Cedros Island, one along the central ridges of the island and the other 15 km away on ridges and cliffs at the northern end of the island (Libby 1997).

Table 1. Estimates of current area of radiata pine populations and protected area as of 1988 census (adapted from Rogers 2004)

	Forest area estimates (ha)	Protected area In ha	Minimum (% of total) ^a	Maximum (% of total) ^b
Population				
Año Nuevo	400	12	2	3
Monterey	6000	911	15	33
Cambria	1200	430	35	48
Guadalupe ^c		All	100	100
Cedros	130	0	0	0

^aThis represents the percent of total forest area that is protected based on the maximum forest area estimates (Burdon 1988)

^bThis is the percent protected based on the minimum forest area estimate

^cOn Guadalupe island, it is more appropriate to measure the pine forest by number of trees (approximately 220) rather than area occupied (Rogers et al. 2004)

Summary of the most significant current and potential threats of each of the native populations of radiata pine are provided in Table 2. Monterey and Cambria are severely being encroached upon by urban development and exotic invasive organisms. In addition, and very importantly, the spread of pitch canker disease internationally means no radiata pine plants or seeds can be brought into Australia or New Zealand. As a result, there is currently a moratorium on importing germplasm from radiata pine native stands to Australia and New Zealand (Gadgil et al. 2003). The stands on Guadalupe Island have been severely impacted by grazing from feral goats, with less than 220 mature trees left based on a 2001 census (Rogers 2004). There is now some documented evidence of recovery of trees on Guadalupe Island (Oberbauer 2003). However, until the seedlings are producing a fair number of cones of their own, there will be great vulnerability to fire which can readily be carried by the grass growth that is now occurring. Efforts to conserve *in situ* are underway. For example, there are efforts to purchase land to protect the remaining forests from degradation or conversion to other uses (Rogers 2002; Rogers et al. 2006).

Table 2. Summary of the most significant and potential threats for each of the native populations of radiata pine (source Rogers 2004)

Population	Most significant current or potential threat
Año Nuevo	Exotic invasive disease, lack of secured ownership with conservation objectives
Monterey	Habitat loss and fragmentation, genetic contamination, exotic invasive organisms
Cambria	Habitat loss and fragmentation, exotic invasive organisms
Guadalupe	Lack of regeneration because of introduced goats, potential for harm from exotic invasive organisms
Cedros	Threat of introduced organisms, particularly pathogens; lack of any reserves or protected status

The introduction, genetic base and state of radiata pine breeding populations in Australia and New Zealand

Radiata pine was introduced into Australia and New Zealand, via England, in the mid 1800's (Wu et al. 2007; Johnson et al. 2008). Initially the species was used for ornamental purposes in botanic gardens. Further seed was imported directly from California to New Zealand in the 1870's with the largest consignment of 11 kg in 1876 probably originating from a large number of trees (Wu et al. 2007). From 1859 into the mid-1860s importations were made basically on a specimen-tree scale, from England (Wu et al. 2007). The species thrived so well that by the late 1860s larger-scale importations had begun from California, and are known to have continued until 1882, by when New Zealand plantings could evidently meet the considerable demand for seed (Raymond and Henson 2009). From the early 1900's seed from these early plantings was used to establish increasing areas of radiata plantation in southern Australia and New Zealand (Moran and Bell 1987; Johnson et al. 2008). However, information on the exact origin and breadth of the original genetic base was unreliable.

In the 1950's structured breeding programs for radiata pine commenced and eventually the issue of the genetic base was raised (Wu et al. 2007; Raymond and Henson 2009). An isozyme study suggested that Australian and New Zealand radiata pine originated from Monterey and Año Nuevo, the two best adapted of the five natural populations (Moran and Bell 1987; Burdon 1992). The isozyme work also showed that the first generation of existing breeding programs had captured a substantial proportion of the genetic diversity (in terms of allelic representation) in Año Nuevo and Monterey provenances (Moran and Bell 1987). However, the three southern provenances (Cambria, Guadalupe and Cedros) did not appear to be represented in breeding programs. As breeding programs became more sophisticated, thought was given to expanding the genetic base by recollecting in the original stands in California. During the 1960's two major collections were carried out. The first one was in 1962 in which cuttings were collected from juvenile trees in the three mainland provenances and planted in California (Hood and Libby 1980) and the second collection was in 1964 where seed was collected in both the mainland and island populations (Forde 1964).

The third collection was in 1978 (referred in this report as the 1978 'Eldridge collection'). The collection was done as a collaboration between New Zealand and Australia with joint funding from United States of America/Australia Cooperative Science Program, CSIRO Division of Forest Research, New Zealand Forest Research Institute, and Forest Resources Division of Food and Agricultural Organisation. Seed was collected from a total of 621 mother trees and at least 22 trees from each provenance and the trees were selected for having

at least 40 collectable cones and being well grown and of good form (Eldridge 1978) (see Fig 1; Table 3). This is the most extensively studied with provenance trials established between 1979 and 1982 at many locations in Australia, New Zealand and other countries (Tables 4 & 5).

Table 3. Seed-origin information for radiata pine populations sampled in California in 1978 (from Eldridge 1978)

Provenance	No. of mother trees	Latitude ⁺ (° N)	Longitude ⁺ (° W)	Altitude (m)	Rainfall (mm/annum) [‡]
Año Nuevo	179	37°08'	122°18'	15-300	700
Monterey	244	36°37'	121°57'	5-580	500
Cambria	99	35°37'	121°09'	30-180	500
Guadalupe*	48	29°10'	118°15'	400-1200	330-510
Cedros	51	28°22'	115°20'	380-640	150
Total	621				

⁺Latitude and longitude are derived directly from map locations and represent approximate centre points of the island and mainland populations

[‡]Annual rainfall figures are approximate and average.

*Substantial occurrence of fog during spring, summer and autumn with fog are especially important in complementing the normal precipitation in drier years. Rainfall figures from Oberbauer (2006)

The 1978 ‘Eldridge collection’ included open-pollinated (OP) seed from individual trees from the Año Nuevo, Monterey, Cambria, Guadalupe and Cedros provenances (Fig 1). This sampling would appear to be a good sampling of the base genetic variation in radiata pine. The 1978 ‘Eldridge collection’ was used to establish provenance trials and large block plantings of genetic conservation trials between 1979 and 1982 at many locations in Australia, New Zealand and Chile. The main objective of the trials was to increase the genetic base of the radiata pine breeding population, which was thought to be mainly of Año Nuevo origin, with less of the ancestry from Monterey and none from Cambria (Burdon et al. 1997; 2008). The details of the base population field trials and *ex situ* gene conservation trials where pedigree information is available are provided in Tables 4 (Australia based trials) and 5 (New Zealand based trials).

The genetic base of the present Australian and New Zealand radiata plantations is sourced from Año Nuevo and Monterey, the two best-adapted of the five native populations: Año Nuevo, Monterey and Cambria on mainland California, USA, and Cedros and Guadalupe islands in Mexico (Burdon and Bannister 1973; Moran and Bell 1987; Burdon 1992; Wu et al. 2007). For example, the original genetic base of the Australian breeding populations may have been of the order of 200 - 300 seed trees from a limited part of the natural range of radiata pine (Wu et al. 2007). Bottleneck or founder effects, through restricted seed collections from ornamental and windbreak plantings for earlier commercial plantations, may have further eroded genetic diversity of the breeding populations (Fielding 1957; Grant 1989). The genetic base for the land race(s) and traditional breeding populations has been a very incomplete and unbalanced sample of the species’ natural range and hence of its total genetic diversity. For example, available evidence suggests that much of New Zealand’s radiata pine plantations originated from Año Nuevo, and that Australia’s plantations originated from the Monterey population with some input from New Zealand’s land race (Moran and Bell 1987; Wu et al. 2007). The effective population size of the individuals in the Australian breeding population managed by STBA that have been grafted for breeding,

Table 4. Details of current base provenance trials and *ex situ* conservation trials (family identities records maintained) in Australia

Trial name	Location	State	Provenances represented	#families	Survival %	Clearfell
Buccleuch	Bondo cpt 869	New South Wales	An, Ca, Gu, Ce	81	>90	2013
Green Hills	Batlow, cpt 777	New South Wales	An, Mont, Cam, Gu, Ce, TSO	506	>92	2012
Green Hills	Batlow, cpt 777	New South Wales	Gu	39	<45	2012
Green Hills	Batlow, cpt 777	New South Wales	Ce	26	<40	2012
Billapallolla	Billapallolla, cpt 1106	New South Wales	Gu, Ce	78	<40	2012
Longford	Salicki, cpt 69	Victoria	An, Mont, Cam, Gu, Ce, GSO	506	<60	Indefinite
Longford	Salicki, cpt 70	Victoria	Gu	36	<30	Indefinite
Longford	Salicki, cpt 70	Victoria	Ce	25	<25	Indefinite
Paramount	Paramount, cpt 28	Victoria	An, Mont, Cam, Gu, Ce, 3 controls	450	>80	2015
Longford	Longs, cpt 9	South Australia	An, Mont, Cam, Gu, Ce, 3 controls	500	>75	2015
Tantanoola	Tantanoola reserve	South Australia	Gu	120	>90	2015
Second Valley	Second Valley	South Australia	An, Mont, Cam, Gu, Ce, 2 controls	324	>90	2015
Wirrabara	Mt Ellen cpt	South Australia	An, Mont, Cam, Gu, Ce, 2 controls	250	<50	2015
West Takone	West Takone, cpt TR04	Tasmania	An, Mont, Cam, Gu, Ce, 5 controls	55	>80	2015
Paramount	Paramount, cpt 27	Victoria	An, Mont	Bulk	>75	2015
Busselton	Baudin, cpt 16	Western Australia	An, Mont, Cam, Gu, Ce, 2 controls	120	>85	2015

An = Año Nuevo; Mont = Monterey; Cam = Cambria; Gu = Guadalupe; Ce = Cedros; TSO = Tallaganda Seed Orchard; GSO = Gippsland Seed Orchard;

Table 5. Details of current base provenance trials (no family identity records) planted in New Zealand

Trial Identity	Location	#Treatments	Status
AK793	Mangaokewa	16	Current
AK844	Aupouri	16	Terminated
AK845	Waitangi	16	Terminated
AK846	Riverhead	16	current
AK865	Pouto	16	Terminated
CY533	Balmoral	16	Terminated
CY534	Waimate	16	Terminated
NN349	Golden Downs	16	Terminated
NN501	Tasman	16	current
NN502	Wairau	16	current
RO664/12	Kaingaroa	16	Terminated
RO1801	Waimihia	16	Terminated
RO1802	Rotoehu	16	Terminated
RO1803	Ruatoria	16	Terminated
SD425	Dean	16	Terminated
SD426	Longwood	16	Terminated
SD427	Naseby	16	Terminated
SD428/1	Berwick	16	Inactive
SD428/2	Berwick	16	Inactive
WD271	Kaniere	16	Terminated
WD400	Nemona	16	Terminated
WN271	Waitarere	16	Terminated
WN272	Ngaumu	16	Terminated
WN273	Mohaka	16	Terminated

and the individuals likely to be considered as potential parents, may be considered too small at this stage. For example, the average coancestry of this combined set, and taking into consideration the long-term contributions to future generations of existing parents and anything that is to be grafted in from the top 20,000, translates to an effective population size that is alarmingly low – perhaps less than 10 (Richard Kerr – pers. comm, 2010). Similarly, the additive genetic coefficient of variation for DBH growth in current breeding populations in Australia and New Zealand is now considered too low for continued gain from selection (Gapare et al. 2011).

However, genetically inferior material is undesirable for breeding purposes, and in some cases infusion of new material may cause loss of genetic gain. The infusion, therefore, needs to be optimised by balancing the genetic gain and relatedness (e.g. Brisbane and Gibson 1995). Several breeding strategies such as sublining (Lowe and van Buijtenen 1981), nucleus breeding, elite breeding (Cotterill et al. 1989; White 1992; White et al. 1999) and multiple-populations breeding strategy (Namkoong et al. 1988; Eriksson et al. 1993) have been developed to allow for infusion of new, unrelated material. For example, the tested F₁ crosses of the infused elements under-perform on average compared with the elite breeding material, they will be outcompeted in progeny tests. They may need specific weighting in any selection

process to ensure they progress through cycles of selection and breeding to allow recombination of alleles to occur and genetic diversity to be maintained. At this stage, it is unclear how the infused elements of the breeding program will be managed in future testing and selection cycles. Future research will include developing strategies to ensure that adequate genetic diversity exists in breeding populations to meet long-term goals. For example, there is no reliable evidence of heterosis in inter-provenance crosses in radiata pine that would favour the process of infusion or outbreeding depression in subsequent generations of inter-race crossing. However, island populations may well have built up unique epistatic interactions following population bottlenecks.

Summary of molecular marker variation and quantitative traits variation in radiata pine

The genetic architecture of radiata pine has been examined extensively using several types of molecular markers and quantitative data (Millar 2000). The literature survey conducted by Millar (2000) and results from this study have shown varying levels of population differentiation, with island populations being more differentiated from the mainland populations and also between themselves. The only exception is the chloroplast DNA diversity study which showed no differences among populations (Hong et al. 1993). Some differences among marker types might be expected through sampling effects given that loci are not a random sample. Given an important distinction between marker diversity and functional genetic diversity, the two must be closely cross-calibrated for the genetic material in question if marker diversity is to be a reliable measure of functional diversity (Burdon and Wilcox 2007). This is increasingly becoming a reality with the advent of functional genomics (e.g. Namroud et al. 2008).

Quantitative traits analysis has shown that the five populations are distinct genetically for morphological and quantitative traits, although different traits show varying degrees of resemblance (e.g. Axelrod 1980; Burdon et al. 1992b; Johnston et al. 1997; Gapare et al. 2012). For example, the Cambria and Guadalupe populations differ relatively little in respect of monoterpane composition, but contrast strongly in cone size and shape and in persistence of juvenile characteristics (Burdon 1992; Burdon et al. 1997). All five populations were differentiated from one another for susceptibility to western gall rust (Kinloch and Libby 1997) and resistance to *Phytophthora cinnamomi* (Butcher and Stukely 1997). Similarly, mainland population effects were highly significant for Dothstroma needle blight infection, with most severe infection in Cambria and least infection in Monterey (Ades and Simpson 1991; Burdon et al. 1992b; Gapare et al. 2011). Large differences between mainland and island populations were apparent for wood density and stiffness (Gapare et al. 2012). In summary, most of the molecular and quantitative traits studies in radiata pine do generally indicate the same genetic architecture, in terms of population differences. We concur with Burdon (1992) who highlighted that the pattern of differences among radiata pine natural populations were highly multi-dimensional, such that resemblances between particular populations depend on the trait or group of traits.

The case for conservation and utilisation of native germplasm

Gene conservation involves several sequential stages, ranging from the initial selection of target taxa and identification of conservation objectives, through field exploration and germplasm collection, to the actual storage and maintenance of that germplasm over extended time periods. *In situ* and *ex situ* gene conservation are two broad groups of methods for genetic conservation; each has different scientific, political and managerial objectives and challenges. However, both methods are complimentary in that they both maintain, and in some instances, create genetic diversity. *In situ* conservation means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surrounds and, in the case of domesticated or cultivated species, in the surrounding where they have developed their distinctive properties. Currently, genetic conservation in the native stands of radiata pine is a minor part of well supported ecological conservation campaigns to conserve the whole ecosystem, not only the pines but also shrubs and wildflowers, birds and animals. Rodgers (2004) emphasised that the most critical *in situ* conservation action is land purchase to protect remaining forests from degradation or conversion to other land uses. *Ex situ* conservation refers to any conservation method that entails removal of individual plants or propagation material (seed, pollen, tissue) from its natural occurrence, i.e., conservation “off-site” in gene banks as seed, tissue or pollen; in plantations; or in other live collections, such as *ex situ* conservation stands.

There is a general consensus among scientists and practitioners that no single conservation method is adequate, and that different methods should be applied in a complementary manner (e.g. Boffa et al. 2000). *In situ* gene conservation, however, has a number of benefits and so often forms the basis of conservation programmes. It allows evolutionary processes to be maintained, including the adaptation of tree populations to changing environmental conditions. This is particularly important for breeding programmes, since future human needs and environmental conditions are difficult to predict. As mentioned earlier, *in situ* and *ex situ* gene conservation should be used in a complementary fashion to conserve forest genetic resources. Both are an integral part of the conservation process, and both can only be effective after genetic diversity has been located and conservation priorities have been set. The main purpose of *ex situ* gene conservation is to capture and maintain a representative sample of the existing genetic diversity of a species. For highly endangered tree species, *ex situ* conservation may be the only approach in the short to medium term. The main pitfalls of collecting germplasm samples for *ex situ* gene conservation are: i) limited coverage of genetic variation; ii) biases in the collected plant material; and iii) samples that are too large to deal with (Brown and Hardner 2000). Because *ex situ* gene conservation is more costly than *in situ* gene conservation, it is particularly important that sampling of populations and germplasm within populations is given special attention to maximise the use of limited financial and human resources.

One of the objectives of gene conservation is to ensure that functionally useful alleles will be available in the future and can be captured in breeding programs within useful genotypic sets. This requires that allelic variation be conserved in adaptationally significant traits such as those that have already been observed to exist in radiata pine. Sustained productivity and long-term profitability of radiata pine forestry requires conservation of species genetic resources (Spencer et al. 1998). Genes of intermediate frequency are relatively easy to conserve as they are commonly expressed. However, low frequency genes (rare alleles) are

more difficult to conserve even though they are likely to be needed for the future to meet new selection criteria such as new pests and diseases (e.g., Eldridge 1978; Yanchuk 2001; Gapare et al. 2005; Burdon 2010).

The near-rotation ages of most provenance trials and conservation-block plantings, and a gradual loss of plantings at more than 20 sites highlighted the need to implement a strategy for re-conservation of radiata pine. The rest of the trials have reached rotation age and are due for clear-felling by 2015. The range-wide radiata pine 1978 'Eldridge collection' also provided an opportunity for boosting the genetic base of the existing Australian and New Zealand breeding populations (Eldridge 1997). To ensure genetic sustainability and further increase genetic gain for radiata pine, infusion of new genetic material from the range-wide radiata pine 1978 'Eldridge collection' was recommended (Burdon 1992; Eldridge 1997; Wu et al. 2007). In order to explore what potential benefits infusions of native-population material into breeding programmes are likely to confer, initial characterisation of quantitative genetic variation of the native provenances would be critical in assessing the size and usefulness of genetic variation and for directing efficient selection of the genetic material. In addition, the 'Eldridge collection' germplasm had not yet been utilised in breeding programs. Infusion of conserved 'wild' genes from natural populations of radiata pine was seen as providing a new opportunity for improvement of Australian and New Zealand radiata pine. However, such expansion of genetic diversity will have a cost as germplasm in the breeding programs are between three (Australia) and five (NZ) generations removed from the wild and have been genetically improved for adaptation to their exotic environments and industrial objectives.

Infusion would extend the future responses to selection by contributing favourable alleles not present in the existing breeding populations. There would also be an opportunity to combine desirable attributes of different populations by genetic recombination. For example, some studies showed that both Guadalupe and Cedros had great potential as inter-provenance hybrid parents (Low and Smith 1997; Burdon et al. 1998; Kennedy 2004). Peripheral populations such as the island population are generally considered to be biologically unique – representing either remnants of a species' former range or its extension to the limit of physiological or ecological extremes and their conservation may present the best opportunity for conserving useful rare alleles (e.g. Gapare et al. 2005; Aitken et al. 2008). Sheer numbers of parents offer the most certain way of retaining genetic variability (Eldridge 1997). This would provide future prospects to combine desirable attributes of different populations by genetic recombination. The real significance of such a boost in genetic diversity from broadening the provenance base may lie in recruiting genetic resistance to various biotic factors and abiotic factors. For example, Cambria performed poorly with respect to *Dothistroma* resistance in previous studies (e.g. Burdon et al. 1992a, Ades and Simpson 1991) but much biotic resistance may be cryptic pending the arrival of new pests or diseases. However, genetically inferior material is undesirable for breeding purposes, and in some cases infusion of new material may cause loss of genetic gain. The infusion, therefore, needs to be optimised by balancing the genetic gain and relatedness (e.g. Brisbane and Gibson 1995). Several breeding-strategy features, such as sublining (Lowe and van Buijtenen 1981), nucleus breeding, elite breeding (Cotterill et al. 1989; White 1992; White et al. 1999) and multiple-populations breeding strategy (Namkoong et al. 1988; Eriksson et al. 1993) have been developed to allow for infusion of new, unrelated material.

While obviously not assisting with genetic resource conservation of three of the provenances not well represented in breeding programs (Cambria, Guadalupe and Cedros), there is the

option of increasing genetic variance in the breeding programs through exchange of selections between breeding programs. Since it has been suggested that much of New Zealand's radiata pine plantations originated from Año Nuevo, and that Australia's plantations originated from the Monterey population with some input from New Zealand's land race (Moran and Bell 1987; Wu et al. 2007), the present study suggests Año Nuevo and Monterey are genetically more different than Cedros and Monterey, in which case one would expect that exchange with Año Nuevo is more likely to have a greater impact on the genetic diversity of a Monterey dominated population.

Methodology

Aims

Overall aims

The project aimed to provide for radiata pine gene conservation for the next 50-60 years and beyond, and to infuse new genotypes into breeding populations for increased genetic gain (increased growth and wood stiffness). Through this project, we were able to integrate genetic conservation with utilisation of native radiata pine resources and demonstrate the effectiveness of a conifer *ex situ* gene conservation as part of a breeding program.

Specific objectives

1. Update the existing catalogue of provenance and genetic conservation trials of radiata pine in Australia;
2. Collect open-pollinated cones and extract seed for long-term storage and for –re-establishment of second-generation *ex situ* conservation blocks;
3. Characterise quantitative genetic variation of *ex situ* native-provenance collections of radiata pine for growth, form and wood property traits in order to explore potential benefits of infusing the native-population material into breeding programs; and
4. Based on an integrated index, select 50 elite trees from each of the mainland provenances and 30 from each of island provenances for grafting into an archive for controlled crossings provenance in order to create relatively “pure” population to maximise genetic diversity for current and future environments and subsequent infusion into breeding populations.

Presentation format

In order to improve the usability of the report, it was split into the following sections:

1. Details of activities, rationale and methodology – which provides background details of the various activities carried out;
2. A Brief summary of major results explicitly cross-referenced to corresponding detailed reports provided in the appendices (via a link). However, there is genuine overlap between the Client technical reports, Scientific manuscripts, References, and Appendices. This arises from a need to present the same material in slightly different forms to different agencies in order to maintain the required level of confidentiality.
3. A Summary that highlights the primary scientific finds which are split into conservation-related findings and infusion-related findings. Major industry adoptions with significant impact are also highlighted.

Activities, rationale and methodology

1. Cataloging the existing provenance and genetic conservation trials and collection of radiata pine in Australia

In order to develop an optimal *ex situ* gene conservation strategy for radiata pine, it was necessary to update the existing catalogue of provenance and genetic conservation plantings. A field inspection of existing provenance and genetic conservation trials of radiata pine in Australia was carried out in order to update the existing catalogue of provenance and genetic conservation trials. A total of 50 provenance trials and genetic conservation trials were inspected across Australia. In addition, working with individual forestry companies, specific management plans were developed with industry for long-term management of specific trials with the individual enterprises.

2. Nucleotide diversity and population genetic structure in disjunct *Pinus radiata* D. Don populations

Gene conservation planning for a species begins with assessments of the genetic diversity and reproductive status of populations and species. In order to recommend an optimal *ex situ* gene conservation strategy, we investigated nucleotide diversity and population structure in radiata pine using single nucleotide polymorphisms (SNPs) from 36 candidate genes previously sequenced in a panel of radiata pine individuals (Tables S1 & S2 in Dillon et al. 2010). For each of the five populations (see Fig 1), 37 seeds from different mother trees were picked at random and haploid megagametophytes were harvested, i.e., one megagametophyte per parental tree (sample size of 37 alleles per population). A sample size of 37 seeds per population was chosen in order to facilitate use of two 96-well plates to accommodate 192 samples, made up of 185 ($37 \times 5 = 185$ samples) and 7 controls. These megagametophytes were regarded as random gamete samples from each population. Genomic DNA was extracted from the megagametophyte tissue of each seed using QIAGEN (Valencia, CA) DNeasy Plant DNA purification kit.

Pair-wise genetic differentiation (F_{ST}) between sampling populations was analysed using Arlequin ver. 3.01 (Excoffier et al. 2005). A Bayesian clustering approach, as implemented in the program STRUCTURE 2.1 (Pritchard et al. 2000) was used to infer population structure and the most likely number of populations (K) that is compatible with the actual populations geographically distributed. The program STRUCTURE 2.1 was also used to perform a population-assignment test for identification of putative migrants between populations.

3. Collection of pollen, open-pollinated (OP) cones and seeds for long-term storage and for re-establishment of second-generation *ex situ* conservation blocks

Pollen was collected from at least 72 mother trees of all five provenances and kept in cold storage at STBA facilities in Mt Gambier. Our goal was to collect seeds from enough trees in each provenance to ensure that our sample represented the genetic diversity of the entire provenance. There was no particular selection done at this stage. At least 10 cones from each

of 15-20 trees in every sub-provenance were collected from five sites (Green Hills, Paramount, Tantanoola in Australia, and Kaingaroa and Rotoehu in New Zealand, Figs. 2, 3, 4 and 5). Generally 75-100 seeds per cone were extracted, cleaned and put in storage envelopes which were properly labelled to indicate family identities of radiata pine. The seeds are being kept in cold rooms at the Australian Seed Centre (ATSC) to ensure long term seed viability. Information recorded on the seed lots includes: species name; pedigree, date of collection; and origin details such as location, latitude, soil type and climatic information. This information is integrated into ATSC information system.

Discrete generations are envisaged for renewing gene resources, but with relatively long generation intervals in order to reduce allele losses (e.g. Burdon and Kumar 2003). Planting sizeable blocks of pure mainland populations will give the numbers that would offer the most secure conservation of all five populations for a long-term conservation programme. The conservation blocks plantings should be made in different sites to ensure security. It is envisaged that three sites in Australia and two sites in New Zealand should be adequate. A minimum of 32 trees per family in each of the provenances would be adequate at each site. However, this approach has its own drawbacks, in the form of opportunity costs for forest owners (e.g. Burdon 2010) and vulnerability to pollen contamination (e.g. Burdon and Kumar 2003). The opportunity costs aspect has become less palatable after privatisations, especially with forest owners representing global interests and strong international risk spread of foreign forest owners; and rapid changes in forest ownership and management (Burdon 2010). The conservation plantings at different locations would offer the most secure conservation of all five populations. It is also known that the maximum genetic diversity of a population (the lowest overall coancestry) is attained in the long-term by subdividing it into as many isolated but large groups as possible (Kimura and Crow 1963), as different allelic variants will become fixed in each group, becoming a genetic reservoir of variation.

Ex situ conservation plantings should be buffered from external pollen contamination which may affect their value as sources of new and unrelated germplasm. External pollen contamination may undermine the value of the resources for their intended purposes, especially if natural and/or silvicultural selection needs to be part of the management system (Burdon and Kumar 2003). For example, Burdon and Kumar (2003) carried out a stochastic simulation over five generations, of which four incurred contamination, using radiata pine in New Zealand as an illustration and highlighted, among other factors, the importance of the choice of planting site. Ideally a planting site should be adjacent to land where there are no pine plantations, preferably on the western side to minimise pollen flow into the conservation planting. Alternatives include use of the 'window design' where a 20 ha block of the conservation planting is surrounded by routine radiata pine plantation. They also recommended the development of a marker set powerful enough to identify individuals resulting from contaminant pollen (candidate parents for seed collections could be checked).



Fig 2. Cone collection at Paramount site in South Australia



Fig 3. Cone collection at Kaingaroa compartment 1104 in New Zealand



Fig 4. Cone collection at Tantanoola site in South Australia



Fig 5. Dried cones in bags ready for seed extraction and cleaning.

4. Study of performance differences among *ex-situ* native-provenance collections of *Pinus radiata* D. Don.: growth and wood property traits

In 1994, plus-tree selections were made in the gene-resource stands of radiata pine in New Zealand. Seed from the plus trees was used to establish tests at two sites in New Zealand and one site in Australia, in 1995. The sites included Kaingaroa and Hauhungaroa in New Zealand and Kangaroovale (Fig 6), New South Wales, in Australia (Table 6). Each trial contained 128 families from three provenances: Monterey had 57, Cambria 34 and Año Nuevo 37 families. Four control seedlots representing different levels of genetic improvement characterised by growth and form rating (GF) were included in the trials. The controls represented two seedlots of unimproved selections (GF6), one older open-pollinated seed orchard stock (GF14) and one current breeding stock (GF28). However, control seedlots were not included in the estimation of genetic parameter estimates as they were considered not to be part of the population. Phenotypic means from “raw data” were used for comparisons of controls to families.

Table 6. Particulars of field trials in New Zealand and Australia

Particulars	New Zealand		Australia
	Kaingaroa	Hauhungaroa	Kangaroovale
Test number	FR259/1	FR259/2	FR259/3
Date planted	6/1995	6/1995	6/1995
Latitude	38° 16'S	38° 50'S	35° 05'S
Longitude	176° 43'E	175° 45'E	148° 15'E
Elevation (m)	330	435	480
Annual rainfall (mm)	1600	1500	840
Soil type	shallow covering of basaltic scoria over layers of rhyolitic pumice	rhyolitic pumice layers	Solodic derived from rangle – volcaniclastic sandstone, polysictic conglomerate
Site type	Undulating terrain; on a 2 nd pine rotation	Gently sloping terrain; Grazing with fertiliser application	Gently sloping terrain, ex-pasture
Spacing (m)	4 x 4	4 x 4	4 x 4

Data were available at age nine years from planting for diameter at breast height (DBH) and several form traits (stem straightness (STR), branching frequency (BRFQ), malformation (MAL), and acceptability (ACC) and *Dothistroma* needle blight (DOT)). Wood density data were available from 640 trees and 586 trees at Kaingaroa and Kangaroovale, respectively.



Fig 6 A trial assessed for survival at Kangaroovale in New South Wales in 2010

Statistical analyses were conducted using ASReml (Gilmour et al. 2009). Preliminary analyses where a separate family within provenance effect was fitted for each provenance indicated that estimates were not statistically different, and therefore, only family effect pooled across provenances was used in subsequent analyses. Likelihood ratio test (LRT) was used to test for any significant differences for these effects (e.g., Gilmour et al. 2009). A combined-site analysis was also conducted for each trait in order to calculate unbiased heritability estimates, estimate an overall Type-B genetic correlation following Yamada (1962), and to test the significance of provenance and the trial x provenance interaction.

5. Study of quantitative genetics of growth, form and wood property traits of radiata pine ‘Eldridge collection’

To ensure genetic sustainability and further increase genetic gain for radiata pine, infusion of new genetic material from the range-wide radiata pine 1978 ‘Eldridge collection’ made in 1978 (Eldridge 1978) was recommended (Burdon 1992; Eldridge 1997; Wu et al. 2007). Eight out of the original 67 provenance trials and large block plantings of genetic conservation trials planted in 1980 in Australia, had seed-parent pedigree records and a combination of assessment ages and traits that allowed characterisation of quantitative genetic variation. The family level pedigrees of most of this material is presumably unknown as samples were from provenance blocks, thus only future molecular work will be able to account for potential relatedness amongst females and males within each provenance and thus allow the effective sample size to be determined.

Table 7 provides general information about the field trials in southeastern Australia planted in 1980 that were examined for growth and form traits. The trial sites were of contrasting growing conditions in terms of altitude, rainfall and soil types. The trials encompassed a variety of designs, plot sizes and replications (Table 7). For the purpose of estimating genetic parameters, the genetic controls were excluded from the analyses. Survival at each trial was assessed at times of assessments between ages 8 and 12 years and expressed as a percentage of the total number of trees planted. Generally, there were juvenile (_J) measurements starting at age 8 years to 12 years and mature (i.e. pre-rotation _M) age measurements starting at age 24 to 30 years. Sherwood, Second Valley and Wirrabara trials had age 16 years measurements and they were regarded as pre-rotation measurements (i.e., _M). Stem diameter at breast height (DBH) was measured in cm. Total height (HT) was measured in metres and was only assessed at Green Hills at juvenile ages. Form traits included stem straightness (STR) on 1 to 6 scale: 1 = most crooked stems, ..., 6 = straightest stems; branch angle (BRA) on a scale of 1 to 6 with 1 = steepest branch angle, ..., 6 = flattest branch angle and flatter branch angles are preferable; branch size (BRS) on a scale of 1 to 6 with 1 = biggest branches, ..., 6 = smallest branches where small branch sizes are preferable; however, a scale of 1 to 4 with 1 = largest branch size and 4 = smallest branches was used at Billapalloola. Nodality (NOD) (intervals between branch clusters) on a scale of 1 to 4 with 1 = uninodal, 4 = highly multinodal.

Modulus of elasticity (MoE) is one of the two key properties of structural sawn timber, the other being dimensional stability. Both wood density and microfibril angle (MfA) determine the MoE, with MfA probably being somewhat more important in juvenile corewood, but density becoming the predominant determinant in outerwood (Cave and Walker 1994; Walker and Butterfield 1995; Ivković et al. 2009; Lachenbruch et al. 2010). Outerwood has more desirable characteristics for structural timber such as high wood density, low MfA, less grain spirality and high stiffness (e.g. Haslett et al. 1991; Young et al. 1991; Walker and Nakada 1999). Wood property traits data included basic density (BD) at age 29 for Green Hills and Salicki trials and at age 27 for Billapalloola trial (Table 7).

Sampling for wood properties

Twelve-millimetre bark-to-bark increment cores were collected at breast height (1.3 m) from 1272 trees at Green Hills on the first three trees in a plot from a total of 424 OP families in four replicates; from 1810 trees at Salicki on first 3 - 4 trees in a plot from 467 OP families in five replicates, and from 202 trees at Billapalloola on first 2 to 4 trees in a plot from a total of 73 OP families in five replicates. All trees were selected on the basis that they were healthy, straight and without defects. In cases where trees appeared to be leaning, cores were extracted at right angles to the direction of stem lean to avoid compression wood. In total 3284 cores were taken between September 2009 and December 2009.

The number of families per provenance and sub-population at each trial and the corresponding number of cores assessed using the SilviScan® instrument are presented in Table 8. Approximately an equal set of common families across Green Hills and Salicki trials were selected per provenance and sub-population. A stratified sampling strategy (e.g. Raymond et al. 2009) was used with families ranked according to breeding values for DBH at mature age (26 to 29 years) (Gapare et al. 2011) and families selected to cover the range of breeding values. Two to four trees per family were selected in each of the Green Hills and Salicki trials. For the Billapalloola trial, all 73 OP families with 2 - 4 trees per family were selected.

In addition, an existing dataset from a previous sampling of 447 mainland-population families with one tree per family for an association study at Green Hills (Dillon et al. 2010) was included in this study. The 447 mainland population families constituted 155, 210, and 82 from Año Nuevo, Monterey and Cambria, respectively. Dillon et al (2010) investigated allelic variation in cell-wall candidate genes affecting solid-wood properties. Their data included density, predicted MoE, and MfA derived from SilviScan® analysis. However, data were only available from 436 cores. This provided an additional core sample per family for almost all the families sampled in the Green Hills trial, and altogether 1678 cores were assessed by the SilviScan® instrument.

In summary, whole-core determinations of basic density (BD) were available at age 29 for Green Hills and Salicki trials and at age 27 for Billapalloola trial. SilviScan measured traits were considered on a cumulative basis weighted according to the sectional areas of individual rings. The cumulative trait for a given ring was calculated as the sum of single-ring measurements weighted by their individual-ring sectional area, up to and including that ring. The purpose of calculating cumulative traits was to simulate the measurement of a whole disc at a particular time. Data for a particular ring number from the pith will therefore represent the area-weighted average(s) of all rings up to that one. These cumulative measurements included area-weighted density (DEN), area-weighted modulus of elasticity (MoE), and area-weighted microfibril angle (MfA). Cumulative area-weighted means at ring 12 were considered to be the upper limit of the transition zone between corewood (following terminology by Burdon et al. (2004)) and outerwood (e.g. Gapare et al. 2006) and therefore represented corewood.

Table 7. Details of radiata pine provenance trials assessed for survival, growth and form traits

Trial name & location	No. families	No. Reps ²	Spacing (m)	Lat-S	Long-E	Altitude (m)	Rainfall (mm/yr)	Soil type/ parent material
Sherwood, ACT ¹	88	2	3 x 3	35°17'	148° 53'	700	700	granitic
Green Hills, New South Wales	467	7	3 x 2.5	35°57'	148° 08'	930	1300	granitic /diorite
Billapaloola, New South Wales	78	6	6 x 4	35°18'	148° 40'	700	1250	granitic /diorite
Salicki, Victoria	467	7	3 x 3	38°17'	147° 02'	30	590	loamy sand
Bong Bong, South Australia	305	5	2.75 x 2.1	38°03'	141° 03'	25	800	sandy rendzina
Longs, South Australia	426	5	2.75 x 2.1	37° 87'	140° 90'	40	700	sandy clay
Second Valley, South Australia	337	2	3 x 2.5	35°53'	138° 32'	330	875	lateritic podzolic sandy
Wirrabara, South Australia	251	2	2.9 x 2.6	33°05'	138° 18'	425	660	podzolic sandy

¹Australian Capital Territory; ²Replicates

Table 8. Total number of families sampled per provenance, sub-population and number of cores assessed using SilviScan® for radiata pine provenances

Site	Provenance	Total # families	families in sub-pop #1	families in sub-pop #2	families in sub-pop #3	families in sub-pop #4	families in sub-pop #5	families in sub-pop #6	Total # cores
Green Hills, NSW	Año Nuevo	50	13	13	12	12			138
	Monterey	50	9	9	8	8	8	8	139
	Cambria	50	17	17	16				138
	AD*	447							436
	Total	513							851
Salicki, VIC	Año Nuevo	52	13	13	13	13			202
	Monterey	54	9	9	9	9	9	9	204
	Cambria	55	19	19	17				220
	Total	161							626
Billapalloola, NSW	Guadalupe	42							116
	Cedros	31							85
	Total	73							201

AD* –Data from Dillon et al. (2010). Of the 447 mainland population families, 155 were from Año Nuevo, 210 from Monterey and 82 from Cambria. Guadalupe and Cedros collections were not classified into subpopulations.

Preliminary individual-trial mixed-model analyses were undertaken with the statistical software ASReml (Gilmour et al. 2009) to identify within-trial trends in spatial variation and neighbourhood effects (Gilmour *et al.* 1997; Costa e Silva *et al.* 2001; Dutkowski *et al.* 2006). Models with both design and spatial components were fitted to each data set: the design components, in which the environmental effects were modelled with only the experimental design features and an independent error, and the spatial components, in which an autoregressive spatial component is added to the design model, as recommended by Costa e Silva et al. (2001) and Dutkowski et al. (2002; 2006). The second analysis was carried out to estimate single-site variance components and genetic correlations between traits within a site. Results from single-trait analyses were used to obtain starting values for the single-site multivariate analyses. As we were interested in additive and provenance variances, both terms were considered as random in the model.

The third analysis was a bivariate, paired-site analysis of within-provenance additive genetic and among provenance effects to assess the magnitude of G×E interaction for a trait by estimating genetic correlations between pairs of sites. Results from single-site analyses were used to obtain starting values for the joint analyses. Both heterogeneous additive and error variances were included in the respective models.

Results

The detailed results were reported in the 12 confidential client technical reports. Only summary results are presented in the following 5 sections. Detailed results that were classified as not commercial-in-confidence are provided in the appendices. The respective appendices may be accessed via the following link:

<ftp://ftp.csiro.au/RadiataGenes/>

1. An annotated catalogue of Australian provenance trials and genetic conservation plantings of *Pinus radiata* D. Don of Californian origin, as at 1st July 2009

Details of previous catalogues including the latest catalogue as at 1st July 2009 were provided in a confidential technical report to all collaborators. There were a total of 50 provenance trials and block plantings in good standing, 16 of which were established between 2004 and 2007. All these trials and block plantings have at least 80% survival. All provenances are largely secure ensuring gene conservation of radiata pine germplasm for the next 50 to 60 years. The latest catalogue as at 1st July 2009 (Appendix 1) may be accessed via the link provided above.

2. Nucleotide diversity and population genetic structure in disjunct *Pinus radiata* D. Don populations: implications for *ex situ* gene conservation in Australia and New Zealand

Detailed literature review, results and discussion are provided in Appendix 2.

All populations had moderate levels of polymorphism, with mean expected heterozygosity (H_e) across all populations of 0.31 (Table 9). Population differentiation estimated with Wright's fixation index F_{ST} (Wright, 1951), was 0.09 ± 0.01 ($P < 0.05$), which reflected moderate genetic differentiation among all five populations. When mainland populations (Año Nuevo, Cambria and Monterey) were analysed separately, F_{ST} estimate was 0.07 ($P < 0.05$), close to that observed by Dillon et al (2010) ($F_{ST} = 0.04$). Similarly, when island populations (Guadalupe and Cedros) were analysed separately, F_{ST} estimate was 0.13 ($P < 0.05$). Jackknifing over populations revealed Cedros made the largest contribution to the overall estimate. Pair-wise F_{ST} estimate ranged from 0.02 to 0.06 (Table 9).

Table 9. Nucleotide diversity estimates in native radiata pine populations

Population	Classification	Nucleotide diversity (θ_w)	Confidence interval (95%) ^a	Expected heterozygosity (H_e) \pm s.e.
Año Nuevo	Mainland	0.0022	0.0017 – 0.0027	0.33
Monterey	Mainland	0.0028	0.0022 – 0.0035	0.37
Cambria	Mainland	0.0023	0.0016 – 0.0029	0.35
Mainland	Mean	0.0024	0.0016 - 0.0034	0.35 \pm 0.07
Guadalupe	Island	0.0003	0.00009 – 0.0008	0.24
Cedros	Island	0.0009	0.0003 – 0.0011	0.25
Island	Mean	0.0006	0.0002 – 0.0021	0.25
Overall		0.0002	0.00005 – 0.0023	0.31 \pm 0.05
Mean				

^a95% confidence interval for θ_w

The two island populations were both quite diverged from the mainland and from each other with pair-wise F_{ST} estimate of 0.21. The likely downward bias resulting from choice of SNPs for, among other things, being polymorphic in the mainland populations which could well explain the very low observed diversity statistics for the island populations (Table 4).

Inspection of the delta- K for models with a range of K values from 1 to 8 for the entire dataset revealed a distinct peak at $K = 2$ and another at $K = 5$ (Fig 7). The peak at $K = 2$ was stronger, indicating that the highest hierarchy of structure had two populations when all samples were considered. The results of simulations assuming more than five populations were highly unstable (Fig 7). The output from STRUCTURE, given as a graphical representation of genotypic profiles, showed that $K = 2$ differentiated mainland from island populations (Fig 8). The alternative structure model with $K = 5$ delineated groups that corresponded with sampling locations (Figs 1 & 8). However, the plot of cluster membership showed that Cambria and Monterey were very similar in their composition. Monterey and Cambria populations appear to comprise a mix of ancestral populations which could relate to sub-population structure. These findings are in contrast to Moran et al (1988) who found Año Nuevo and Monterey to be most similar to one another. Additionally, each geographic population had at least a small proportion of individuals assigned to every cluster (Fig 8). Assignment tests indicated that the majority of individuals had recent ancestry with only their predefined populations. Significant genetic divergence was not detected between stands within Monterey and Cambria. Divergence was detected between stands within Año Nuevo ($F_{ST} = 1.4\%$, $P = 0.004$), although this was not significant between stands 1 (Last Chance Road) and 2 (Swanton) (Dillon et al. in review). Detailed results are described in Appendix 2.

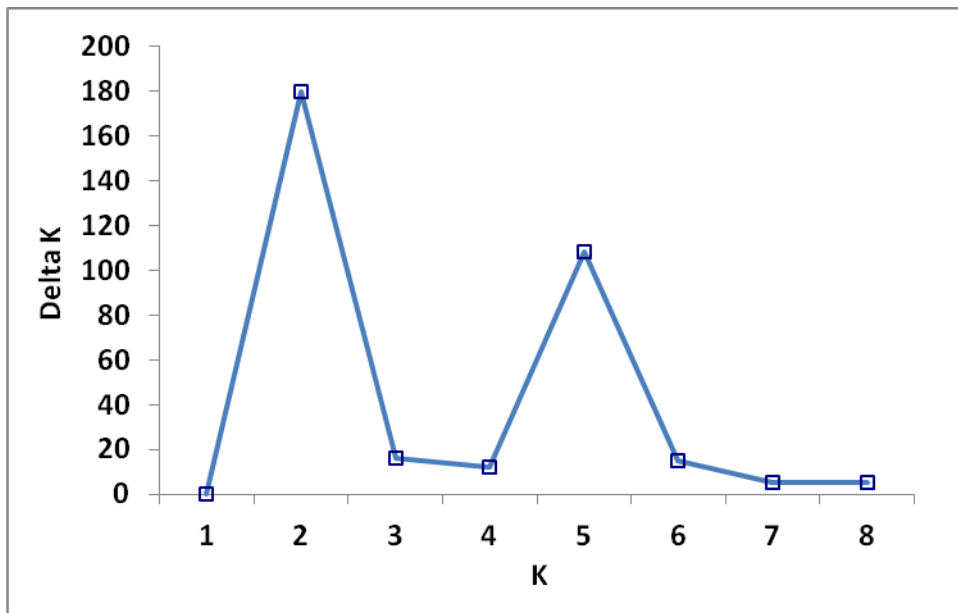


Fig 7. Magnitude of ΔK as a function of K (mean \pm SD over 10 replicates (except for $K = 1$ where only one run was performed) calculated using the procedure outlined in the methods. K is a model representing the most likely number of clusters, while delta K is the optimal probability of the data for a given K .

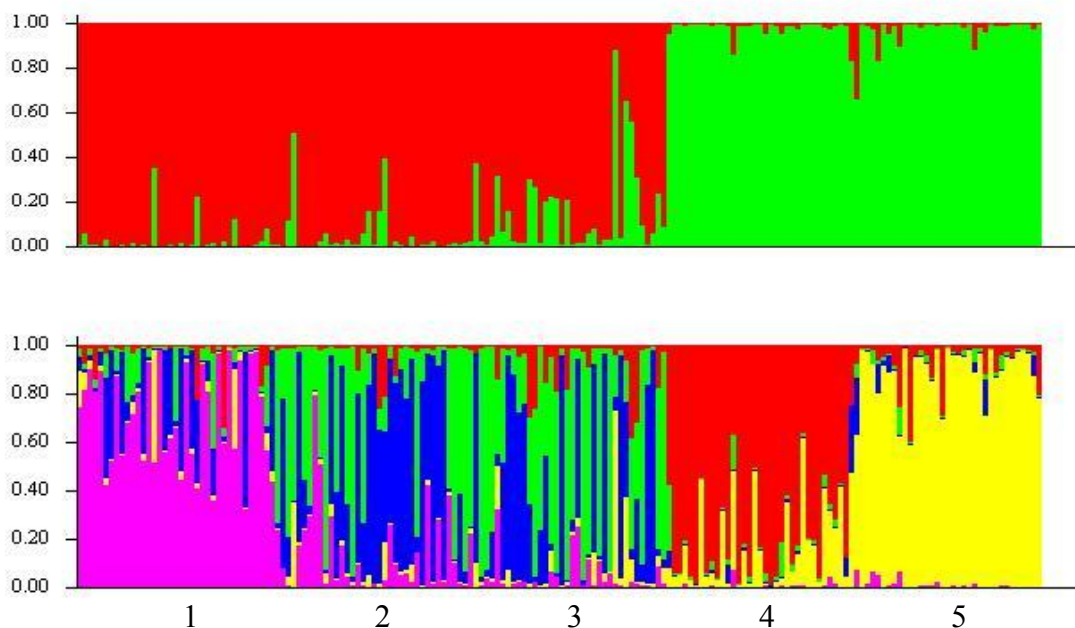


Fig 8. Graphical representation of the genotypic profiles based on $K = 2$ (top) and $K = 5$ (bottom) population clusters. The different colours represent ancestry from the hypothetical genetic populations. 1 = Ano Nuevo; 2 = Monterey; 3 = Cambria; 4 = Guadalupe; 5 = Cedros

Results from molecular data and previous juvenile wood initiative (JWI) studies were useful in guiding our conservation thinking and strategy for radiata pine. The distinctiveness of two island populations (higher F_{ST}) from mainland populations, combined with the fact that no genotypes from island populations were used in current breeding population indicate these two populations need to be conserved and utilised for infusion into breeding population.

There has been some effort on this through hybridisation of Guadalupe crosses with mainland populations (Low and Smith 1997). Lower genetic diversity of two island populations meant that the conservation scale may not be as extensive as for mainland populations. A study using 149 SNP markers from 38 candidate genes to understand adaptive genetic variation in radiata pine for mainland populations is ongoing (Dillon et al. in review). Potentially adaptive genetic variation from ten candidate genes indicated there was some adaptive structure to water availability of different sites for the three mainland California populations (Dillon et al. in review). Further examination from all five populations including island populations or examination of diversity variation among various functional genes (for example, water resistance and cell-wall wood quality gene) relative to neutral genes will shade more light on adaptive genetic variation in radiata pine. With genome sequencing of radiata pine, there will be more information available to detect selective sweeps and adaptive genetic variation.

3. Collection of open-pollinated (OP) cones and extraction of seed for long-term storage and for re-establishment of second-generation *ex situ* conservation blocks

Details on the amounts (weights) of seed collected and kept by individual family identities are provided in Appendix 3.

Pollen was collected from at least 72 plus trees from different provenances (Table 10). The plus trees were selected based on an integrated index DATAPLAN® and TREEPLAN®. The pollen was tested for viability and generally germination/viability tests exceeded 70%. Cones were collected at five sites (Green Hills, Tantanoola, Paramount, Kaingaroa and Rotoehu) and seeds were extracted. Cones were collected from 992 trees from five provenances (Table 11). No cones were available from any Cedros trees. Details of cone collection sites (latitude, longitude, elevation, rainfall) and number of trees from which cones were collected were included in a database stored in the CSIRO Australian Tree Seed Centre records. Scions were collected from 210 trees and grafted at the radiata pine National Germplasm Resource Centre (NGRC) in Mount Gambier (Table 12; Fig 9). The crossings will be carried out to create relatively “pure” provenance to maximise genetic diversity for current and future environments. Cones and seeds were collected at sites in New Zealand and Australia. Seed was kept by family identity and packed for long-term cold storage. Sub-provenance classification was disregarded in light of field trial results (Burdon et al. 1992).

Table 10. Number of genotypes per provenance from which pollen was collected and attained > 70% viability

Provenance	#genotypes from which pollen was collected
Año Nuevo	19
Monterey	16
Cambria	16
Guadalupe	5
Cedros	16
Total	72

Table 11. Number of trees per provenance from which cones were collected

Provenance	#trees collected
Año Nuevo	284
Monterey	180
Cambria	133
Guadalupe	395
Cedros	-
Total	992

Table 12. Number of scions collected for each provenance at various provenance trials

Source/Trial	Año Nuevo	Monterey	Cambria	Guadalupe	Cedros	Total
Green Hills	37	33	27	-	-	97
Salicki	8	13	21	-	-	42
Wirrabara		1	2	7	10	20
Second Valley	4	2				6
Bong Bong	1	1				2
Billapalloola	-	-	-	23	20	43
Total	50	50	50	30	30	210



Fig 9. Scions collected from Guadalupe selections and grafted for subsequent controlled crosses in the NGRC arboreta in 2010

4. Performance differences among *ex-situ* native-provenance collections of *Pinus radiata* D. Don.: growth, form and wood property traits

Detailed description of performance differences and genetic variation among *ex-situ* native provenances are provided in Appendices 4 and 5.

The provenance of origin for these trials at least on the female side is maintained, but on the male side could be subject to some contamination from surrounding plantings, but is mainly assumed to be of the same provenance. The open-pollinated seed collected from these *ex situ* plantings would be effectively the same generation/ type as that which has been collected during this study for the conservation plantings, thus are effectively an advanced insight into the genetic architecture of the next generation of conservation plantings.

Growth and form traits from a series of three provenance trials of *Pinus radiata* D. Don planted in New Zealand and Australia were analysed at age 9 years from planting for growth and form traits and at 14 years from planting for wood density. Monterey and Cambria performed better than Año Nuevo at two New Zealand sites but Monterey and Año Nuevo were almost identical in growth, whereas Cambria grew less vigorously at the Australian site (FR259_3) (Fig 10). We detected significant provenance differences for DBH growth and stem straightness across countries ($P < 0.001$) (Figs 10 & 11, respectively and Table 13). Monterey was significantly different from Año Nuevo and Cambria at Kaingaroa ($P < 0.05$), for wood density and had slightly higher density, whereas all provenances were almost identical and not significantly different at Kangaroovale. However, there were significant differences for wood density at family level for Año Nuevo and Cambria at Kangaroovale.

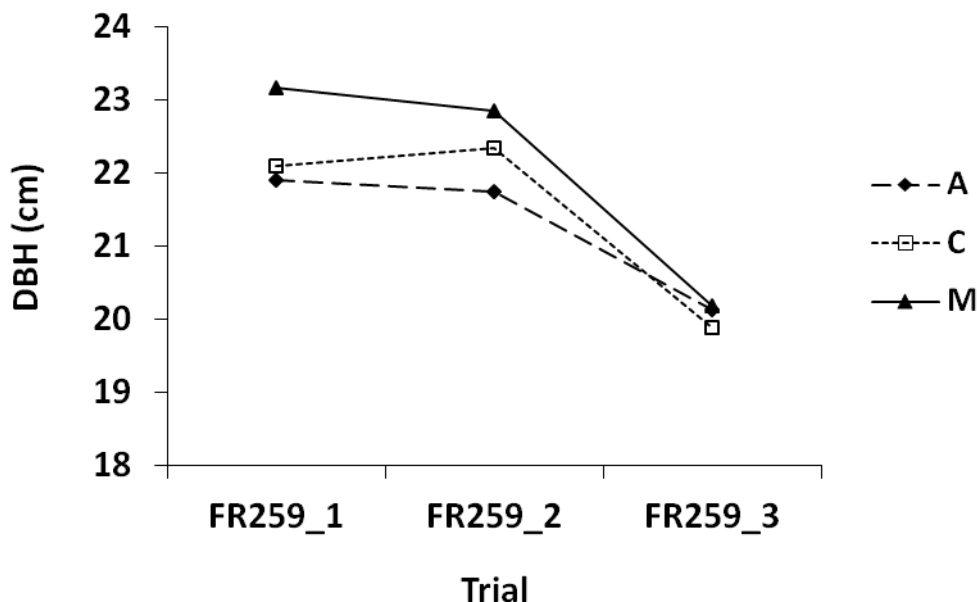


Fig 10. Trial, Provenance and Trial x Provenance effects for diameter growth (DBH). A = Año Nuevo; C = Cambria; M = Monterey; FR259_1 =Kaingaroa; FR259_2 = Hauhungaroa; FR259_3 = Kangaroovale. Trial significant ($p < 0.001$), Provenance significant ($p < 0.001$), trial x provenance significant ($p < 0.001$).

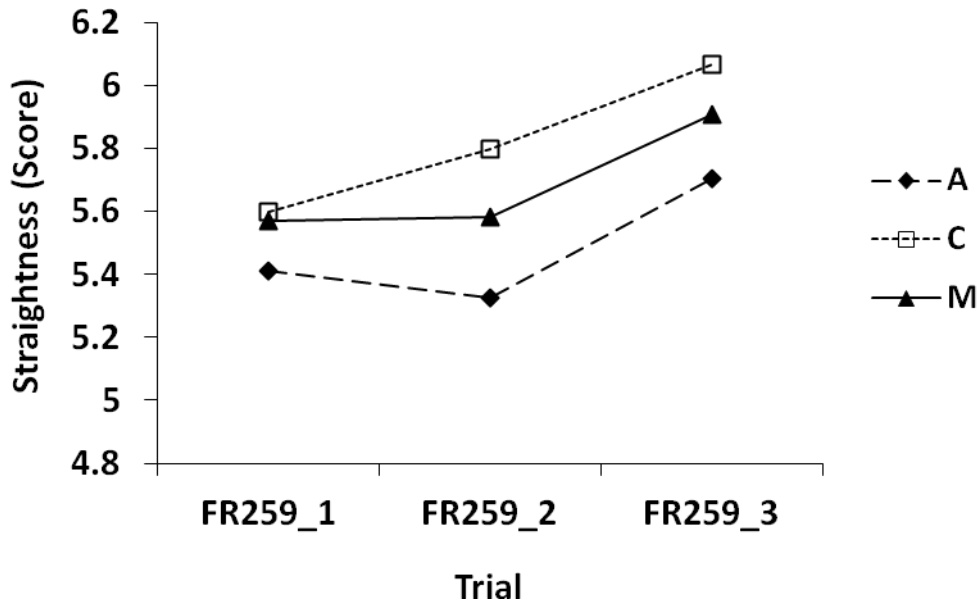


Fig 11. Trial, Provenance and Trial x Provenance effects for stem straightness (STR), A = Año Nuevo; C = Cambria; M = Monterey; FR259_1 = Kaingaroa; FR259_2 = Hauhungaroa; FR259_3 = Kangaroovale; Trial significant ($p < 0.001$), Provenance significant ($p < 0.001$), trial x provenance not significant ($p = 0.37$)

Table 13. Provenance averages for wood density and core length and associated standard errors for radiata pine. The same letter across a row indicates no significant difference between means at $p \leq 0.05$ (experiment-wise error)

Trial/Trait	Año Nuevo	Monterey	Cambria
FR259/1 (Kaingaroa)			
Wood density (kg/m^3)	364.9 ± 1.6^b	368.9 ± 1.4^a	362.4 ± 2.1^b
FR259/3 (Kangaroovale)			
Wood density (kg/m^3)	357.2 ± 1.2^a	355.6 ± 1.4^a	357.0 ± 1.6^a
Across sites			
Wood density (kg/m^3)	360.9 ± 1.6^a	362.1 ± 1.0^a	359.4 ± 1.3^a

Estimated heritability for DBH ranged from 0.19 to 0.26 within sites, while heritability estimates for stem straightness and branching frequency ranged from 0.10 to 0.24 (Table 14). Estimated type-B (between-sites) genetic correlations for DBH were always higher between the two trials in New Zealand trials than between pairs of trials in New Zealand and the Australian site. The estimated additive genetic coefficient of variation (CV_A) for DBH was around 8-10% compared to ca 5% for the current breeding population.

The estimates of heritability for wood density were all above 0.50 and generally higher at Kaingaroa than at Kangaroovale (Table 15). Estimates of additive genetic correlations between wood density and core length were imprecise. Genotype \times site interactions for density appeared minor (estimated type B genetic correlation = 0.70) despite substantial differences in rainfall and soils (see Table 6). The similarity of Cambria to Año Nuevo for density is an interesting result because the genetic base of the present Australian and New Zealand plantations has been shown to be from Año Nuevo and Monterey. Infusion of Cambria material would increase the overall genetic base of the radiata pine breeding programs, with potential long-term benefits, despite the often disappointing growth performance of material collected from Cambria.

Table 14. Mean single-site and pooled-site estimates of within-population heritability and associated standard errors, and of coefficients of additive genetic variation (CV_A) for the three trials.

Trial	Trait	Heritability	CV_A (%)
Kaingaroa	Diameter at breast height (DBH)	0.23 \pm 0.05	10.1
	Stem straightness (STR)	0.10 \pm 0.03	8.5
	Branch frequency (BRFQ)	0.10 \pm 0.03	8.4
	Malformation (MAL)	0.10 \pm 0.03	13.2
	Dothistroma needle blight (DOT)	0.41 \pm 0.07	13.0
Hauhungaroa	Diameter at breast height (DBH)	0.19 \pm 0.04	7.4
	Stem straightness (STR)	0.23 \pm 0.04	15.0
	Branch frequency (BRFQ)	0.22 \pm 0.04	14.6
	Malformation (MAL)	0.07 \pm 0.03	14.9
Kangaroovale	Diameter at breast height (DBH)	0.26 \pm 0.05	5.6
	Stem straightness (STR)	0.25 \pm 0.05	14.9
	Branch frequency (BRFQ)	0.26 \pm 0.05	20.5
	Malformation (MAL)	0.08 \pm 0.03	12.4
Pooled-site	Diameter at breast height (DBH)	0.10 \pm 0.02	
	Stem straightness (STR)	0.10 \pm 0.02	
	Branch frequency (BRFQ)	0.14 \pm 0.03	
	Malformation (MAL)	0.01 \pm 0.01	

Table 15. Estimates of genetic parameters for wood density from sib-analysis by provenances, assuming half-sib families

Trial/Provenance	No. of families	Among-family variance ($\hat{\sigma}_{fam}^2$)	Narrow-sense heritability (\hat{h}^2)	Phenotypic variance ($\hat{\sigma}_P^2$)	CV_A (%)
FR259/1 (Kaingaroa)					
Año Nuevo	37	112.17**	0.89 ± 0.17	392.10	5.8
Monterey	57	56.92*	0.51 ± 0.25	447.48	10.7
Cambria	34	112.12*	0.86 ± 0.19	518.25	5.7
Pooled	128	95.68***	0.83 ± 0.18	460.68	5.4
FR259/3 (Kangaroovale)					
Año Nuevo	37	65.42*	0.56 ± 0.25	465.43	4.5
Monterey	57	43.48 ^{ns}	0.51 ± 0.36	369.20	3.4
Cambria	34	46.18*	0.56 ± 0.22	331.55	3.8
Pooled	128	57.38***	0.57 ± 0.16	402.68	4.2
Across-sites	128	44.80*	0.43 ± 0.13	422.19	3.7

*P<0.05; **P<0.01; ***P<0.001; ^{ns}not significant; significance levels based on one-tailed LR tests that were used to test the departure of σ_{fam}^2 from zero; Statistical significance for estimated heritability is same as for additive genetic variance.

In our study, six unrelated families from Monterey, one from Cambria and one from Año Nuevo ranked above a control seedlot rated at GF14 (Fig 12). Two additional families from Monterey ranked just below GF14. The best individual trees from those 10 unrelated families would be potential candidates for infusion in line with the current breeding strategy. However, consideration should also be given to unrelated families from Cambria that ranked above GF6 (Fig 12). Cambria is unique because the genetic base of the present Australian and New Zealand plantations is almost if not entirely derived from Año Nuevo and Monterey.

These results suggested that there is appreciable genetic variation in the native populations and infusion of these materials would increase the genetic variation in current breeding populations. The promising performance of the Cambria material is an important result because Cambria is believed not to have contributed to the genetic base of the present Australian and New Zealand plantations.

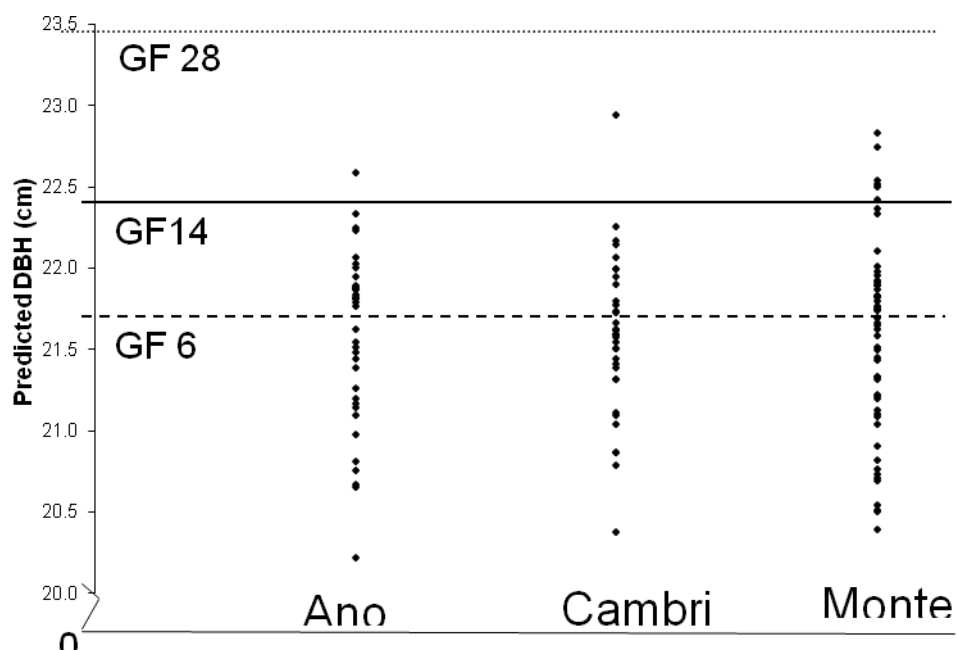


Fig 12. Benchmarking of across-sites family performances relative to the genetic controls (GF6 = unimproved selections; GF14 = older open-pollinated seed orchard stock; GF28 = current breeding stock)

5. Quantitative genetics of growth, form and wood property traits of radiata pine ‘Eldridge collection’ in Australia

Genetic material from eight of the trials in Australia were from effectively unselected germplasm collected from the wild and the results best reflect the genetic variation in the native population and the fact that females can be assumed unrelated probably provide some of the best estimates of genetic parameters due to the large number of founder parents (506).

Detailed description of results and discussion on estimation of genetic parameters of the native populations is presented in Appendices 6 and 7.

Genetic parameters (heritability, additive variance and covariance and genetic correlations) and breeding values are critical population parameters to understand genetic control of growth, form and wood quality traits in order to make decisions about possible infusion of new germplasm as a way of increasing the effective population size of radiata pine breeding populations in Australia and New Zealand. In this project, genetic parameters for growth, form, wood density, microfibril angle (MfA), and modulus of elasticity (MoE) (stiffness) were estimated using progeny of the 1978 ‘Eldridge collection’. These included (1) estimation of heritabilities and genetic correlations diameter at breast height (DBH), stem straightness, branch size, branching angle, wood density, MfA, and MoE; (2) study of genotype by environment interaction for all traits; (3) prediction of breeding values based on an integrated index for growth, form and wood property traits and selection of the best individuals within best families for possible infusion into breeding populations.

Early survival of all provenances was around 90%, except for Cedros (<60%). Monterey and Año Nuevo were the best performers for growth at almost all sites (Table 16). However, good growth performance of Cambria and good stem straightness of Guadalupe on some sites are important results, because the genetic base of the present Australian plantations evidently originated from only Monterey and Año Nuevo.

The control seedlots significantly outperformed all provenances at almost all sites for DBH at juvenile and mature ages. For example, the TSO and GSO seedlots (first-generation controls) were 18% and 11% better than the best provenance at Green Hills and Salicki, respectively. Similarly, a second-generation SAG2 seedlot that was included at Second Valley and Wirrabara was 11% better than Monterey and Año Nuevo. Two improved seedlots at Longs were 5% superior to all provenances. An unimproved control seedlot, MTBURR was only 5% superior to the best provenance, Año Nuevo at Bong Bong. The control lot (Guadalupe-Australia land race (GA)) at Sherwood was also 11% better than the best provenance (Cambria) and 26% better than Guadalupe.

The average estimated single-site heritability for diameter at breast height was 0.22 and 0.32 at juvenile and mature ages, respectively. Heritability estimates for stem straightness and branching ranged from 0.23 to 0.55. Heritability estimates of density, predicted MoE and MfA were significant and $\bar{h}^2 > 0.45$, suggesting moderate to strong genetic control (Table 17). The estimated genetic correlations between DBH and wood properties were weaker (less negative) than the mean estimated from the current generation in radiata pine. Of the wood properties, density had the strongest negative correlations with DBH. Selection for MoE may

produce greater gain than selection for density because MoE had almost twice \widehat{CV}_A compared to density. Genetic correlation estimates between DBH at juvenile and rotation ages were all > 0.80 .

Table 16. Percent survival and means for growth and stem form traits for provenances. The same letter within a sub-column indicates no significant difference between means at $p \leq 0.05$ (experiment-wise error)

Site	Provenance	‡Survival (%)	HT_03 (m)	HT_09 (m)	DBH_08 (cm)	DBH_12 (cm)	DBH_16 (cm)	DBH_24 (cm)	DBH_26 (cm)	NOD_26 (1-4)	STR_08 (1-6)	BRA_08 (1-6)	BRS_08 (1-6)
Sherwood, ACT	Año Nuevo	92 a	-	-	15.5 c	-	24.5 b	-	-	-	3.3 a	3.4 b	3.5 a
	Monterey	93 a	-	-	15.1 c	-	24.5 b	-	-	-	3.4 a	3.4 b	3.6 a
	Cambria	80 b	-	-	16.5 b	-	24.3 b	-	-	-	3.1 a	3.4 b	3.1 b
	Guadalupe	83 b	-	-	13.7 d	-	23.9 b	-	-	-	3.3 a	3.6 a	3.7 a
	Cedros	60 c	-	-	14.3 d	-	24.3 b	-	-	-	3.2 a	3.6a	3.5 a
	Control	84 b	-	-	18.5 a	-	27.8 a	-	-	-	3.5 a	2.9 c	2.3 c
Green Hills, NSW	Año Nuevo	95 a	1.6 a	8.5 a	12.0 b	-	-	-	30.2 b	2.6 a	3.6 a†	2.1 a*	-
	Monterey	96 a	1.6 a	8.4 a	12.0 b	-	-	-	30.1 b	2.7 a	3.6 a†	2.1 a*	-
	Cambria	95 a	1.6 a	8.5a	12.0 b	-	-	-	25.1 c	2.3 a	3.6 a†	2.1 a*	-
	Guadalupe	91 b	1.0 a	6.6 b	7.4 c	-	-	-	-	-	-	-	-
	Cedros	59 c	0.8 a	4.4 c	5.3 c	-	-	-	-	-	-	-	-
	Control	96 a	1.8 a	9.2 a	13.7 a	-	-	-	37.3 a	3.3 a	-	-	-
Billapaloola, NSW	Guadalupe	41 a	-	-	-	-	-	-	27.1 a	2.4 a	3.4 a†	1.9 a*	2.5 a**
	Cedros	36 a	-	-	-	-	-	-	28.4 a	2.2 a	3.1 a†	1.9 a*	2.3 a**
Salicki, VIC	Año Nuevo	44 bb	-	-	-	21.3 bc	-	-	34.5 bc	-	3.6 b††	-	-
	Monterey	48 bb	-	-	-	22.4 b	-	-	36.0 b	-	3.4 b††	-	-
	Cambria	54 ba	-	-	-	21.2 c	-	-	33.1 c	-	3.4 b††	-	-
	Guadalupe	55 ba	-	-	-	16.0 e	-	-	28.1 d	-	3.9 a††	-	-
	Cedros	26 bc	-	-	-	18.0 d	-	-	27.6 d	-	3.4 b††	-	-
	Control	38 bc	-	-	-	24.5 a	-	-	40.3 a	-	4.2 a††	-	-
Bong Bong, SA	Año Nuevo	-	-	-	-	-	-	-	32.3 ab§	-	4.4 a†††	-	-
	Monterey	-	-	-	-	-	-	-	31.5 b§	-	4.3 a†††	-	-
	Cambria	-	-	-	-	-	-	-	31.3 b§	-	4.5 a†††	-	-
	Guadalupe	-	-	-	-	-	-	-	27.9 c§	-	4.7 a†††	-	-
	Control	-	-	-	-	-	-	-	34.7 a§	-	4.8 a†††	-	-
	Control	-	-	-	-	-	-	-	28.9 b§	-	4.7 a†††	-	-
	Control	-	-	-	-	-	-	-	34.0 a§	-	4.8 a†††	-	-
Longs, SA	Año Nuevo	-	-	-	-	-	-	-	34.9 ab	-	3.7b†††	-	-
	Monterey	-	-	-	-	-	-	-	35.5 ab	-	3.8b†††	-	-

	Cambria	-	-	-	-	-	-	-	35.5 ab	-	4.1 a†††	-	-
	Guadalupe	-	-	-	-	-	-	-	31.2 c	-	4.6 a†††	-	-
	Cedros	-	-	-	-	-	-	-	27.0 d	-	3.4 b†††	-	-
	Control	-	-	-	-	-	-	-	37.3 a	-	4.6 a†††	-	-
	Control	-	-	-	-	-	-	-	34.4 ab	-	4.3 a†††	-	-
Second Valley, SA	Año Nuevo	92 a	-	-	19.1 b	-	28.3 b	36.5 b	-	-	-	-	-
	Monterey	94 a	-	-	19.4 b	-	28.3 b	36.4 b	-	-	-	-	-
	Cambria	96 a	-	-	18.9 b	-	27.3 b	34.9 b	-	-	-	-	-
	Control	93 a	-	-	21.0 a	-	32.0 a	41.3 a	-	-	-	-	-
	Control	90 a	-	-	19.8 b	-	30.8 a	39.7 a	-	-	-	-	-
Wirrabara, SA	Año Nuevo	81 a	-	-	13.4 c	-	22.6 b	25.8 b	-	-	-	-	-
	Monterey	91 a	-	-	15.2 a	-	24.4 b	27.9 b	-	-	-	-	-
	Cambria	89 a	-	-	14.4 b	-	22.9 b	26.4 b	-	-	-	-	-
	Guadalupe	86 a	-	-	13.1 c	-	21.5 b	24.6 b	-	-	-	-	-
	Cedros	80 b	-	-	13.6 c	-	22.9 b	26.5 b	-	-	-	-	-
	Control	93 a	-	-	15.3 a	-	26.3 a	29.2 a	-	-	-	-	-

†survival assessed at juvenile ages; †STR_26; ††STR_29; †††STR_30; §DBH_30

*BRA_08 scored on a scale 1-3; **BRS_08 scored on a scale 1-4

Table 17. Estimates of variances (σ_A^2 , σ_P^2) for additive and phenotypic variances, respectively, heritabilities (h^2) \pm approximate standard errors, genetic coefficient of variation (\widehat{CV}_A %), type-B genetic correlations (r_B) \pm approximate standard errors (s.e) and type-B among-provenance (r_{BPR}) correlations for growth, stem and wood quality traits from joint-site analyses (Green Hills and Salicki). Numbers in the trait names are denoting age or ring number of cumulative expression

Traits analysed	$\hat{\sigma}_A^2$	$\hat{\sigma}_P^2$	$\hat{h}^2 \pm \text{s.e}$	\widehat{CV}_A (%)	$r_B \pm \text{s.e}$	r_{BPR}
DBH_12	5.93***	35.98	0.16 \pm 0.02	15.9	0.57 \pm 0.09**	0.87 ^{ns}
DBH_29	15.1	117.1	0.13 \pm 0.03	NE	0.69 \pm 0.15**	0.69*
BD_29	404.98***	814.09	0.50 \pm 0.07	6.9	0.92 \pm 0.12 ^{ns}	0.68*
DEN_12	2006.5***	2394.6	0.59 \pm 0.09	9.0	0.84 \pm 0.14 ^{ns}	0.90 ^{ns}
DEN_29	1019**	1588.3	0.64 \pm 0.12	8.2	0.89 \pm 0.11 ^{ns}	0.70*
MoE_12	1.45*	2.78	0.52 \pm 0.08	15.2	0.92 \pm 0.13 ^{ns}	0.40*
MoE_29	2.05**	4.34	0.47 \pm 0.11	14.1	0.95 \pm 0.12 ^{ns}	#
MfA_12	27.57*	44.14	0.62 \pm 0.09	19.0	0.87 \pm 0.13 ^{ns}	0.42*
MfA_29	13.47*	20.42	0.49 \pm 0.11	17.6	0.92 \pm 0.10 ^{ns}	0.51*
STR_29	0.50***	1.022	0.49 \pm 0.04	20.1	0.90 \pm 0.19 ^{ns}	0.95 ^{ns}

*P<0.05; **P<0.01; ***P<0.001; ^{ns}not significant, #failed to converge; NE not estimated; significance levels for σ_A^2 , r_B and r_{GPR} were based on one-tailed LR tests that were used to test the departure of σ_A^2 from zero and r_B and r_{BPR} from +1. Approximate standard errors tend towards zero as the point estimates tend toward 1. Statistical significance for estimated heritability is same as for additive genetic variance.

Estimates of between-site genetic and provenance correlations for DBH were often low, indicating high genotype by environment interaction across trials, consistent with previous Australian studies. Trial sites were then grouped into three classes, based on environmental variables, rainfall and altitude (Fig 13). The classes included high-altitude (Green Hills, Sherwood and Billapalloola), low-altitude (Salicki, Longs and Bong Bong) and dry sites (Wirrabara and Second Valley). The differences among inter-site genetic correlation estimates among and within classes were tested using ANOVA. The objective was to investigate the general pattern of resemblance among sites in order to identify possible key drivers for G×E interactions. For this, DBH was used, which was deemed the most informative trait. DBH was also measured in each trial over the longest time span (8 to 30 years).

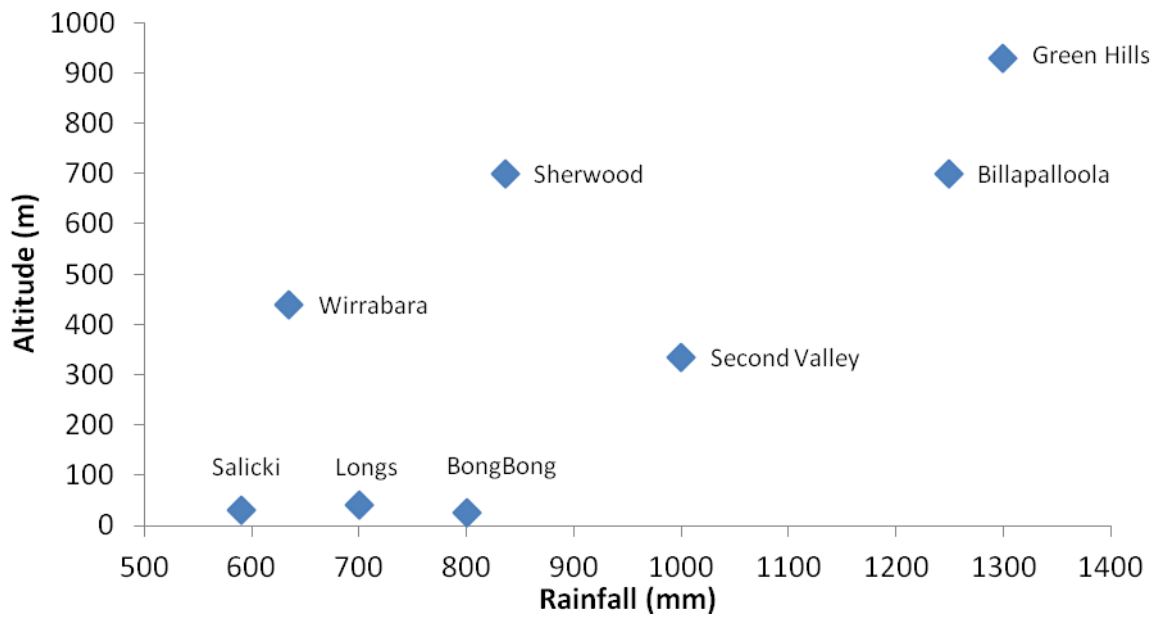


Fig 13. Plot of trial sites based on rainfall and altitude. Trial sites were grouped into three classes: high-altitude (Green Hills, Sherwood and Billapalloola), low-altitude (Salicki, Longs and Bong Bong), and dry sites (Wirrabara and Second Valley).

High-altitude sites had also high rainfall, while low-altitude sites had low rainfall. There was minimal G×E between trials located in New South Wales including Sherwood in the Australian Capital Territory on high-altitude (>700 m), and high-rainfall (>800 mm/annum) sites. Similarly, low-altitude (<700 m), low-rainfall (<800 mm/annum) sites (Salicki, Bong Bong, and Wirrabara) formed a group on their own based on additive and provenance inter-site correlations (Tables 18 & 19). This grouping is consistent with the first observation that higher-altitude, higher-rainfall sites were distinctly different from lower-altitude, the warmer, and drier sites in other States (Wu and Matheson 2005) and a recent study by Raymond (2011) within New South Wales sites. Baltunis et al. (2010) also reported evidence of genotype by environment interaction (G×E) between radiata pine trials in Tasmania and mainland Australia. The trials sites were distinctly different in terms altitude, rainfall and soil types.

Table 18. Estimated within-provenance additive genetic (r_G) \pm approximate standard errors (above diagonal) and provenance (r_{GPR}) (below diagonal) correlations for tree diameter at juvenile age (DBH_J) (assessment age in parentheses) between pairs of provenance trials

	Sherwood	Green Hills	Salicki	Second Valley
Sherwood (age 8 yrs)		0.97 ± 0.03^{ns}	$0.65 \pm 0.13^{***}$	$0.51 \pm 0.10^*$
Green Hills (age 9 yrs)	0.75^{**}		$0.57 \pm 0.09^{**}$	$0.60 \pm 0.11^{***}$
Salicki (age 12 yrs)	0.67^{***}	0.87^{ns}		0.91 ± 0.07^{ns}
Second Valley (age 9 yrs)	0.60^{***}	0.72^{**}	0.93^{ns}	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ^{ns}not significant. Significance levels based on one-tailed LR tests that were used to test the departure of r_G or r_{GPR} from +1.

Table 19. Estimated within-provenance additive genetic (r_G) correlations (type-B) \pm approximate standard errors for tree diameter at mature age (DBH_M) (assessment age in parentheses) between pairs of provenance trials

	Green Hills	Salicki	Bong Bong	Second Valley	Wirrabara
Sherwood (age 16 yrs)	0.84 ± 0.09^{ns}	$0.62 \pm 0.09^{**}$	$0.69 \pm 0.12^{**}$	$0.53 \pm 0.19^{**}$	$0.38 \pm 0.27^{**}$
Green Hills (age 26 yrs)		$0.69 \pm 0.15^{**}$	$0.19 \pm 0.28^{***}$	$0.59 \pm 0.27^{**}$	$0.29 \pm 0.51^{**}$
Salicki (age 29 yrs)			0.87 ± 0.12^{ns}	0.93 ± 0.05^{ns}	$0.57 \pm 0.34^{**}$
Bong Bong (age 30 yrs)				0.90 ± 0.03^{ns}	0.93 ± 0.10^{ns}
Second Valley (age 24 yrs)					0.95 ± 0.09^{ns}

** $P < 0.01$; *** $P < 0.001$; ^{ns}not significant. Significance levels based on one-tailed LR tests that were used to test the departure of r_G from +1.

Monterey and Año Nuevo had higher density and modulus of elasticity (at one site) than the Cambria provenance. At the site containing Guadalupe and Cedros provenances, basic density and modulus of elasticity (MoE) were 20% higher than at the site containing mainland provenances (Figs 14 & 15), but whatever the real difference, it was confounded with any site differences and therefore the results would need to be interpreted with caution. However, control seedlots were not sampled for the wood traits and therefore we could not make comparisons with the provenances.

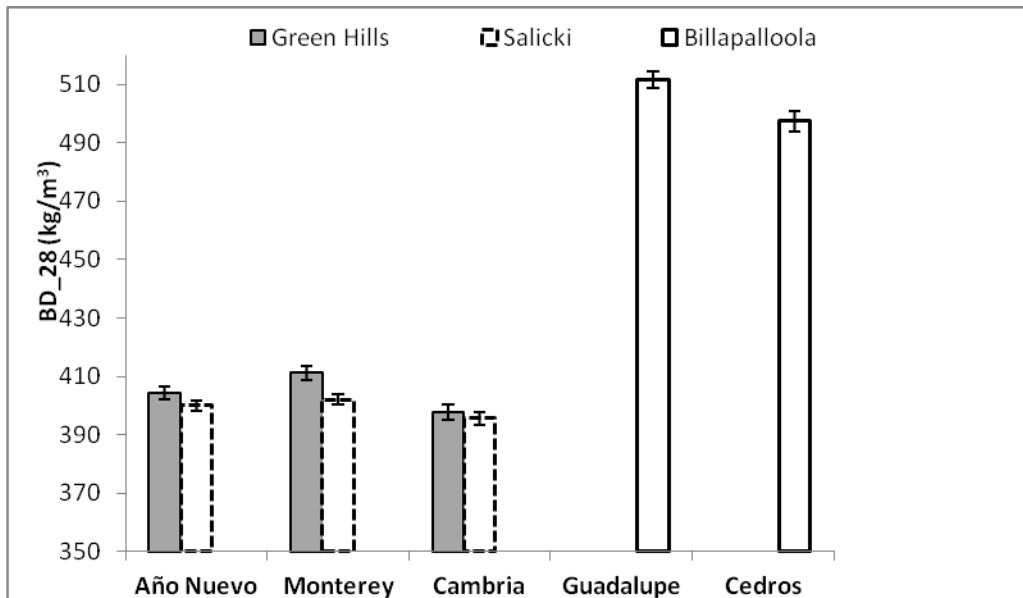


Fig 14. Mean whole-core basic density (BD) of each provenance at Green Hills, Salicki and Billapalloola. Vertical bars at tops of the histogram plots denote standard errors

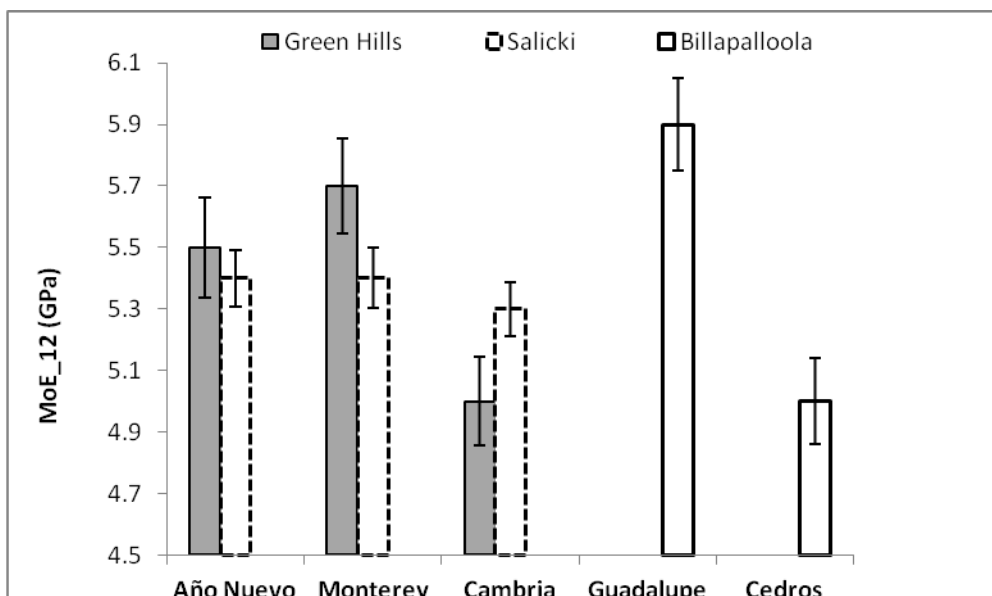


Fig 15. Mean cumulative area-weighted MoE at ring 12 (MoE_12) for each provenance at Green Hills, Salicki and Billapalloola. Vertical bars at tops of the histogram plots denote standard errors

Cumulative area-weighted pith-to-bark trajectories for mainland provenances showed that Monterey was consistently the highest for DEN, reaching 546 kg/m^3 at ring 27 and Año Nuevo the lowest, reaching at 531.2 kg/m^3 at Green Hills. However, Cambria was the lowest in the corewood zone but exceed Año Nuevo towards ring 27. For example, cumulative area-weighted density was 471.9 kg/m^3 at ring 12 for Cambria and 481.8 kg/m^3 for Año Nuevo. Monterey was consistently the highest for DEN and Cambria, the lowest at Salicki. Differences in cumulative area-weighted pith-to-bark trajectories for MfA among mainland provenances were not large but Cambria was initially the highest in rings close to the pith but Año Nuevo was marginally the highest at ring 27 at both sites. For predicted MoE, cumulative area-weighted pith-to-bark trajectories showed that Año Nuevo was slightly lower than Monterey and Cambria in the outerwood, presumably reflecting a combination of provenance differences for DEN and MfA. Cumulative area-weighted pith-to-bark trajectories for island provenances diverged steadily after about ring 5 (ring 8 for MfA), e.g. 600 kg/m^3 vs 560 kg/m^3 for DEN, 24.2° vs 20.8° for MfA, and 11.7 GPa vs 8.6 GPa for predicted MoE, for Guadalupe and Cedros, respectively, at ring 25.

Average cumulative SilviScan density (DEN) at successive rings in the radial cores at three sites for mainland provenances is presented in Fig 16. Average DEN for mainland provenances at Green Hills was found to rise from 457 kg/m^3 at rings 7 to 483 kg/m^3 at ring 12, before rising steadily to above 530 kg/m^3 in the outer rings. At Salicki, the average DEN for mainland provenances rose from about 451 kg/m^3 in ring 7 to 465 kg/m^3 in ring 12, and then rose steadily to above 512 kg/m^3 in the outer rings. Average DEN for the island provenances at Billapaloola was found to rise from 513 kg/m^3 in ring 7 to about 530 kg/m^3 in ring 12 and rose steadily to above 550 kg/m^3 in the outer rings.

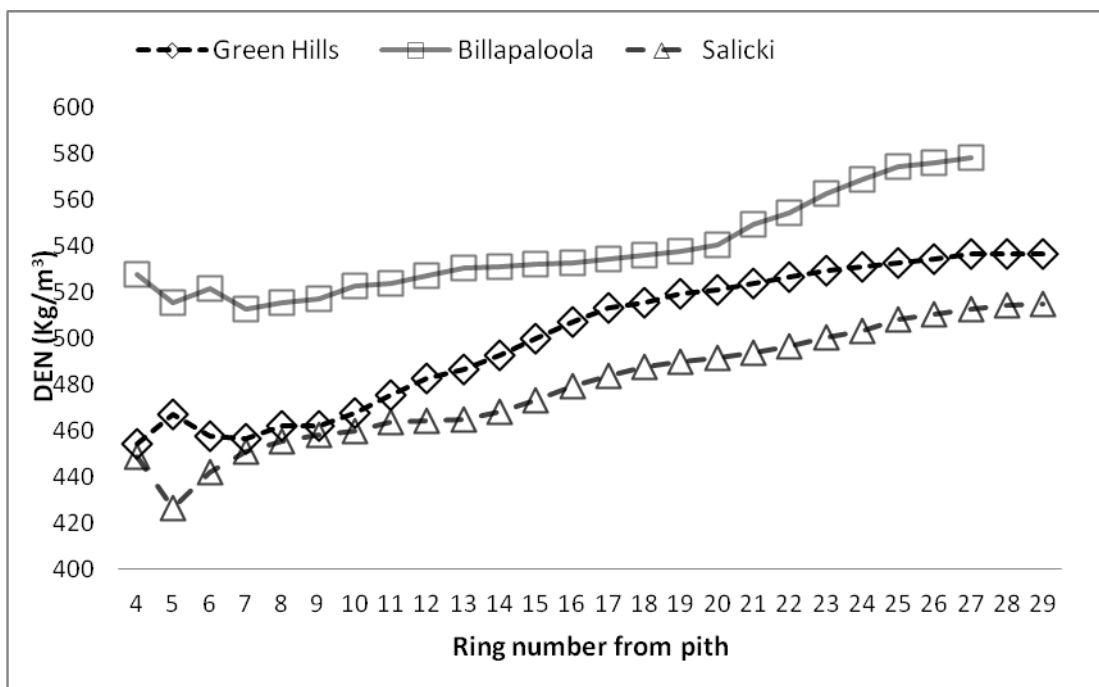


Fig 16. Cumulative ring-by-ring means (weighted by sectional areas of rings) for SilviScan density (DEN), from single pith-to-bark cores taken at breast height, from trees in Green Hills, Billapaloola and Salicki. Approximate average standard error of the ring means for Green Hills and Salicki is ± 17.1 and that for Billapaloola is ± 38

Mean predicted MoE for mainland provenances at Green Hills and Salicki was about 2.9 to 3.3 GPa close to the pith, then began to rise with successive rings to a maximum of approximately 10 to 12 GPa close to the bark (Fig 17). Mean predicted MoE for island provenances rose from 4.5 to 6.3 GPa between rings 5 and 12 and increased to ~ 10.4 GPa near the bark.

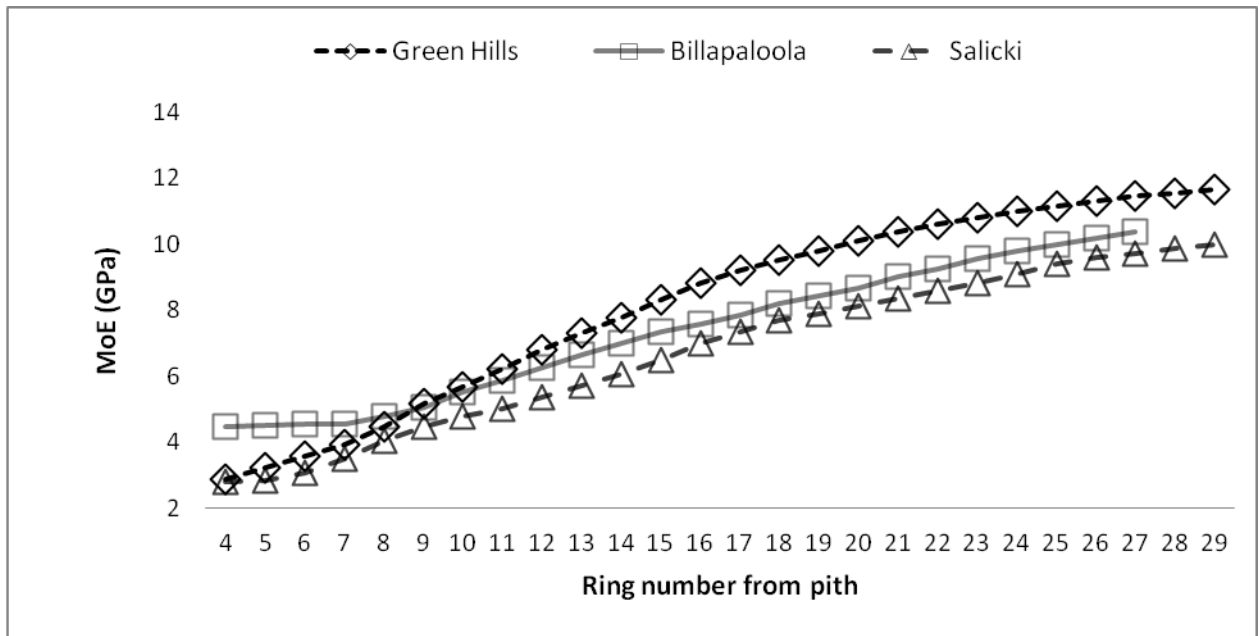


Fig 17. Cumulative ring-by-ring means for SilviScan-predicted modulus of elasticity (MoE), from single pith-to-bark cores taken at breast height from trees in Green Hills, Billapaloola and Salicki. Approximate average standard error of the ring means is ± 0.3

Microfibril angle (MfA) for mainland populations at Green Hills and Salicki was about 38° in rings close to the pith (Fig 18). By ring 12, MfA at Green Hills had dropped to 28° whereas MfA at Salicki had only dropped to 31° . In rings close to the bark, MfA at both sites were below 25° . MfA values for island provenances were much lower than for mainland provenances at rings close to the pith (32°) and continued to drop with successive rings to 22° in rings close to the bark. These differences were of course confounded with site differences.

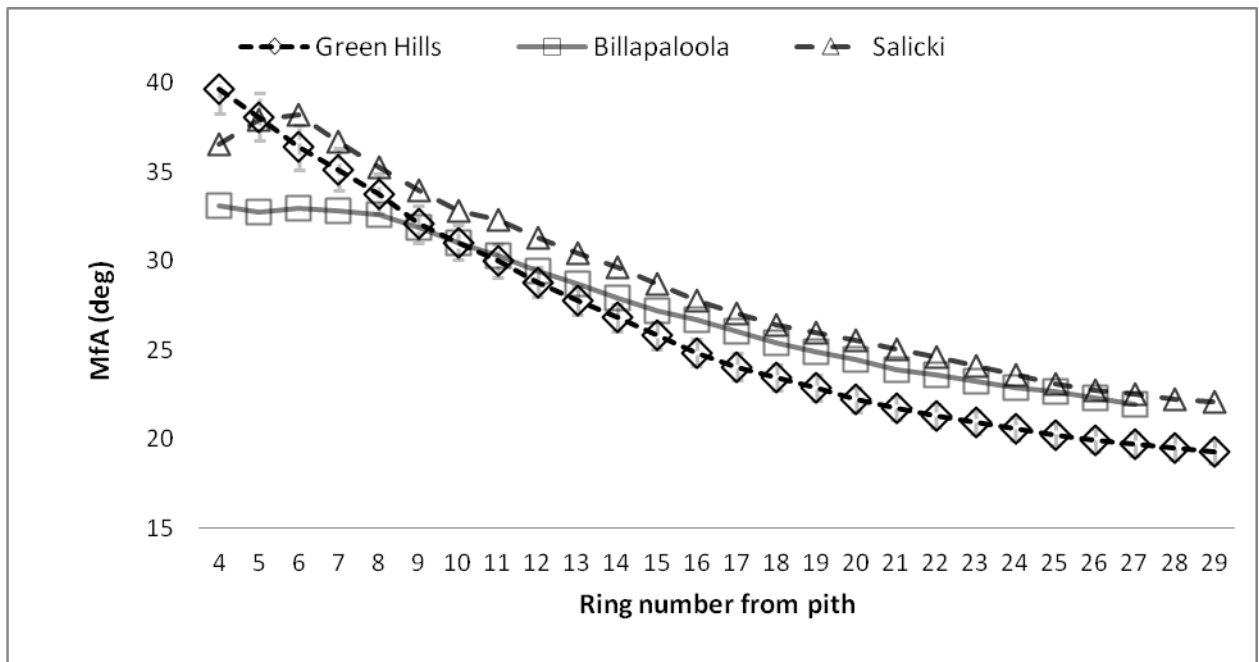


Fig 18. Cumulative ring-by-ring means for microfibril angle (MfA), from single pith-to-bark cores taken at breast height from trees in Green Hills, Billapaloola and Salicki. Approximate average standard error of the ring means is ± 1.2

Type-B genetic correlations for all wood quality traits were typically high, conforming to the trend that wood properties have low $G \times E$ (Table 20). Significant differences in growth, form and wood properties among provenances, families and/or individual trees provide an opportunity for breeding programs to select superior trees for solid wood production that will combine superior growth with desirable wood traits.

Further analyses of the ring-by-ring wood property data are planned (subject to funding) as mainly focuses on site effects and genetic variation in the cumulative effects across ages. A key issue which needs to be address with this extremely valuable data is the genetic control of the actual age trajectory itself and how much of the genetic variation in the final harvest age properties between provenances and families can be explained by genetic differences in these trajectories. In addition, it may be worthwhile splitting the samples into core- and outer-wood and treating these measurements as different traits.

Data for 28 845 trees from 609 families in seven provenance trials were used to predict breeding values for 22 selection criteria traits (see Appendices 6 and 7). Based on an integrated index, 50 elite trees from each of the mainland provenances and 30 from each of island provenances were selected for grafting into an archive. The selections were as follows:

- For each of the mainland provenances:
 - Family rank 1-10 = +3 selections per family = 30 selections;
 - Family rank 11-20 = +2 selections per family = 20 selections.
- For each of island provenances:
 - Family rank 1-10 = +3 selections per family = 30 selections

Table 20. Estimates of additive (r_A) genetic correlations and approximate standard errors in parentheses between pairs of traits from Green Hills and Salicki trials (joint-site analyses). Numbers in the trait names are denoting age or ring number of cumulative expression

	DBH_29 [§]	BD_29	DEN_12	DEN_29	MoE_12	MoE_29	MfA_12	MfA_29	STR_29
DBH_12 [§]	0.91 (0.03)**	-0.17 (0.11)*	-0.23 (0.13)*	-0.21 (0.08)*	-0.14 (0.06)*	-0.12 (0.06)*	0.26 (0.15)*	0.26 (0.09)*	0.28 (0.08)*
DBH_29		-0.12 (0.13)*	-0.23 (0.09)*	-0.26 (0.07)*	-0.13 (0.05)*	-0.13 (0.07)*	0.20 (0.19)*	0.23 (0.10)*	0.61 (0.08)*
BD_29			0.79 (0.07)***	0.80 (0.07)*	0.23 (0.12)***	0.15 (0.14)***	-0.16 (0.13)**	-0.17 (0.14)**	0.06 (0.09) ^{ns}
DEN_12				0.96 (0.04)**	0.73 (0.08)***	0.78 (0.11)***	-0.48 (0.13)*	-0.48 (0.15)*	-0.18 (0.11)*
DEN_29					0.74 (0.09)***	0.82 (0.07)***	-0.49 (0.14)***	-0.51 (0.14)***	-0.12 (0.14) ^{ns}
MoE_12						0.90 (0.09)**	-0.94 (0.02)*	-0.79 (0.11)**	#
MoE_29							-0.84 (0.11)**	-0.94 (0.04)**	0.20 (0.14) ^{ns}
MfA_12								0.84 (0.09)**	-0.02 (0.13) ^{ns}
MfA_29									-0.13 (0.16) ^{ns}

*P<0.05; **P<0.01; ***P<0.001; ^{ns}not significant; #failed to converge; Significance levels based on two-tailed LR tests that were used to test the departure of r_A from zero

[§]exact ages of assessment for DBH at Green Hills and Salicki are provided in Gapare et al (2011)

Discussion and conclusions

It is evident from this work that a successful gene conservation strategy will require a reasonable quantitative framework, and we suggest that allele frequencies, as well as expectations of quantitative genetic variances from empirical and theoretical work, should be used in an integrated approach (e.g. Yanchuk 2001). Breeding and gene conservation need to be elevated to a stage where tree breeders can state what types of genes or genetic variances they are expecting to conserve, and what vehicles they will use to conserve them. The Active genetic conservation and utilisation of native radiata pine germplasm project has made significant progress in the management, conservation and utilisation of native radiata pine germplasm. The significance and benefits of the outcomes of the project were documented in 10 client technical reports to funding organisations and industry clients and in 5 scientific manuscripts.

This project has demonstrated the strong role that scientific research can play in solving national and global forest genetic resource management issues. The radiata pine *ex situ* gene conservation stands in Australia and New Zealand not only played a significant role in conserving genetic diversity, but also generated new selections to increase genetic base and genetic gains for existing and future breeding populations. For example, if there is a shift in breeding objectives, including what might be imposed by a biotic crisis caused by arrival of arrival of new pests or diseases, the real significance of such a boost in genetic diversity from broadening the provenance base may lie in recruiting genetic resistance to various biotic factors and abiotic factors. For example, our results showed Cambria proving more tolerant than other provenances of *Phytophthora cinnamomi*. The importance of this work has been clearly demonstrated for the long-term maintenance of the genetic resource base, and for the benefit of the people and industries that these genetic resources sustain.

Primary scientific findings

Nine major scientific findings related to conservation, utilisation of native radiata pine germplasm, and basic genetic knowledge are outlined below:

1. Conservation related findings

(i). A catalogue of existing provenance and genetic conservation trials of radiata pine in Australia was updated and distributed to member organisations after a thorough inspection of existing provenance and genetic conservation trials in Australia. There were a total of 50 provenance trials and block plantings, 16 of which were established between 2004 and 2007. All these trials and block plantings have at least 80% survival. All provenances are largely secure ensuring gene conservation of radiata pine germplasm for the next 50 to 60 years (see Appendix 1).

(ii) Nucleotide diversity estimated using SNPs indicated that genetic diversity was higher in mainland than island populations. However, observed low diversity statistics for the island populations may be biased downward because of the choice of SNPs used, among other things, being polymorphic in the mainland populations. The average differentiation among

the populations ($F_{ST} = 0.09$) was low relative to other conifers. A search for the optimal number of populations (clusters (K)) in radiata pine revealed two relative strong peaks at $K = 2$ and another at $K = 5$, corresponding to the strong differentiation between island and mainland populations, and to the five geographic populations. However, the cluster suggested that the mainland populations were an admixture of two or three ancestral populations with the genetic composition of Cambria and Monterey being very similar, in contrast to some previous studies (see Appendix 2).

(iii) An *ex situ* gene conservation strategy, based on the observed adaptive structure, was recommended, with the aim of preserving population genetic diversity and maintaining the distinctness of each provenance in conservation plantings (see Appendix 2).

(iv) The 1978 ‘Eldridge’ conservation collection captured as much diversity as expected from stratified sampling; therefore, all provenances of radiata pine appear to be adequately conserved (see Appendices 1 to 3).

2. Infusion related findings

(i). Monterey and Año Nuevo were the best growth performers at almost all sites. However, Cambria had some individual trees with better growth than Monterey and Año Nuevo while Guadalupe had good stem straightness trees of on some sites. These new genotypes could be infused into existing breeding population which is largely derived from Monterey and Año Nuevo populations (see Appendices 4 to 7).

(ii). The control seedlots significantly outperformed all provenance means at almost all sites in Australia for DBH at juvenile and mature ages. However, at individual-tree levels, ten unrelated parents ranked above control seedlots from the older open-pollinated seed orchard stock for DBH growth in 3 trials in New Zealand and would be potential candidates for infusion (Appendices 4 & 6).

(iii). The genetic coefficient of variation (CV_A) for DBH for native populations was around 8-10% compared to ca 5% for the current breeding population. These results suggested that there is appreciable genetic variation in the native populations and infusion of these materials would increase the genetic variation in current breeding populations (see Appendix 4).

(iv). Large differences between mainland and island provenances were apparent for wood density and stiffness, with Guadalupe and Cedros being 20% higher than the mainland provenances with potential infusion of genotypes with favourable wood quality characteristics (see Appendix 7) but whatever the real difference, it was confounded with any site differences and therefore the results would need to be interpreted with caution.

(v). The estimated genetic correlations between DBH and wood properties from native population collections in the current study were weaker (less negative) than the mean estimated from the current generation in radiata pine, with good prospects of being able to infuse alleles with less negative pleiotropic effect (see Appendix 7).

3. Major industry adoptions with significant impact

(i) The updated catalogue has been adopted by forest managers to keep track of all radiata pine genetic resource plantings established in Australia. This new catalogue provides forest managers with a one-stop-shop that lists details of trial establishment, location, owners and collaborators of the trials, and proposed schedule of activities recommended for each trial including possible prolonged rotation and seed collected at time of clearfell (see Appendix 1).

(ii). Three genetic conservation trials of the island material were established at two sites in South Australia and one site in the Australian Capital Territory. Two other block plantings are earmarked for Victoria and Tasmania. The island populations are not represented in current breeding programs in Australia, yet they may represent the best-adapted seed sources for range expansion and adaptation to drier climates. The Guadalupe provenance is of considerable interest for producing F₁ hybrids with local stock, to give superior stem straightness and wood density combined with high growth vigour (see Appendices 6 and 7).

(iii). Growth and stem-quality traits data from 32 trials, wood density, modulus of elasticity (MoE) and microfibril angle (MfA) data from 3 trials, and trial-specific additive genetic, and error variances, and correlations were deposited into DATAPLAN®. Data for 28 845 trees from 609 families in seven trials of the native provenance material were used to predict breeding values for 22 selection criteria traits (see Appendices 6 and 7). It is anticipated that the investment in these data sets will be rewarded by the use of the information into the breeding programs as well as provision of a unique long-term research data base to further improve our understanding of the genetic and environmental control of age-trajectories in wood properties and use in association genetic studies.

(iv). Based on an integrated index, 50 elite trees from each of the mainland provenances and 30 from each of island provenances were selected for grafting into an archive for controlled crossings and subsequent infusion into breeding populations. The infusion would extend the future responses to selection by contributing favourable alleles not present in the existing breeding populations (see Appendices 6 and 7).

(v) Approximately 200 crosses per provenance are being carried out. In addition, 100 random trees from each of the island provenances were selected from several trials and block plantings and scions collected for grafting into the National Genetic Resource Centre (NGRC) arboreta. The crossings will be used to create relatively “pure” population to maximise genetic diversity for current and future environments (see Appendix 2).

List of client confidential technical reports

MS2. An annotated catalogue of Australian provenance trials and genetic conservation plantings of *Pinus radiata* D. Don of Californian origin, as at 1st July 2009. (FWPA, STBA & RPBC)

MS3. Collection of open-pollinated (OP) cones and extracting seed for long-term storage and for –re-establishment of second-generation *ex situ* gene conservation blocks (FWPA, STBA & RPBC)

- MS4 Field assessments of survival, growth and form traits in selected radiata pine provenance trials in Australia and New Zealand. (FWPA, STBA & RPBC)
- MS5. Sampling of increment cores for wood quality assessments in radiata pine in Australia and New Zealand. (FWPA, STBA & RPBC)
- MS6. Genetic analysis of *ex situ* native-provenance collections of *Pinus radiata* D. Don. : Potential for infusion into breeding populations in Australia and New Zealand. (FWPA, STBA & RPBC)
- MS7. Genetic analysis of *ex situ* native-provenance collections of *Pinus radiata* D. Don. : wood quality traits in Australia and New Zealand. (FWPA, STBA & RPBC)
- MS8. Nucleotide diversity and population genetic structure in disjunct *Pinus radiata* D. Don populations: implications for *ex situ* gene conservation in Australia and New Zealand. (FWPA, STBA & RPBC)
- MS9. Collection of open-pollinated (OP) cones and extracting seed for long-term storage in New Zealand
- MS10. Selection of elite trees and collection of scions from mainland and island provenances for grafting at two sites for controlled crosses. (FWPA, STBA & RPBC)
- MS 11. Complete conservation selection of 100 random trees from each of Guadalupe and Cedros population, scion collection and grafting for controlled cross for conservation
- MS12. Evolutionary history and conservation of native radiata pine populations: a simulation approach. (FWPA, STBA & RPBC)
- MS13. Long-term storage of seed and selection of block planting sites in Australia and New Zealand and Final Project Report (FWPA, STBA & RPBC)

List of scientific manuscripts

1. Zhang L, Huanqiong N, Dillon SK, Li X, Gapare WJ, Wu HX. Comparison of allelic diversity between gene resource plantings and selections in progeny tests of radiata pine. (In preparation)
2. Gapare WJ, Ivkovich M, Dillon SK, Chen F, Evans R, Wu HX. 2012. Genetic parameters and provenance variation of *Pinus radiata* D. Don. ‘Eldridge collection’ in Australia 2: wood properties *Tree Genetics and Genomes* DOI: 10.1007/s11295-012-0475-x (Appendix 7)
3. Gapare WJ, Ivkovich M, Dutkowski GW, Spencer DJ, Buxton P, Wu HX. 2012. Genetic parameters and provenance variation of *Pinus radiata* D. Don. ‘Eldridge collection’ in Australia 1: growth and form traits. *Tree Genetics and Genomes* 8: 391-407. DOI: 10.1007/s11295-011-0449-4 (Appendix 6)

4. Bian L, Gapare WJ, Ivkovich M, Jefferson P, Wu HX. 2011. Genetic variation between and within *ex-situ* native-provenance collections of *Pinus radiata* D. Don planted in Australia and New Zealand. *Silvae Genetica* 60: 276-285 (Appendix 5)
5. Gapare WJ, Baltunis BS, Ivkovich M, Low CB, Jefferson P, Wu, HX. 2011. Performance differences among *ex-situ* native-provenance collections of *Pinus radiata* D. Don. 1: Potential for infusion into breeding populations in Australia and New Zealand. *Tree Genetics and Genomes* 7: 409-419 (Appendix 4)

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Appendices

Appendix 1. An annotated catalogue of Australian provenance trials and genetic conservation plantings of *Pinus radiata* D. Don of Californian origin, as at 1st July 2009. 7 pp

<ftp://ftp.csiro.au/RadiataGenes/>

Appendix 2: Nucleotide diversity and population genetic structure in disjunct *Pinus radiata* D. Don populations: implications for *ex situ* gene conservation in Australia and New Zealand. 36 pp

<ftp://ftp.csiro.au/RadiataGenes/>

Appendix 3. Collection of open-pollinated (OP) cones and extracting seed for long-term storage and for –re-establishment of second-generation *ex situ* gene conservation blocks. 6 pp

<ftp://ftp.csiro.au/RadiataGenes/>

Appendix 4. Performance differences among *ex-situ* native-provenance collections of *Pinus radiata* D. Don. 1: Potential for infusion into breeding populations in Australia and New Zealand. 27 pp

<ftp://ftp.csiro.au/RadiataGenes/>

Appendix 5. Genetic variation for wood density between and within *ex-situ* native-provenance collections of *Pinus radiata* D. Don planted in Australia and New Zealand. 37 pp

<ftp://ftp.csiro.au/RadiataGenes/>

Appendix 6. Genetic parameters and provenance variation of *Pinus radiata* D. Don. ‘Eldridge collection’ in Australia 1: growth and form traits

<ftp://ftp.csiro.au/RadiataGenes/>

Appendix 7. Genetic parameters and provenance variation of *Pinus radiata* D. Don. ‘Eldridge collection’ in Australia 2: wood properties. 42 pp

<ftp://ftp.csiro.au/RadiataGenes/>