

# **J. W. GOTTSTEIN MEMORIAL TRUST FUND**

The National Educational Trust of the Australian Forest Products Industries



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## **DESIGN OF *EX SITU* GENE CONSERVATION PLANTATIONS TO MINIMIZE INBREEDING IN RADIATA PINE**

**WASHINGTON GAPARE**

2010 GOTTSTEIN FELLOWSHIP REPORT

## **JOSEPH WILLIAM GOTTSTEIN MEMORIAL TRUST FUND**

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Bill Gottstein was an outstanding forest products research scientist working with the Division of Forest Products of the Commonwealth Scientific Industrial Research Organization (CSIRO) when tragically he was killed in 1971 photographing a tree-felling operation in New Guinea. He was held in such high esteem by the industry that he had assisted for many years that substantial financial support to establish an Educational Trust Fund to perpetuate his name was promptly forthcoming.

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## Executive Summary

Radiata pine has become one of the most important commercial tree species in the world and is currently the most extensively planted conifer species in Australia and New Zealand. The radiata pine domestication process of selection and breeding has occurred almost entirely in regions far removed from its native forests in California and Mexico. The wood of this species is remarkably versatile and is used for both structural and appearance-grade wood products, and for pulp. The conservation of radiata pine germplasm has become an important component of long-term forest management for its sustained productivity and profitability. Potential gene conservation benefits include disease and insect damage alleviation, an increase in volume, improved wood stiffness and extending the current suitable plantation area by breeding for drought tolerance. Maintaining such resources to serve their intended purpose needs to be made as efficient and cost effective as possible. This depends on measures of the underlying genetic diversity of traits of interest.

Guadalupe and Cedros island radiata pine populations are the most drought resistant populations but have small effective population sizes. Inbreeding is unavoidable in such small isolated populations, as all individuals will be related by coancestry over time. This report presents a design layout for an *ex situ* gene conservation plantation that minimizes the global probability of generating inbred offspring for the whole population. The design layout was based on the integration of information on coancestry between individuals and pollen dispersal kernels, which allowed for particular crosses to be banned or encouraged. The plantation design put forward in this report will play an important role in developing sound strategies for gene conservation of radiata pine.

The design layout would accommodate a total of 5000 plants, at a spacing of 5 x 5 metres covering a total area of 12.5 hectares. The plants could be produced commercially and made available at a cost commensurate with the cost of production to the following organisations: Hancock Victoria Plantations (HVP), Auspine, ForestrySA, Green Triangle Forest Products, Australia Capital Territory Dept of Forests and

Environment, Timberlands Forests, Gunns Limited, ArborGen Australia and Forest Products Commission. The conservation plantations would be allowed to grow to rotation age and cones collected as and when they produce seed for further conservation and utilisation as and when needed.

This work has demonstrated the strong role that scientific research can play in solving national and global forest genetic resource management issues. Although *ex situ* gene conservation will not yield short to medium term benefits, the significance of *ex situ* gene conservation certainly becomes increasingly important, as the global forest science community comes to terms with the need to manage, conserve and utilize forest genetic resources effectively, especially in the face of a rapidly changing environment. 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.

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## 1. 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.

Radiata pine has become one of the most important commercial tree species in the world and is currently the most extensively planted conifer species in Australia and New Zealand (Burdon 2001; ABARE 2009). Its domestication process of selection and breeding have occurred almost entirely in regions far removed from its native forests in California and Mexico (Rogers 2004). The wood of this species is remarkably versatile and is used for both structural and appearance-grade wood products and pulp. This outcome has largely been achieved through long-term radiata pine national breeding programs in Australia and New Zealand aiming at improving both its productivity and quality (Jayawickrama and Carson 2000; Wu et al. 2008; Dungey et al. 2009). However, a recent review by Forest & Wood Products Australia (FWPA) (2010) highlighted several challenges for the future and among them included the need for gene conservation of radiata pine to meet potential biotic and abiotic risks. The achievements of intensive breeding programs continue to increase the opportunity costs of maintaining essentially unimproved but broadly-based gene resources of radiata pine. Conservation of radiata pine germplasm has become an important component of long-term management for its sustained productivity and profitability. Furthermore, new germplasm is required that maintains productivity and is able to tolerate increased risks (drought, insect and disease) due to anticipated changes in climate. Conservation and infusion of Guadalupe and Cedros genotypes into the radiata pine breeding populations will increase the genetic diversity and variation needed for long-term breeding. Potential conservation benefits include disease and insect damage alleviation, and a possible increase in volume and wood stiffness.



## 1.1. Purpose of Research

In 2006, the CSIRO forest genetics group embarked on work to make use of some seeds from the 1978 “Eldridge” collection. These were seeds that were retained and had been in cold storage for nearly 30 years. The threat of disease that has arisen since the collections were made means no further seed collections from Mexico are currently allowed into Australia. Further, the conservation plantings established from the Eldridge collection have reached rotation age and some have largely been cut down. Seeds from the remnants in store will no longer germinate and were sent to New Zealand for the embryos to be rescued and placed in tissue culture. The objective was to establish conservation stands from the plants to fulfill two objectives:

- (i) Conserve the two valuable drought-resistant island populations of radiata pine on several sites in Australia (they may go extinct if we do not).
- (ii) Set up an experimental planting which can be used for teaching of both conservation genetics and tree breeding at the International Arboretum in Canberra in collaboration with the ACT government, Australia National University and CSIRO.

The cultures were derived from approximately 130 mother trees in Mexico, about 50 from Cedros and about 80 from Guadalupe. To fulfil objective 1) above, we should have representatives of as many mother trees as possible. To fulfil objective 2) above, we should plant about 20 plants from each mother tree, leading to about 2600 plants in total. Depending on the spacing between adjacent plants, this would require 2-2.5 hectares of land for each site. To date, Australian Capital Territory (ACT) Forests have set aside land for establishment of a Guadalupe and Cedros conservation plantation in 2011.

The only cost-effective way to control inbreeding in *ex situ* forest tree plantations is often to allocate trees in such a way that the possibility of close relatives mating is small and, consequently, inbreeding does not increase too much over time. In order to avoid inbreeding in Guadalupe and Cedros conservation plantings, a plantation layout based on coancestry and pollen dispersal kernels would be the best option. The objective of this work was two-fold: (i) gain an understanding of inbreeding in plants; (ii) design a plantation layout based on coancestry data and pollen dispersal kernels in radiata pine in order to minimize inbreeding in future generations. The plantation design would be used in the establishment of *ex situ* gene conservation populations.

## **2. RESEARCH SCOPE**

### **2.1. Rationale for *ex situ* gene conservation**

Preservation of genetic diversity and avoidance of inbreeding are primary issues in conservation programs for threatened and endangered species. A loss of genetic diversity and an increase in inbreeding may result in inbreeding depression, lack of adaptation to changing environmental conditions, and population extinction (Hedrick 2001; Frankham et al. 2002). There is an increasingly general consensus on the best way to preserve genetic diversity and to avoid inbreeding in *ex situ* conservation programs. The most widely accepted strategy minimizes the global coancestry (kinship) in the population through optimization of contributions of parents to the next generation (e.g., Lacy 2000; Caballero & Toro 2000).

*Ex situ* gene conservation is appropriate when the tree species is rare and grows only as small patches, when the site is threatened or when regeneration is uncertain. The principal method for the *ex situ* conservation of forest tree species is the establishment of tree collections. The individual trees are either propagated by grafting and the grafts moved to clone collections or seed is collected from the original trees and so-called family collections are established with seedlings. In family collections, several seedlings of one family are planted near each other, and thinning is carried out so that only one tree, representing the family is finally left growing. The families representing the same forest are distributed within the collection so that it can also be used for seed production after thinning. One of the benefits of tree collections is that some selection takes place within them. They produce well-adapted and genetically variable seed that can be used in forest regeneration or in landscaping. The trees selected for genetic resource collections are not necessarily the best trees from the point of view of forestry, as the aim has been to collect a random sample of the existing genetic variation. The aim is usually to propagate all the genetic resource collections and to establish duplicate reserve collections in different localities for genetic security and future use.

### **2.2. Challenges of small population sizes**

Plant populations are that often targeted for *ex situ* conservation have small effective population sizes. As such, inbreeding is unavoidable in small isolated populations, as all individuals will be related by coancestry over time, a fact that is exacerbated by fine-scale spatial genetic structure and restricted dispersal. If populations remain small and isolated for many generations, they face two genetic threats. As alleles are randomly fixed or lost from the population by drift, levels of quantitative genetic variation necessary for adaptive evolution erode (Lande 1995). Simultaneously, deleterious mutations will tend to accumulate, because selection is less

effective in small populations (Lynch and Walsh 1995). This could eventually lead to a 'mutational meltdown' for populations with an effective size ( $N_e$ ) of  $<100$ . Both processes tend to be gradual, however, and thus do not threaten populations in the short term. By contrast, inbreeding can act swiftly. By restricting opportunities for mating, small populations foster inbreeding via mating among relatives. Simultaneously, small populations tend to fix an appreciable fraction of the genetic load by drift, resulting in among-population inbreeding. Both forms of inbreeding increase the frequency of individuals that are homozygous for alleles identical by descent. Such inbreeding results in a reduction in fitness (inbreeding depression). Although any of these genetic mechanisms could threaten population persistence, inbreeding poses the most immediate risk for small size populations. For example, plant studies, based mostly on comparing populations that differ in size or levels of genetic variation, also reveal significant inbreeding effects on seed set, germination, survival and resistance to stress.

### **2.3. Inbreeding in small populations**

Inbreeding, which is defined as mating between individuals that are more genetically similar than individuals drawn at random from the population, has attracted the attention of scientists for decades. As a result of its relevance in a range of diverse scientific disciplines, such as evolutionary biology, conservation genetics and animal and plant breeding, numerous studies have been performed to estimate the level of inbreeding from pedigree or marker information and to analyse the consequences of inbreeding on fitness traits and variance components.

It is important to understand the genetic basis of these effects. Inbreeding depression is caused by increased homozygosity of individuals. There are two genetically distinct ways in which increased homozygosity can lower fitness: increased homozygosity for partially recessive detrimental mutations and increased homozygosity for alleles at loci with heterozygote advantage ('overdominance'). Deleterious alleles will generally be present in populations at low frequencies (mutation–selection balance), whereas over-dominant alleles at a locus are maintained at intermediate frequencies by balancing selection.

The harmful effects of inbreeding were first documented in detail and quantified by Charles Darwin, who carried out experiments on 57 plant species that involved self-fertilization and outcrossing between unrelated individuals (Darwin 1876). Darwin's laborious study was motivated by the desire to explain why reproduction by outcrossing is prevalent in nature, what maintains the sometimes complex outcrossing mechanisms of flowers (Darwin 1862), and why numerous plant species have systems that prevent self-fertilization (Darwin 1877). Darwin's experiments supported his hypothesis that

self-fertilization must be strongly disadvantageous for the progeny produced — it lowered vigour and fertility in most of his study species (Darwin 1876).

Repeated inbreeding in a population can lead to the exposure of deleterious recessive traits and the decreased fitness of a population (inbreeding depression). An important issue for research on inbreeding is the extent to which the deleterious alleles that are associated with inbreeding depression can be reduced by selection (Byers & Waller 1999; Crnokrak & Barrett 2002; Ferreira & Amos 2006; Swindell & Bouzat 2006). This can happen by purging of deleterious alleles expressed because of increased homozygosity in inbred individuals; such purging would reduce the genetic load (Crnokrak & Barrett 2002), or by balancing selection, which favours heterozygote individuals over homozygotes (Charlesworth & Charlesworth 1987; Ferreira & Amos 2006). This latter issue is of general interest in evolutionary biology because it is related to the maintenance of genetic variation (Aguilar et al. 2004; Asthana et al. 2005; Ferreira & Amos 2006).

Many studies, using different genetic markers, have reported a positive correlation between fitness and the level of heterozygosity (see references in Hedrick & Kalinowski 2000; Hedrick 2005). The genetic background for these positive correlations is still under debate. Some studies attribute the positive relationship between heterozygosity and fitness, to heterozygosity at a few specific loci that are linked to fitness-related genes (Hansson 2004; Tiira et al. 2006), whereas others attribute it to increased heterozygosity across the entire genome (Hansson & Westerberg 2002). Furthermore, balancing selection may explain why heterozygosity is sometimes lost more slowly than expected based on neutral expectations during inbreeding (Rumball et al. 1994; Kaeuffer et al. 2007). Thus there is evidence that balancing selection plays a role in explaining the level of inbreeding depression (Charlesworth & Charlesworth 1987). Purging, or selection against recessive deleterious alleles that are expressed as a consequence of inbreeding, has also been suggested as a mechanism that can reduce the level of inbreeding depression (Crnokrak & Barrett 2002; Swindell & Bouzat 2006). Both balancing selection and purging may reduce the deleterious consequences of inbreeding as a result of selection for heterozygotes and or selection against recessive deleterious alleles (Charlesworth & Charlesworth 1987).

Inbreeding lowers fitness-related characters in many species of plants and animals. Major abnormalities are more frequent in inbred (consanguineous) families than in outcrosses. These abnormalities include mutant phenotypes that are lethal early in life, such as chlorophyll- deficient albino seedlings in plants and developmental defects in fish, or genetic diseases in humans. Even when there are no overt major abnormalities, inbreeding depression is detectable by the lower fertility, survival and growth rates of individuals with high inbreeding coefficients. The survival and fertility of individuals in experimentally produced inbred lines is frequently so low that many such lines go extinct. When surviving lines are intercrossed, the 'hybrids' often have higher

quality than their inbred parents and frequently exceed the best parent values for several characters (Zhang et al. 2008). This increased performance of first generation (F1) hybrids is called heterosis, or hybrid vigour. In many crops, heterosis has been extensively studied for characteristics of economic interest, such as drought tolerance, disease resistance and yield, and some of these characteristics may also be important for fitness in populations targeted for ex situ conservation.

Historically, much of the interest in inbreeding depression has come from either animal and plant breeders or researchers investigating the evolution of mating systems, local adaptation, or how inbreeding contributes to selection among sub-populations. At the same time, the historical focus of conservation biologists has been demographic and ecological events. A review by Keller & Waller (2002) on inbreeding suggested that demography, ecology, and genetics of small populations all interact to affect population persistence. None should be ignored. Inbreeding within and among populations can strongly affect both individual and population viability. Although it is often asserted that inbreeding depression is greater under stress or field conditions, this pattern is neither universal nor theoretically resolved. It also would be of interest to know how variance in the degree of inbreeding among individuals within a population (Crow & Kimura 1970) affects the expression of inbreeding depression and subsequent population dynamics. We need to learn more about how genetics and metapopulation dynamics interact (Richards 2000) if we are to understand just when and how inbreeding contributes to the 'extinction vortex' of fragmented populations. A population's ability to avert extinction during rapidly changing environmental conditions, or the magnitude of response to selection of a trait, depend on the ability of the genome to maintain potentially adaptive genetic variation in the face of random genetic drift.

## 2.4. Population history of island populations

Prior to introduction of goats, the stands of radiata pine on Guadalupe island were approximated to have covered almost 650 ha (Oberbauer 2003; 2006). However, according to a 2001 census, there were approximately 220 mature trees remaining on Guadalupe island (Rogers et al. 2006). Karhu et al. (2006) was able to detect the effects of changing population sizes and bottlenecks in the island populations. Small and/or isolated island populations are likely to be experiencing high levels of genetic drift, because the extent of drift is inversely related to effective population size. The island populations were getting more fragmented and infected with pitch canker. These effects continue to reduce both census size and effective population size (Rogers et al. 2006). These effects are expected to negatively affect fitness due to inbreeding (e.g., Charlesworth and Charlesworth 1999).

## 2.5. Potential inbreeding in island populations

Inbreeding is unavoidable in small isolated populations, as all individuals will be related by coancestry over time, a fact that is exacerbated by fine-scale spatial genetic structure and restricted dispersal. For example, most temperate forest trees lack prezygotic self-incompatibility systems (e.g. *Pinus* spp.), although they retain considerable heterozygosity because of early inbreeding depression. For instance, pines, a widespread genus comprising over 100 species, typically have a high number of lethal equivalents and suffer strong inbreeding depression (Williams and Savolainen 1996), which results in the purge of inbred individuals at early stages of development (Savolainen et al. 1992). Lethal and near-lethal alleles often result in early inbreeding depression in conifers (i.e., embryo abortion or mortality), while moderately deleterious alleles tend to affect quantitative characters such as growth and fecundity at later life stages (Sorensen 2001). Even low levels of close inbreeding such as sib-mating are predicted to be effective in purging deleterious genes of large effect, as homozygous recessive genotypes are exposed to selection (Fu et al. 1998).

A review by Williams and Savolainen (1996) suggested that radiata pine, like most other conifers, displays inbreeding depression upon selfing, manifested as reduced growth rate and seed set. However, Wu et al. (1988) suggested that the level of inbreeding in radiata pine is very low compared with other conifers and may indicate that many deleterious alleles have already been purged. It should be noted that the material studied by Wu et al (2008) was from the mainland populations. Similarly, Vogl et al. (2002) reported a limited amount of inbreeding in the mainland populations but significant inbreeding in island populations. Vogl *et al.* (2002) noticed that in the island populations of radiata pine pollination patterns have changed moderately towards selfing, while the mainland populations have pollination patterns close to those

observed in the other species (Muona 1990). Vogl *et al.* (2002) estimated outcrossing rates from seeds in five natural populations of radiata pine. The selfing rate was about 0.1 for the mainland populations and about 0.5 to 0.7 for the island populations. It is known that radiata pine populations have undergone bottlenecks due to fragmentation and colonization (Axelrod 1980, Millar 2000). Bottlenecks increase the probability of selfing but even more the probability of weak inbreeding, e.g., mating of distant cousins. Thus, the weak inbreeding may have been a first step in purging in radiata pine. Once the level of inbreeding depression had decreased, closer inbreeding and nowadays selfing would have become possible. Consequently, the mating system of radiata pine would have changed from almost completely outcrossing to mixed mating with an intermediate level of selfing (Karhu 2001).

From the above it is easy to see that avoiding mating among relatives in the management of island populations is especially important. Different solutions have been developed to avoid mating among relatives in conservation or breeding programs. For example, directed mating schemes that avoid a certain degree consanguineous matings are generally the more suitable to accomplish conservation goals (e.g. circular mating, Kimura and Crow 1963; compensatory mating, Caballero *et al.* 1996). In forest trees directed mating schemes are rarely used, given the high level of management required, and are generally restricted to breeding programs of commercial species where conservation goals are only secondary.

Managers of open-pollinated tree plantations usually tend to avoid mating among relatives through physical isolation (e.g. using “barriers” of other species) or, more commonly, by using plantation designs that separate like-individuals in space. Indeed, the larger the distance that separates two trees, the less likely they mate (see reviews in Sork & Smouse 2006). In addition, some tree plantations managed for quality seed production (i.e. seed orchards) incorporate management criteria, such as fertility control, selective thinning or selective harvest, aiming at reducing inbreeding in seed crops (Kang & Lindgren 1999; Kang *et al.* 2001).

### 3. Radiata pine gene conservation

#### 3.1. Current status of radiata pine in its native habitats in California and Mexico

Radiata pine 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 five locations differ substantially from each other with respect to soil, elevation, temperature, rainfall and ecosystem associates (Libby 1997). Unlike many other commercially significant forest tree species—including *Picea abies*, *Pseudotsuga menziesii*, *Pinus taeda*, and several *Populus* species—radiata pine has little economic importance within its native countries.

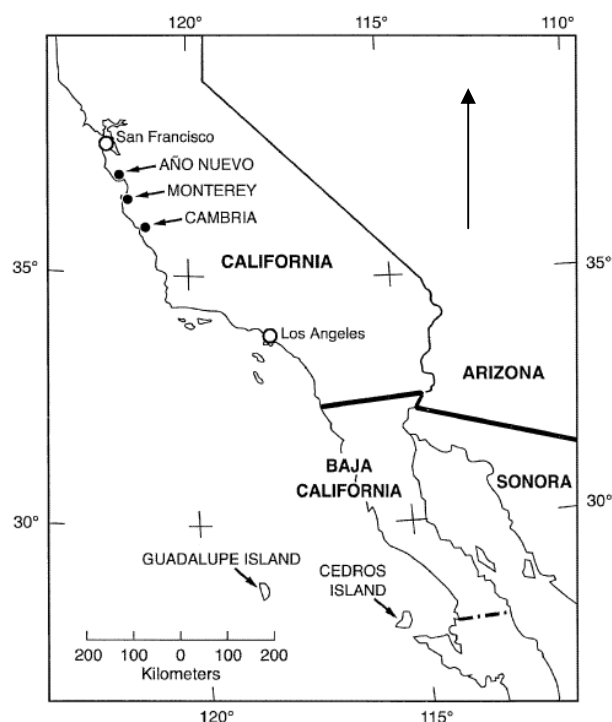


Fig 1. Location of *Pinus radiata* populations: Año Nuevo, Monterey and Cambria native to California and Guadalupe and Cedros Islands in Mexico. Figure reprinted with permission from (Ledig *et al.* 1998), Copyright 1998 by the Society of American Foresters.

In Mexico, radiata pine has never had any significant economic value. In California, the radiata pine forests were periodically harvested, particularly in the Cambria and Monterey areas, in the 18th and 19th centuries for local construction, fuel, and export (Libby 1997). During the 20th century it had some horticultural value, being



used in revegetation projects along transportation corridors, as a landscaping tree in parks and residential areas, and grown for the retail and wholesale Christmas tree markets. However, increased mortality from a canker disease (caused by an introduced fungal pathogen) has all but stopped its use in any revegetation or landscaping projects since the mid-1980s, although it continues to be grown for the Christmas tree market.

The Guadalupe and Cedros Island populations are listed as endangered according to the International Union of Conservation of Nature and Natural Resources (IUCN) Red List Categories (Hilton-Taylor, 2000). Although an attempt was made in 1999 to have the California populations of radiata pine listed as 'threatened' under the California Endangered Species Act (CNPS, 1999), that effort was halted because of political pressure. These three populations are listed as 'rare or endangered in California or elsewhere' (category '1B') within the Rare Plant Program of the California Native Plant Society (CNPS, 2001; Tibor, 1999). 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 severe in the native stands. For example, Monterey and Cambria are severely being encroached upon by urban development and exotic invasive organisms. As a result, there is currently a moratorium on importing germplasm from radiata pine native stands to Australia and New Zealand due to diseases (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). On one island (Guadalupe), there were only 220 trees left in 2001 and on the other (Cedros) the pines were under threat from repeated fires. Seed collections were made in 1978 from both islands and conservation plantings set up from the seeds. Both islands are extremely inhospitable environments. Guadalupe has an annual rainfall of less than 120 mm and Cedros is an extension of the Sonoran desert flora of Baja California. The establishment and continued existence of radiata pine on these islands is something of a mystery, but is probably related to the fog drip caused by complex meteorology of the region. Published studies on the origins of these populations suggests that the Cedros island population is very different from Guadalupe in that it has a different number of needles in each fascicle and was once called *Pinus cedrosensis*. Molecular studies also show that the populations are distinct and deserve separate attention (Moran et al. 1988; Burdon et al. 1992). Genes for increased resistance to pests, diseases and drought are likely to be in much higher frequencies in these populations than others of the species. However, there is now some documented evidence of recovery of trees on Guadalupe island (Oberbauer 2003). 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).

### 3.2. Current status of radiata pine gene conservation in Australia

Given the commercial significance of radiata pine—particularly in Australia, New Zealand, Chile and Spain—some recent and continuing international collaborations have used this interest to support genetic conservation activities for the native populations. A collaboration among Mexican, American, and Australian scientists and volunteers resulted in a successful collection of seed from the Mexican island populations in 2001 for conservation, restoration, and research purposes (Rogers 2004). Continued collaboration, particularly between Australia, New Zealand and American participants, should result in a strategy and agreement for long-term seed conservation that potentially could serve other species also. The Food and Agriculture Organization of the United Nations has shown interest, offered encouragement, and provided some financial support for these collaborative conservation activities.

The range-wide radiata pine “Eldridge” collection made in 1978 (Eldridge 1978) provided an opportunity for boosting the genetic base of the existing Australian and New Zealand breeding populations (Eldridge 1997). The number of mother trees from which seed was collected is presented in Table 1. Several provenance trials were established using seeds from the 1978 “Eldridge” collection. The 1978 “Eldridge” collection is unique because it has not yet been utilized for breeding purposes. It is anticipated that infusion of this material will provide a good opportunity for broadening the genetic base of the breeding populations which are now in the third generation selections. They could also be of special conservation interest because they may represent the best-adapted seed sources for range expansion and adaptation to new climates. Radiata pine is being altered through selective breeding, it is therefore important to maintain representative “wild populations” as genetic reserves to provide a benchmark of original genetic condition and to allow for the discovery of new alleles of use in the breeding program. Many of the provenance trials and *ex situ* block plantings have reached rotation age and plans are underway for a re-conservation strategy (Gapare et al. 2011).

Table 1. Seed-origin information for radiata pine populations sampled in California in 1978. (from Eldridge 1978)

Population	# mother trees	Latitude <sup>+</sup> (° N)	Longitude <sup>+</sup> (° W)	Elevation (m)	Rainfall (mm/annum) <sup>‡</sup>
Año Nuevo	179	37°	122°	15-300	700
Monterey	244	36°	121°	5-580	500
Cambria	99	35°	121°	30-180	500
Guadalupe*	48	29°	118°	400-1200	330-510
Cedros	51	28°	115°	380-640	150

<sup>+</sup>Latitude and longitude are derived directly from map locations and represent approximate centre points of the islands and mainland populations <sup>‡</sup>Annual rainfall figures are approximate and average. \*Substantial occurrence of fog during spring, summer and autumn with fog complementing the normal precipitation in drier years. Rainfall figures from Oberbauer (2006)

### 3.3. Recommended conservation strategy for radiata pine

The conservation strategy for renewal of conservation populations would be by re-propagation through seed from current *ex situ* stands, followed by planting at different sites in Australia and New Zealand to ensure security. The conservation plantings would be kept separate by population because most studies revealed some unique differences among populations for both molecular and quantitative traits. 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 plantings would be in the form of multiple population breeding system (MPBS) (e.g. Namkoong et al. 1980; Eriksson et al. 1993). MPBS emphasizes interpopulational diversity within an array of populations both in traits targeted for improvement and in environmental adaptabilities. The approach will generate the sizeable genetic variance that is necessary to cope with future uncertainties regarding environmental conditions and trait values (Eriksson et al. 1993).

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.

Deliberate crossing will be done to maximize diversity, nominally using up to five unrelated males in a pollen-mix. It is anticipated that up to 200 crosses per provenance will be carried out, subject to flowering and pollen availability. Such matings would not have to follow any particular mating scheme, but would increase the effective population size and nucleotide diversity of the next generation of conservation population while maintaining relatively 'pure' populations. In addition, controlled crosses would be carried out for radiata pine populations in a single-pair mating design involving as many parents as possible to retain the genetic integrity of the conservation collections. Again, such matings would not have to follow any particular mating scheme, but would increase the effective population size and nucleotide diversity of the next generation of conservation population while maintaining relatively 'pure' populations. The strategy would conserve not only existing genetic diversity, but increase the genetic base necessary to adapt to changing environment and market demands.

### **3.4. Conservation of Guadalupe and Cedros populations**

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. In contrast, the breeding population in New Zealand has a separate, pure Guadalupe population and some selections have been incorporated into the New Zealand breeding population (Low & Smith 1997). The Guadalupe population is of considerable interest for producing F1 hybrids with local stock, to give superior stem straightness and wood density combined with high growth vigour (Burdon 1992). Boardman and McGuire (1997) reported that some individuals from Guadalupe had a marked ability to survive and grow well on some low rainfall sites in South Australia. We recommend collection of open-pollinated cones from existing conservation plantings, followed by new plantings, cold storage of seed for future use and implementation of controlled crosses.

## **4. Conservation plantation designs**

### **4.1. Traditional designs**

Traditionally, plantations designed to avoid mating among relatives have relied in (i) random designs, (ii) incomplete block designs or (iii) permuted neighborhood and related methods (e.g. Bell & Fletcher 1978). These methods allocate individuals avoiding ramets of the same clone in neighbouring positions. For example, with the COOL software (Bell & Fletcher 1978) the number of rows of neighbours that separate two ramets of the same clone can vary from 1 to 8, depending on the user requirements. Permuted neighborhood methods have been extensively applied to conservation plantation designs. However, these methods have several limitations. As their name suggests, the allocation algorithm only considers the closest trees, which is not adequate for most forest species because they often fit highly leptokurtic (i.e. fat-tailed) pollen dispersal kernels (e.g., Lucas et al. 2008). A further limitation of permuted neighbourhood methods is that they do not allow for the incorporation of any information about the relationship between individuals, which might be a severe limitation in *ex situ* conservation plantations, in particular when considering the high levels of inbreeding and coancestry usually found in small and isolated populations.

In natural populations of forest trees, genealogies are usually lacking (at least for the paternal lineage) and, therefore, it is not possible to construct a pedigree-based coancestry matrix. However, the recent development of DNA screening techniques have provided with an increasingly large number of molecular markers that can be used to estimate the genetic relationships between individuals. Different methods have been

developed to infer the coancestry between pairs of individuals (see, for example, Lynch 1988; Ritland 1996) or for the whole population at a time (Butler et al. 2004).

In order to avoid inbreeding in Guadalupe and Cedros conservation plantings, a plantation layout based on coancestry and pollen dispersal kernels would be the best option. An ideal design would allocate trees in the plantations so as to avoid, as much as possible, the increase of inbreeding in the following generation (with no management).

## **4.2. Materials and methods used in this study**

We selected a total of 130 samples (80 from Guadalupe and 50 from Cedros) for a DNA study. These samples were considered to be a good representative of the Eldridge collection. The trees from which the seeds came from were sampled randomly avoiding close neighbours following standard procedures used to collect plant material for *ex situ* conservation (Eldridge 1978). Diploid genomic DNA was extracted from seeds (megagametophytes) using a modified CTAB protocol (Doyle & Doyle 1990), and further purified on QIAquick® PCR purification columns according to the manufacturer's instructions. DNA concentrations were measured using a fluorometer (Qubit™ Quantitation Platform).

Single nucleotide polymorphisms (SNPs) data were available from a previous study on nucleotide diversity of radiata populations (Gapare et al 2010). Briefly, the SNPs were chosen from a set of 36 candidate genes that were previously sequenced in a panel of radiata pine individuals (Tables S1; Dillon et al. 2010). The candidate genes were chosen on the basis of their known or likely involvement in development of the vascular tissue and wood. A full description of the candidate gene selection process and SNP discovery can be found elsewhere (Dillon et al. 2010). From the set of 36 candidate genes, 111 haplotype tagging SNPs were genotyped in parallel across 130 individuals using the Goldengate universal bead arrays (Illumina Inc, San Diego) (Shen et al. 2005).

## **4.3. Coancestry estimation**

Coancestry can be used, among others, in estimating relatedness and inbreeding coefficients for comparing the performances of different estimators using simulations or empirical datasets, and testing for differences between groups (populations) in average inbreeding or relatedness coefficients. Genetic marker data are widely used to estimate the relatedness between individuals in a population in which pedigree records are either lacking or unreliable. Such marker-based relatedness is valuable in many areas of research in behaviour, evolution and conservation in natural populations (Blouin 2003).

Example applications include estimating heritabilities (e.g. Ritland 2000; Visscher et al. 2006), minimizing inbreeding in captive populations (e.g. Sekino et al. 2004), and studying spatial structure and isolation by distance (e.g. Hardy & Vekemans 2002; Vekemans & Hardy 2004).

The software program COANCESTRY (Wang 2011) was used to estimate the pairwise relatedness between individuals using their multi-locus genotypes. This method gives relatedness estimates from the two likelihood estimators with inbreeding taken into account, and also inbreeding coefficients. A simulation module is built in the program to simulate multilocus genotype data of individuals with a predefined relationship, and to compare the estimators and the simulated relatedness values to facilitate the selection of the best estimator in a particular situation (Wang 2011). Bootstrapping and permutations are used to obtain the 95% confidence intervals of each relatedness or inbreeding estimate, and to test the difference in averages between groups.

The software SOFSOG (Fernandez & Gonzalez-Martinez 2010) which includes three programs: *Coancestry*, to estimate molecular coancestry between individuals; *Divide*, to distribute candidates among different available sites; and SOFSOG, to determine the plantation scheme within plots to avoid inbred offspring was used. SOFSOG (Fernandez & Gonzalez-Martinez 2010) was then used to allocate individuals following the Fernandez & Gonzalez-Martinez (2009) method. Details of the optimization technique are provided in Fernandez & Gonzalez-Martinez (2009). Briefly, the program generates the optimum allocation of  $N$  plants in a conservation plantation in order to minimize the global probability across the whole population of generating inbred offspring. The relationships between individuals are defined via the coancestry matrix obtained from the software program COANCESTRY (Wang 2011). The method is based on a simulated annealing algorithm (Kirkpatrick et al. 1983) to search across the feasible space of plantation designs through a combination of real coancestry and pollen dispersal functions. Alternate solutions were constructed by exchanging the positions of many couples of trees at a time in order to perform a broad search across the space of solutions. The number of trees changed per solution is automatically decided by the algorithm and depends on the number of rejections/acceptances along the previous step.

## 5. Plantation design layout

Plant conservation programs often focus on species or populations with a low effective population size, and, normally, with severely threatened native environments. Avoiding mating among relatives in plant population management, in particular in plantations established as *ex situ* conservation units, is important to reduce inbreeding

and its deleterious consequences (i.e. inbreeding depression) in subsequent generations. Usually, managers of open-pollinated plantations use physical isolation to avoid mating among relatives, (e.g. implementing 'barriers' of other species) or, more commonly, plantation designs that separate like-individuals in space. For example, a 'Window' design was used for large block plantings of Guadalupe provenance (Fig 2). This was a 20 ha block surrounded by routine radiata pine plantation. The design was aimed at reducing pollen contamination of the Guadalupe provenance material. However, the allocation of trees within a 4.5 ha block followed routine randomized complete block designs. However, such designs do not allow for the incorporation of any information about the relationship between individuals, which might be a severe limitation in *ex situ* conservation plantations, in particular when considering the high levels of inbreeding and coancestry usually found in small and isolated populations. Furthermore, such designs would require subsequent thinning before trees start producing flowers and cones in order to avoid pollinations among relatives.



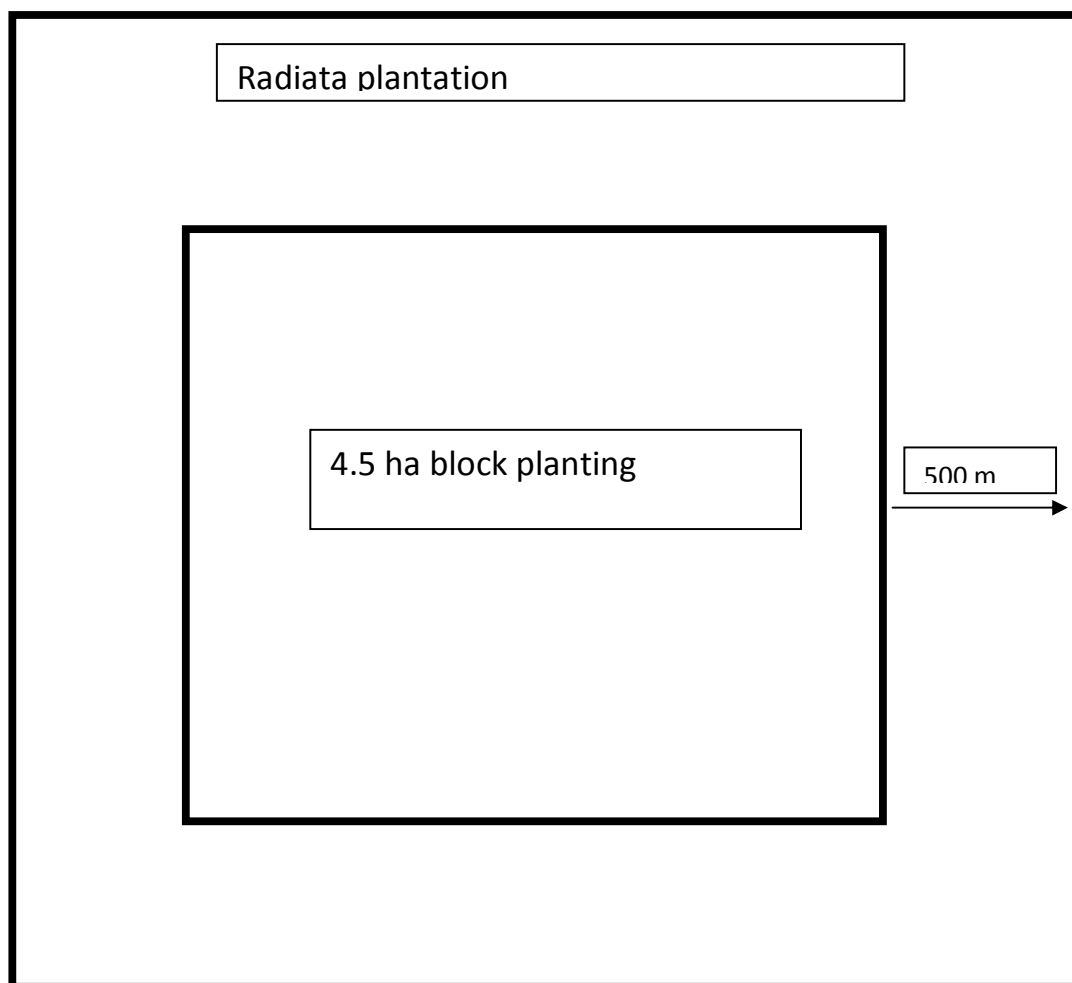


Fig 2. Example of a 'Window' design – 4.5 ha block surrounded by routine radiata pine plantation.

Our plantation design (Fig 3) (not showing full layout of total area to be planted) based on Fernandez & Gonzalez-Martinez (2009) method allowed for the integration of coancestry between individuals and pollen dispersal kernels, which allowed for particular crosses to be banned or encouraged. Each number represents a genetic identity. This design is different from commonly used designs for seed orchards and or provenance/progeny trials where a plot of trees from the same family will be in a row or a square plot. The design minimizes the global probability of generating inbred offspring for the whole population. For example, the method was efficient in spreading the different members of the same genet (e.g., genotype IDs 72, 120, 122). Because coancestry within genets is generally higher than between families, the method was able to separate individuals from the same genet and yield lower global probability of

inbreeding in next generation. On average, each genotype is represented 27 times in the plantation design in Fig 3.



232	23	122	16	20	13	57	26	50	47	60	5	16	46	33	1	24	17	31	57	10	34	41	107	37	23	36	2	
72	8	6	5	58	47	2	14	59	53	23	36	44	125	29	14	18	4	49	13	30	6	22	4	36	60	72	37	
	41	125	50	36	17	72	24	29	41	22	34	43	13	15	53	47	19	23	16	26		18	33	61	59	46	13	
		107	44	38	18	31	33	60	16	52	58	122	10	61	9	39	43	52	42	44		50	17	58	46	60	27	
				232	8	8	26	18	60	5	122	49																
				125	45	45	36	43	57	46	17	22																
			9	22	47	13	20	1	2	45	34	7															232	
			57	7	2	17	5	14	52	41	42	18	27	9													35	125
			72	18	6	34	60	50	31	23	33	6	43	125	29	60	3	18	45	29	41							
	60	58	122	46	16	44	24	10	38	39	4	16	47	57	37	72	122	22	6	107								
49	5	23	12	37	35	36	57	12	55	107	17	13	31	14	23	46	12	23	44	58								
19	20	43	18	50	27	53	40	72	59	47	19	2	232	38	24	36	49	8	5	35								
122	26	44	14	41	31	48	20	46	45	42	15	36	26	50	61	60	43	20	125	72								
107	24	34	52	38	16	22	33	41	3	37	52	10	58	16	44	33	52	50	17	9								
43	1	33	125	55	7	1	61	122	2	50	34	56	20	122	55	34	46	41	2	3								
15	45	42	17	39	10	4	13	29	60	43	6	18	51	5	31	6	57	1	107	122								
31	16	57	36	12	35	46	59	47	57	31	24	53	44	7	42	12	22	23	27	49								
20	56	30	44	6	5	49	28	36	232	17	33	9	46	14	10	15	13	26	18	232								
47	51	46	23	24	22	16	26	1	55	12	45	16	40	43	125	39	45	47	35	58								
61	58	8	43	60	125	122	48	72	27	24	35	30	2	47	20	33	16	60	36	2								
18	59	45	38	39	2	15	31	20	41	107	37	23	3	50	36	14	17	5	45	72								
4	53	17	41	57	50	34	10	53	22	4	36	60	22	29	19	31	107	57	38	41								
14	42	31	5	33	13	6	55	7	18	33	61	59	8	57	122	37	12	42	18	7								
50	125	47	12	18	36	16	37	42	50	17	58	46	34	55	46	20	24	44	6	232								
2	34	43	20	9	29	14	19	43	38	5	2	52	44	14	1	5	49	43	22	34								
57	122	58	17	10	49	1	24	12	45	47	23	16	41	13	43	45	17	60	3	23								
7	52	44	60	22	107	8	46	48	57	122	31	125	72	26	2	122	18	27	9	125								
37	45	23	35	125	6	72	3	44	232	107	35	29	9	6	72	58	41	107	35	232								
232	19	1	43	24	19	39	15	1	59	36	16	34	47	2	14	59	42	55	16	26								
					72	35	3	107	9	232	72	23	42	47	36	12	26	1	23									
					41	23	29	6	44	58	125	7	57	52	55	9	43	41	30									
				232	8	8	26	18	60	5	122	1	26	44	6	13	33	4	24									
				125	45	45	36	43	57	46	17	20	49	22	46	16	31	29	20									
			9	22	47	13	20	1	2	45	34	7															232	
			57	7	2	17	5	14	52	41	42	18	27	9													35	125
			72	18	6	34	60	50	31	23	33	6	43	125	29	60	3	18	45	29	41							
	60	58	122	46	16	44	24	10	38	39	4	16	47	57	37	72	122	22	6	107								
49	5	23	12	37	35	36	57	12	55	107	17	13	31	14	23	46	12	23	44	58								
19	20	43	18	50	27	53	40	72	59	47	19	2	232	38	24	36	49	8	5	35								
122	26	44	14	41	31	48	20	46	45	42	15	36	26	50	61	60	43	20	125	72								

107	24	34	52	38	16	22	33	41	3	37	52	10	58	16	44	33	52	50	17	9
43	1	33	125	55	7	1	61	122	2	50	34	56	20	122	55	34	46	41	2	3
15	45	42	17	39	10	4	13	29	60	43	6	18	51	5	31	6	57	1	107	122
31	16	57	36	12	35	46	59	47	57	31	24	53	44	7	42	12	22	23	27	49
20	56	30	44	6	5	49	28	36	232	17	33	9	46	14	10	15	13	26	18	232
47	51	46	23	24	22	16	26	1	55	12	45	16	40	43	125	39	45	47	35	58
61	58	8	43	60	125	122	48	72	27	24	35	30	2	47	20	33	16	60	36	2

Fig 3. Plantation design obtained using a coancestry matrix with ones for relationships between ramets, of the same genet (i.e., banning the location of two ramets near each other, irrespective of their level of self-coancestry).

## 6. Potential implementation

The proposed design would accommodate a total of 5000 plants per site. Each planting site would be approximately 4.5 ha. Trees would be planted at a spacing of 3 x 3 metres in order to avoid thinning at later years before the trees start producing flowers and cones. Multiple sites per organisation would be recommended for security reasons. The plants could be produced commercially and made available at a cost commensurate with the cost of production. The establishment and maintenance costs would be borne by individual organisations and timber harvested at rotation age would be owned by the individual organisations. However, CSIRO owns the intellectual property (IP) associated with the genetic material. The 4.5 ha plantings would act as conservation stands for Guadalupe and Cedros populations. Top quality trees should be selected and STBA may choose to archive some for infusion into the breeding program. Organisations that have been asked to provide land for establishment of the conservation plantations include Hancock Victoria Plantations (HVP) and Auspine in Victoria; ForestrySA and Green Triangle Forest Products in South Australia, Australia Capital Territory Dept of Forests and Environment in Canberra, Timberlands Pvt Limited and Gunns Limited in Tasmania, ArborGen Australia in New South Wales, and Forest Products Commission in Western Australia. For the 2011 planting season, ForestrySA and ACT Govt will each establish 4.5 ha. It is expected that other organisations will follow suit in the coming years.

The conservation plantations would be allowed to grow to rotation age and cones collected as and when they produce seed. Cedros is generally a shy cone and seed producer. The ideal environment for Cedros to produce cones and seed is not clear. Therefore, the stands established at various sites may be used to study the reproductive cycles of Cedros provenance, from cone bud initiation to shedding mature cones. The buds and cones would be investigated at characteristic developmental stages. The study would compare the reproduction of the trees in the geographic areas in Australia with regards to cone production and cone survival, seed potential, filled-seed production, types of seeds produced (e.g., full or insect-damaged), and causes for the different types of seeds under varying pollination times and methods. Potential funding for such a study would be sought from FWPA and STBA members.

## 7. Conclusions

Whereas our understanding of how inbreeding affects individuals has improved, we still know little about the effects of inbreeding on population dynamics in the presence of strong selection. In such circumstances, the magnitude of inbreeding depression would not necessarily directly affect population dynamics. When selection is 'hard', however, inbreeding could substantially affect population dynamics. Inbreeding and inbreeding depression do occur commonly in nature and can be severe enough to affect the viability of small and isolated populations. Methods that allow us to understand the genetic basis of inbreeding are improving all the time, and we should be

able to better incorporate details into models that try to predict future distributions, population size and patterns of inbreeding, and further consider the potential impact on functional genes. For example, methods that aim to assess the genome-wide level of functionally important genetic variation have been recommended (e.g., Kohn et al. 2006; Avise 2010; Ouborg et al 2010). These may include the application of single nucleotide polymorphisms (SNPs) (e.g., Neale and Savolainen 2004; Thornton et al. 2007; Namroud et al. 2008).

Optimization of contributions of parents to progeny by minimizing the average coancestry of the progeny is an effective strategy for maintaining genetic diversity in *ex situ* conservation programs. Contributions of minimum coancestry determined from pedigree information can be an effective method for maintaining allelic frequencies in conservation populations. Plantation designs for *ex situ* gene conservation can also facilitate the integration of ecological, life history and pedigree information in *ex situ* conservation strategies, and encourage managers to adopt objective-oriented plantation designs. However, as illustrated by many of the examples provided in this report, we can already use inference gained from this study to identify true risks of small size populations and this will facilitate the development of broad gene conservation strategy of forest tree species. In an increasingly complex and rapidly changing world, this type of improved insight will be essential to the design of lasting, effective gene conservation strategies. Although emphasis has been placed on radiata pine native populations, the results may be generalized for other tree breeding programs involving other tree species and those species valuable to commercial forestry, ecology as well as to forest science.

The design layout for the conservation plantings would cover an area of 4.5 ha at a tree spacing of 3 x 3 metres. The plants will be available from ArboGen nursery in Colac, Victoria at a cost commensurate with the cost of production. A request will be put to several STBA members to provide at least 4.5 ha of land for establishing a conservation planting. SCION in New Zealand is also coordinating similar conservation plantings. Costs associated with establishment and maintenance of the conservation planting will be borne by individual organisations. The conservation plantations would be allowed to grow to rotation age and cones collected as and when they produce seed for further conservation and utilisation as and when needed.

## 8. References

- ABARE (2009) Australian forest and wood products statistics, September and December quarters 2008, Canberra, May 2009. Available at: [http://www.abare.gov.au/publications\\_html/afwps/afwps\\_09/afwps\\_may09.pdf](http://www.abare.gov.au/publications_html/afwps/afwps_09/afwps_may09.pdf)
- Aguilar A, Roemer G, Debenham S et al. (2004) High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proceeding of the National Academy of Sciences of the United States of America*, 101, 3490– 3494
- Asthana S, Schmidt S, Sunyaev S (2005) A limited role for balancing selection. *Trends in Genetics* 21: 30–32
- Awise JC (2010) Perspective: conservation genetics enters the genomics era. *Conserv Genet* 11: 665-669
- Axelrod DI (1980) History of maritime closed-cone pines, Alta and Baja California. University of California Publications in Geological Sciences 120
- Bell G, Fletcher AM (1978) Computer organised orchard layouts (COOL) based on the permuted neighbourhood design concept. *Silvae Genet* 27:223–225
- Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology and Evolution* 18: 503–511
- Boardman R, McGuire DO (1997) Responses of *Pinus radiata* provenances near the warmth-dry limit of their potential range in a mediterranean-type climate. Pp. 62-69 *in*: Proceedings of the NZFRI – IUFRO Conference: IUFRO '97 Genetics of Radiata Pine, 1-4 December 1997, Rotorua, NZ, R.D. Burdon and J.M. Moore (Eds.). New Zealand Forest Research Institute, FRI Bull. No. 203
- Burdon RD (1992) Genetic survey of *Pinus radiata*. 9: General discussion and implications for genetic management. *N Z J For Sci* 22:274-98
- Burdon RD (2001) *Pinus radiata*. In: F. T. Last (ed.) *Ecosystems of the world: Tree crop ecosystems*. Amsterdam: Elsevier 99 -161
- Burdon RD, Bannister MH, Low CA (1992) Genetic survey of *Pinus radiata*. 2: Population comparisons for growth rate, disease resistance, and morphology. *N Z J For Sci* 22:138-159



- Butler K, Field C, Herbinger CM, Smith BR (2004) Accuracy, efficiency and robustness of four algorithms allowing full sibship reconstruction from DNA marker data. *Mol Ecol* 13:1589–1600
- Byers DL, Waller DM (1999) Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Genetics* 30: 479–513
- Caballero A, Toro MA (2002) Analysis of genetic diversity for the management of conserved subdivided populations. *Conserv Genet* 3: 289–299
- Caballero A, Santiago E, Toro MA (1996) Systems of mating to reduce inbreeding in selected populations. *Anim Sci* 62:431–442
- Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268
- Charlesworth B, Charlesworth D (1999) The genetic basis of inbreeding depression. *Genet Res* 74: 329-340
- CNPS (1999) A Petition to the State of California Fish and Game Commission: Monterey Pine. California Native Plant Society, Sacramento, CA
- CNPS (2001) Inventory of Rare and Endangered Vascular Plants of California, 6th ed. California Native Plant Society, Sacramento, CA
- Crnokrak P, Barrett SCH (2002) Perspective: purging the genetic load: a review of the experimental evidence. *Evolution* 56: 2347–2358
- Crow JF, Kimura M (1970) *An Introduction to Population Genetics Theory*, Burgess Pub. Co
- Darwin CR (1876) *The Effects of Cross and Self Fertilization in the Vegetable Kingdom* (John Murray, London)
- Darwin CR (1862) *The Various Contrivances by which Orchids are Fertilised by Insects*. (John Murray, London)
- Darwin CR (1877) *The Different Forms of Flowers on Plants of the Same Species*

(John Murray, London)

- Dillon SK, Nolan M, Li W, Bell C, Wu HX, Southerton SG (2010) Allelic variation in cell wall candidate genes affecting solid wood properties in natural populations and land races of *Pinus radiata*. *Genetics* 185: 1477-1487
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 23: 13-15
- Dungey HS, Brawner JT, Burger F, Carson M, Henson M, Jefferson P, Matheson AC (2009) A new breeding strategy for *Pinus radiata* in New Zealand and New South Wales. *Silvae Genet* 58: 28-38
- Eldridge KG (1978) Refreshing the genetic resources of radiata pine plantations In. *Division of Forest Research: Genetics Section Report Number 7* CSIRO. 1-120
- Eldridge KG (1997) Australian radiata provenance trials. Client Report 146, CSIRO Forestry and Forest Products, Canberra, 23pp
- Eriksson G, Namkoong G, Roberds JH (1993) Dynamic gene conservation for uncertain futures. *For Ecol Manag* 62: 15-37
- FAO (2001) International action in the management of forest genetic resources: status and challenges. Based on the work of C. Palmberg-Lerche. *Forest Genetic Resources Working Papers No. 1*. Rome
- Fernandez J, Gonzalez-Martinez SC (2009) Allocating individuals to avoid inbreeding in *ex situ* conservation plantations: so far, so good. *Conserv Genet* 10: 45-57
- Fernandez J, Gonzalez-Martinez SC (2010) SOFSOG: a suite of programs to avoid inbreeding in plantation designs. *Molecular Ecology Resources* 10: 393-396
- Ferreira AGA, Amos W (2006) Inbreeding depression and multiple regions showing heterozygote advantage in *Drosophila melanogaster* exposed to stress. *Molecular Ecology* 15: 3885-3893
- Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge
- Fu YB, Namkoong G, Carlson JE (1998) Comparison of breeding strategies for purging inbreeding depression via simulation. *Conserv Biol* 12:856-864

- FWPA (2010) Investment plan for genetic improvement to increase productivity and wood quality and manage risks. Melbourne, Australia, 23pp.
- Gadgil P, Dick M, Simpson J, Bejakovich D, Ross M, Bain J, Horgan G, Wylie R (2003) Management plan response to an incursion of pine pitch canker in Australia or New Zealand. Commissioned and published by the Forest Health Committee on behalf of the Forestry and Forest Products Committee, Canberra
- 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 Genet and Genomes* 7: 409-419
- Hansson B (2004) Marker-based relatedness predicts egg hatching failure in great warblers. *Conservation Genetics*, 5, 339–348.
- Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology* 11: 2467–2474
- Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2: 618–620
- Hedrick PW (2001) Conservation genetics: where are we now? *Trends in Ecology & Evolution* 16:629–636
- Hedrick PW (2005) *Genetics of Populations*, 3rd edn. Jones and Bartlett Publishers, London
- Hedrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, 31, 139–162
- Hilton-Taylor C. (compiler) (2000) 2000 IUCN Red List of Threatened Species. IUCN, Gland, Switzerland and Cambridge, UK, xviii +61p.
- IUCN (2009) IUCN Red list of threatened species. Version 2009.1.  
<[www.iucnredlist.org](http://www.iucnredlist.org)>
- Jayawickrama KJS, Carson MJ (2000) A breeding strategy for the New Zealand radiata pine breeding cooperative. *Silvae Genet* 49 (2): 82-90

- Kaeuffer R, Coltman DW, Chapuis JL, Pontier D, Reale D (2007) Unexpected heterozygosity in an island mouflon population founded by a single pair of individuals. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 274: 527–533
- Kang KS, Lindgren D (1999) Fertility variation among clones of Korean pine (*Pinus koraiensis* S. et Z.) and its implications on seed orchard management. *For Genet* 6:191–200
- Kang KS, Lindgren D, Mullin TJ (2001) Prediction of genetic gain and gene diversity in seed orchard crops under alternative management strategies. *Theor Appl Genet* 103:1099–1107
- Karhu A (2001). Evolution and applications of pine microsatellites. PhD theses. Faculty of Science, Department of Biology, University of Oulu
- Karhu A, Vogl C, Moran GF, Bell JC, Savolainen O (2006) Analysis of microsatellite variation in *Pinus radiata* reveals effects of genetic drift but no recent bottlenecks. *J Evol Biol* 19: 167-175
- Keller LK, Waller DM (2002). Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17: 230-240.
- Kimura M, Crow JF (1963) On the maximum avoidance of inbreeding. *Genet Res* 4:399–415
- Kirpatrick S, Gelatt CD, Vecchi MP (1983) Optimization by simulated annealing. *Science* 220:671
- Kohn MH, Murphy WJ, Ostrander EA, Wayne RK (2006) Genomics and conservation genetics. *Trends Ecol Evol* 21: 629-637
- Lacy RC (2000) Should we select genetic alleles in our conservation breeding programs? *Zoo Biology* 19:279–282.
- Lande R (1995) Mutation and conservation. *Conserv. Biol.* 9, 782–791
- Ledig T (1988) The conservation of diversity in trees. *Bioscience* 38: 471-479

- Libby WJ (1997) Genetic diversity for the future: Conservation or creation and capture? Pp. 9-21 *in*: Proceedings of the NZFRI – IUFRO Conference: IUFRO '97 Genetics of Radiata Pine, 1-4 December 1997, Rotorua, NZ, R.D. Burdon and J.M. Moore (Eds.). New Zealand Forest Research Institute, FRI Bull. No. 203
- Lynch M (1988) Design and analysis of experiments on random drift and inbreeding depression. *Genetics* 120: 791-807.
- Lynch M. Walsh B. (1995) Mutation accumulation and the extinction of small populations. *Am. Nat.* 146, 489–518
- Low CB, Smith T (1997) Use of the Guadalupe provenance in *Pinus radiata* improvement in New Zealand. Pp. 57-61 *in*: Proceedings of the NZFRI – IUFRO Conference: IUFRO '97 Genetics of Radiata Pine, 1-4 December 1997, Rotorua, NZ, R.D. Burdon and J.M. Moore (Eds.). New Zealand Forest Research Institute, FRI Bull. No. 203
- Lucas AI (de), Robledo-Arnuncio JJ, Hidalgo E, González-Martínez SC (2008) Mating system and pollen gene flow in Mediterranean maritime pine. *Heredity* 100: 390-399
- Moran GF, Bell JC, Eldridge KG (1988) The genetic structure and the conservation of the five natural-populations of *Pinus radiata*. *Can J For Res* 18: 506-514
- Muona O (1990) Plant populations, genetics, breeding and genetic resources. *In*: Population Genetics in Tree Improvement, pp. 282-298. Sinauer Assoc., Sunderland, MA, USA
- Namkoong G, Barnes RD, Burley J (1980). A philosophy of breeding strategy for tropical forest trees. Tropical Forest Papers No. 16, Oxford Forestry Institute, University of Oxford, UK. 67 p
- Millar CI (2000) Evolution and biogeography of *Pinus radiata* with a proposed revision of the Quaternary history. *NZ J For Sic* 29: 335-365
- Namroud MC, Beaulieu J, Juge N, Laroche J, Bousquet J (2008) Scanning the genome for gene single nucleotide polymorphisms involved in adaptive population differentiation in white spruce. *Mol Ecol* 17: 3599–3613

- Neale DB, Savolainen O (2004) Association genetics of complex traits in conifers. *Trends Plant Sci* 9: 325-330
- Oberbauer T (2003) A comparison of estimated historic and current vegetation community and structure on Guadalupe Island, Mexico. 143-153 *Proceedings of the Sixth California Islands Symposium*. 2005. D. K. Garcelon and C. A. Schwemm (editors). National Park Service Technical Publication CHIS-05-01, Institute for Wildlife Studies, Arcata, California
- Oberbauer T (2006) La Vegetacion de isla Guadalupe. Entonces Y Ahora. *Gaceta Ecologica* 081. Instituto Nacional de Ecologia. Distrito Federal, Mexico pp 47-58
- Ouborg NJ, Angeloni F, Vergeer P. (2010) An essay on the necessity of conservation genomics *Conserv Genet* 11: 643-653
- Raddi S, Stefanini FM, Camussi A, Giannini R (1994) Forest decline index and genetic variability in *Picea abies* (L.) Karst. *For Genet* 1: 33-40
- Richards, C.M. (2000) Inbreeding depression and genetic rescue in a plant metapopulation. *Am. Nat.* 155, 383–394
- Ritland K (1996) Estimators of pairwise relatedness and inbreeding coefficients. *Genet Res* 67: 175-186
- Ritland K (2000) Marker-inferred relatedness as a tool for detecting heritability in nature. *Molecular Ecology* 9: 1195–1204
- Rogers DL (2002) *In situ* genetic conservation of Monterey pine (*Pinus radiata* D. Don): information and recommendations. Genetic resources conservation program, report no. 26. University of California, Davis, CA, USA. Also available at <http://www.grcp.ucdavis.edu/publications/MPinedex.htm>
- Rogers DL (2004) *In situ* genetic conservation of a naturally restricted and commercially widespread species, *Pinus radiata*. *For Ecol Manag* 197:311–322
- Rogers DL, Matheson AC, Vargas Hernández, JJ, Guerra Santos, JJ (2006) Genetic conservation of insular populations of Monterey pine (*Pinus Radiata* D. Don). *Biod Conserv* 15: 779-798

- Rumball W, Franklin IR, Frankham R, Sheldon BL (1994) Decline in heterozygosity under full-sib and double first-cousin inbreeding in *Drosophila melanogaster*. *Genetics* 136: 1039–1049.
- Savolainen O, Kärkkäinen K, Kuittinen H (1992) Estimating numbers of embryonic lethals in conifers. *Heredity* 69:308–314
- Sekino M, Sugaya T, Hara M, Taniguchi N (2004) Relatedness inferred from microsatellite genotypes as a tool for broodstock management of Japanese flounder *Paralichthys olivaceus*. *Aquaculture* 233: 163–172
- Shen R, Fan JB, Campbell D, Chang WH, Chen J, Doucet D, Yeakley J, et al (2005) High-throughput SNP genotyping on universal bead arrays. *Mut Res-Fund Mol Mec Mutagenesis* 573: 70-82
- Sorensen FC (2001) Effect of population outcrossing rate on inbreeding depression in *Pinus contorta* var. *murrayana* seedlings. *Scand J For Res* 16:391–403
- Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. *Landsc Ecol* 21:821–836
- Swindell WR, Bouzat JL (2006) Ancestral inbreeding reduces the magnitude of inbreeding depression in *Drosophila melanogaster*. *Evolution* 60: 762–767.
- Thornton KR, Jensen JD, Becquet C, Andolfatto P (2007) Progress and prospects in mapping recent selection in the genome. *Heredity* 98: 340-348
- Tibor, D., 1999. The CNPS rare plant program. *Fremontia* 27: 4–6.
- Tiira K, Laurila A, Enberg K, Piironen J, Aikio S, Primmer CRR (2006) Do dominants have higher heterozygosity? Social status and genetic variation in brown trout, *Salmo trutta*. *Behavioural Ecology and Sociobiology*, 59, 657–665.
- Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* 13: 921–935
- Visscher PM, Medland SE, Ferreira MAR, Morley KI, Martin NG (2006) Assumption-free estimation of heritability from genome-wide identity-by-descent sharing between full siblings. *PLoS Genetics* 2: e41
- Vogl C, Karhu A, Moran G, Savolainen O (2002) High resolution analysis of

- mating systems: inbreeding in natural populations of *Pinus radiata*. *J Evol Biol* 15: 433-439
- Wang J (2011) COANCESTRY- A program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources* 11: 141-145
- Williams CG, Savolainen O (1996) Inbreeding depression in conifers: implications for breeding strategy. *For Sci* 42:102–117
- Wu HX, Ivković M, Gapare WJ, Baltunis BS, Powell MB, McRae TA (2008) Breeding for wood quality and profit in radiata pine: a review of genetic parameters. *N Z J For Sci* 38: 56-87
- Wu HX, Spencer, DS, Matheson AC (1988). Evolution of inbreeding depression with tree age and effects on growth curve in radiata pine. Pp. 92-96 *in*: Proceedings of the NZFRI – IUFRO Conference: IUFRO '97 Genetics of Radiata Pine, 1-4 December 1997, Rotorua, NZ, R.D. Burdon and J.M. Moore (Eds.). New Zealand Forest Research Institute, FRI Bull. No. 203
- Zhang, H.-Y, Hang HE, Man-Zhong L, Xing WD (2008). A genome-wide transcription analysis reveals a close correlation of promoter INDEL polymorphism and heterotic gene expression in rice hybrids. *Mol. Plant* 1: 720–731



## 9. Itinerary

Dates	Destination	Activity
16 April 2010	Depart Canberra	
19 <sup>th</sup> April to 30 May 2010	North Carolina State University, Raleigh, NC	<ul style="list-style-type: none"> <li data-bbox="715 539 1281 680">(i) Meet with CAMCORE scientists and discuss CAMCORE <i>ex situ</i> gene conservation strategy for pines.</li> <li data-bbox="715 680 1281 754">(ii) Literature search and review of inbreeding in plants</li> <li data-bbox="715 754 1281 828">(iii) Coancestry estimation and simulations</li> <li data-bbox="715 828 1281 902">(iv) Design model building and testing different algorithms</li> <li data-bbox="715 902 1281 938">(v) Write-up (on-going)</li> <li data-bbox="715 938 1281 969">(vi) Seminar presentation</li> </ul>
31 <sup>st</sup> May 2010	Return Canberra	