Specialty Wood Products Research Partnership

A population-genomic and taxonomic study of *Eucalyptus argophloia* and *E. bosistoana*.

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FOREST GROWERS RESEARCH

Doctoral Research Proposal

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1. Title

A population-genomic and taxonomic study of *Eucalyptus argophloia* and *E. bosistoana.*

2. Summary

The New Zealand Dryland Forests Initiative (NZDFI) aims to create plantations of high-value *Eucalyptus* timber species in dry environments on the east coast of New Zealand. This would enable the sustainable production of naturally-durable hardwood in New Zealand as a substitute for CCA-treated pine and unsustainably harvested tropical hardwoods. For this purpose, Australian seed collections of five promising *Eucalyptus* species have been used since 2009 to establish progeny trials in New Zealand. These trials are used to select and breed plant lines with growth and wood properties that are desirable for the New Zealand environment. As part of this effort, NZDFI is interested in understanding how genomic and environmental variation interact to influence commercially important traits in the NZDFI progeny trials. My PhD research project is a component of this project. Its specific research questions are: 1) what is the taxonomic identity of a morphologically deviating population of *E. bosistoana,* 2) what is the patterns of genetic diversity and structure of *E. argophloia* and *E. bosistoana*, and 3) what is the mating system of *E. bosistoana*. I aim to address these questions using morphological, DNA sequence and Single Nucleotide Polymorphism (SNP) data. To be able to compile the latter data set, we joined the 'Eucalyptus 65kSNP Axiom array production and deployment initiative'.

3. Significant prior research (literature review)

The Radiata pine monoculture in New Zealand forestry plantations and its disadvantages

The targeted breeding and establishment of plantations of exotic species in New Zealand started in the mid-20th century when indigenous supplies of domestic timber were becoming exhausted as a result of continued deforestation (Mead 2013; Roche 2017). Since then, *Pinus radiata*, commonly known as Radiata pine or Monterey pine (originally from the Monterey peninsula in California), has played a pivotal role in fostering industrial plantations in the country. Today, most regions of New Zealand have Radiata pine plantation forests of various sizes, except for Central Otago and Fiordland. The total estimated net stocked plantation forest area in New Zealand was about 1,706,429 ha in April 2017, of which 90% was composed of Radiata pine trees (New Zealand Forest Owners Association 2018). As a plentiful, versatile, and readily available timber, *P. radiata* provides source material for a broad range of products for export and domestic use, such as wood chips, logs and poles, sawn timber and sleepers, pulp, paper and paperboard, reconstituted panels,

and plywood (Cown et al. 1996; Jayawickrama and Carson 2000; New Zealand Trade and Enterprise 2008; New Zealand Forest Owners Association 2018).

Pinus radiata forestry is a profitable industry in New Zealand. However, despite its versatility and good economic performance, concerns over the excessive reliance on *P. radiata* have been frequently raised (Sweet and Burdon 1983; Burdon and Miller 1995; Treeby 1997; Maclaren 2005; Millen et al. 2018). For example, Maclaren (2005) pointed out that: (1) the lack of biodiversity as a consequence of monocultures of *P. radiata* may make the industry vulnerable to introduced pests and diseases, (2) overspecialization in a single market of softwood may hinder the prevision for future business opportunities of hardwood, (3) the diverse demands of domestic consumers might not be met due to a lack of product choice, and that (4) people prefer to see more variety in the landscape rather than landscapes dominated by a single species, given that they value its aesthetic qualities and biodiversity. There are also worries about climate change which will bring a warmer environment to the eastern part of New Zealand, but will also result in more frequent and severe droughts and intense storms, leaving the single species forestry industry of Radiata pine vulnerable to it (Millen et al. 2018).

Radiata pine is not naturally durable and needs preservative treatment when it is used for exterior uses and structural purposes (Cown et al. 1996). Copper Chrome Arsenate (CCA) is a cost-effective preservative that protects non-durable wood like *P. radiata* against decay caused by fungi and insects. CCA is toxic and excessive exposure to it may cause various cancers, cardiovascular disease and neurological disorders (Jomova et al. 2011). For that reason, the use of CCA is prohibited in Europe and its use is partly restricted in Australia and USA (Ramage et al. 2017). Burning CCA waste releases its active elements into the atmosphere and soil and is therefore only permitted in approved incinerators in many countries (Stephan et al. 1996; Humar et al. 2004). In addition, several cases of leaching of copper, chromium and arsenic from preservative-treated-wood have been reported in New Zealand, for instance from CCA-treated *P. radiata* vineyard posts in Marlborough and kiwifruit orchards in the Bay of Plenty region (Barlow and Prew 2005; Robinson et al. 2005). While the impact of CCA leachate on the environment may differ depending on rainfall conditions and most of the reported cases in New Zealand showed relatively moderate leaching, diffusion of heavy metal can result in significant environmental damage (Stephan *et al.*, 1996; Hedley, 1997; Humar *et al.*, 2004; Barlow and Prew, 2005; Apiolaza *et al.*, 2011).

Because of the aforementioned concerns about the Radiata pine monoculture and the adverse effects of chemically-treated wood on human health and the environment, naturally durable timber species of certain eucalypts, cypresses and redwoods have been suggested as an alternative to CCA-treated radiata pine timber (Sutton, 1998; Hocking, 2003; Maclaren, 2004, 2005; Robinson *et al.*, 2005; Nicholas and Garner, 2007; Cown, 2008; Apiolaza *et al.*, 2011; Millen *et al.*, 2018). Although some naturally durable hardwood timber-species have been used in forestry plantations in New Zealand for quite some time, they have thus far only received limited market interest (Roper 2006; Page and Singh 2014). This is because of the abundant availability of relatively cheap and treated radiata pine products (Apiolaza et al. 2011a). Furthermore, Radiata pine remains popular with foresters, because of the versatility of its timber, its relatively short harvesting rotation, and the availability of extensive information about *P. radiata* silviculture which has accumulated as a result of a century of research and industry experience (Hocking 2003; Nicholas and Garner 2007). However, despite of these advantages, the market is now much more aware of the range of environmental and safety concerns caused by chemical-treated timbers and international consumer resistance is increasing (Millen et al. 2018). As a result, treated timbers are becoming less attractive

to many sectors and markets. This has resulted in a gap in the market that can be filled by treatment-free timbers (Nicholas and Garner 2007; Apiolaza et al. 2011a; Millen et al. 2018).

Market opportunity for sustainably produced hardwood timber products

There is now diverse international and domestic market demand for sustainably produced high-value and naturally-durable hardwood (Nicholas and Garner 2007; Grealy 2008; Millen 2009, 2018). Comprehensive market research for various wood products from, for example, durable eucalypt species suggests a high potential for the establishment of more financially viable plantations of hardwood species and attracting investors (Grealy 2008). Several durable hardwood species are popular because of their aesthetic value and high stiffness. These have traditionally come from primeval forests, but demand for them is now outstripping natural supplies. The sustainable supply of timber products from plantations is actively supported and required by many governments including those of Australia, EU countries, New Zealand, and USA, because of a global effort to combat the trade in highly sought-after illegally harvested durable timbers of threatened species and ecosystems (Millen 2018). In addition, people are increasingly likely to pay more for environment-friendly and sustainably produced timbers (Vlosky et al. 1999).

Grealy (2008) distinguished three market groups for durable hardwood timbers: round timbers, sawn timbers and pulpwood residues. The market for round timbers, which are used for poles, piles, girders and landscaping, is the most valuable as it offers considerable economic benefits because of their high value and the moderate processing costs. Sawn timber is important as not all log products are suitable for producing round timber. The quality of sawn timber significantly relies on the natural durability of the timber (e.g. quality of heartwood) because the preservative treatment is less effective in the heartwood of the hardwood species. The pulpwood residues market is equally important. Although residues from durable hardwood are usually less suitable as pulpwood than softwood, a number of products and markets are available.

According to the Food and Agriculture Organization of the United Nations (FAO), the total volume of imported industrial round and sawn hardwood timber has been steadily increasing, except at the time of the global economic recession (Figure 1). The fastest hardwood market recovery and growth is observed in Asia (Mantau et al. 2018). Up to 15 million m^3 of hardwood logs and 10 million m^3 of hardwood sawn timber worth 8.4 billion USD are annually imported by China, indicating a huge market opportunity for foresters (FAO 2016; Millen 2018). Also Europe remains a big market for hardwood lumber, although the net import quantity has been decreasing since 2008 (Luppold and Bumgardner 2015).

Burdon and Miller (1995) categorized three groups of plantation-forestry species that can be used as naturally-durable alternatives to *P. radiata* in New Zealand: special-purpose species, extreme-site species, and contingency species. Special-purpose species are species that can be used for purposes for which *P. radiata* is not well suited, such as high-value furniture, veneer timbers, specialty industrial wood and many more. Where environmental conditions are too harsh to grow radiata pines (e.g. at high elevation or because of the presence of snow, or aridity) extreme-site species can be used. Lastly, contingency species can be used when the use of *P. radiata* cannot be continued for any reason. These species should be easy to establish and suitable for a wide range of end uses.

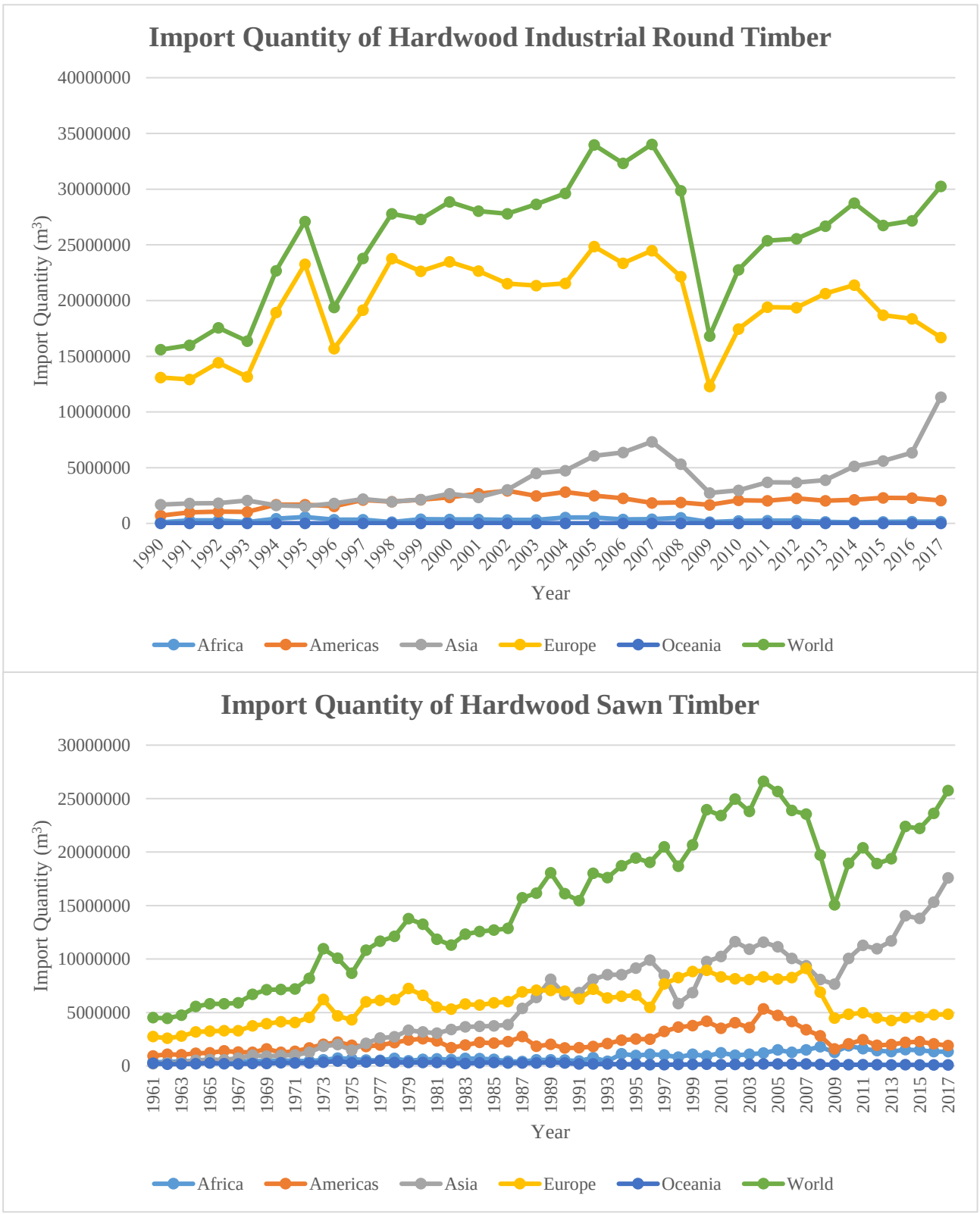

Figure 1. Import Quantity of Hardwood Industrial Round and Sawn Timber (Source: UN FAO).

Naturally durable *Eucalyptus* as alternative to Radiata pine

Some *Eucalyptus* species show significant potential as substitutes of *P. radiata* in plantations in New Zealand, because they are widely suitable for targeting the aforementioned categories of both hardwood timber markets groups and high-potential alternative species (Burdon and Miller 1995;

Nicholas and Garner 2007; Apiolaza et al. 2011a; Millen et al. 2018). Since 1976, comprehensive trial tests and breeding research have been conducted for several non-durable *Eucalyptus* timber species, including *E. delegatensis*, *E. fraxinoides, E. fastigata, E. nitens*, and *E. regnans,* targeted mainly for pulpwood production (Johnson and Wilcox 1989). Currently, *E. nitens* is grown at industrial-scale plantations in the South Island and ongoing breeding research involving this species aims to improve its wood quality (Apiolaza *et al.*, 2011; Suontama *et al.*, 2018).

For the durable timber *Eucalyptus* species, The New Zealand Dryland Forests Initiative (NZDFI) is leading a breeding program. Although numerous eucalypt species have been tested in New Zealand for solid wood production, there was no long-term, systematically funded and organized breeding program until the NZDFI (Ballekom and Millen 2017). Since its inception in 2008, companies collaborating with the NZDFI have been producing and selling ground-durable eucalypt timbers for poles and posts in vineyard, kiwifruit orchards, organic farms and for general farm uses, although their production is still at a small scale (Millen 2018).

The naturally durable eucalyptus species selected by the NZDFI (*E. argophloia, E. bosistoana, E. globoidea, E. macrorhyncha, E. quadrangulata*, *E. saligna,* and a few others) show good drought tolerance in New Zealand's eastern dryland region while producing highly durable timber (Millen et al. 2018). Much of the east coast of New Zealand has low rainfall and rainfall levels are likely to be less predictable in the future due to climate change. A study showed that *P. radiata* plantations are not performing as well and their productivity is lower in dry regions compared to other areas in New Zealand and in comparison with the dairy industry in the same area, considering equivalent amounts of investment (Palmer et al. 2010). This has resulted in the decision to remove many of the radiata pine plantations in Canterbury. There is therefore an opportunity to instead use environmentally marginalized regions such as dryland in the eastern part of New Zealand for growing better adapted alternative species (Apiolaza 2009; NZDFI 2013; Ballekom and Millen 2017).

When it comes to high-strength engineered wood products such as laminated veneer lumber (LVL), there is a market premium of 30% for super-stiff timber products that have a hardness of 16 GPa and above (Millen et al. 2018). Wood with this strength cannot be manufactured from radiata pine as it does not produce wood of high stiffness (Page and Singh 2014; Sharma and Altaner 2017; Millen et al. 2018). However, this desired quality and quantity of timber products can be obtained from some durable eucalypts. NZDFI-selected *Eucalyptus* species for the New Zealand environment including *E. bosistoana, E. cladocalyx, E. globoidea, E. quadrangulata, E. sideroxylon* and *E. tricarpa* have these high durability values (Sharma and Altaner 2017). Additionally, amongst potential alternative species, only *Eucalyptus* species can challenge *P. radiata* in terms of growth rates and productivity on ordinary plantation forestry sites (Burdon and Miller 1995).

In conclusion, durable eucalypt timber is hard, strong, and naturally resistant to decay, so it can be used without any chemical treatment. On sites that are optimal for *Eucalyptus* species that are adapted to low-rainfall habitats such as New Zealand's eastern dryland regions, eucalypts can achieve high growth rates, even exceeding those of radiata pine, while producing highly durable timber. However, there has been very little formal genetic improvement work on any durable eucalypts to date. This proposed PhD project aims to conduct research to help establish a newly emerging hardwood forest industry based on naturally durable eucalyptus species by informing their breeding programs. It is titled 'A population-genomic and taxonomic study of *Eucalyptus argophloia* and *E. bosistoana*' and carried out in collaboration with the NZDFI.

The breeding program of the New Zealand Dryland Forests Initiative (NZDFI)

Numerous *Eucalyptus* species from several Southern Hemisphere countries such as Brazil, Chile and Uruguay were tested in the 1970s to identify suitable species for large-scale use in commercial forestry plantations (FAO 1981). The purpose of the plantations was mainly to service the pulp industry and breeding programs were established using the most promising species, for example, *E. urophylla* x *E. grandis* hybrids in Brazil, *E. globulus* and *E. nitens* in Chile, and *E. globulus* in Uruguay (NZDFI 2017a).

In the same period, governmental institutes in New Zealand such as the Ministry of Works and Development Plant Division and the New Zealand Forest Research Institute (FRI), later Scion, began similar research (NZDFI 2017a). Only *Eucalyptus nitens* became a source of commercial forestry for producing pulp in New Zealand.

In 2008, the NZDFI was established to advance commercially-oriented research and the development of naturally durable eucalypt timber plantations. It aims to develop genetically improved planting stock and management systems for high-value *Eucalyptus* species that are suited for dry environments on the east coast of the South Island in New Zealand (NZDFI 2013). For this purpose, the initiative collected seeds of promising species from Australia, and established progeny trials in various environments in New Zealand to determine if they show desirable growth and can produce wood of high quality. The NZDFI aims to establish 100,000 hectares of durable eucalypt plantation by 2050 (Ballekom and Millen 2017).

In conventional breeding programs, genetic gain is a core concept that describes the genetic improvement (Xu et al. 2017). The genetic gain per year for selection, commonly known as the "breeder's equation," can be described as $\Delta G = (\sigma_a)(i)(r) / L$, where ΔG is genetic gain per year (also called response to selection), σ_a is additive genetic variation of the trait that the breeder wants to improve within the population, i is selection intensity (a standardized selection differential, which relates both the proportion of the population that is being selected and its variability), r is selection accuracy (correlation between what the breeder is assessing when selecting and what the breeder wants to breed for), and L is number of years per breeding cycle (Lush 1937; Eberhart 1970). In summary, genetic gain per year is directly proportional to the genetic variability, selection intensity and selection accuracy and inversely proportional to the time required for the breeding. As improving genetic gain is a major objective of a breeding program, measuring the breeder's equation is one of the primary tasks in the NZDFI breeding program. The key traits of eucalypt trees that the NZDFI breeding program selects for are the quantity and quality of heartwood, growth-strain, growth and eucalypt health such as pest tolerance.

To maximize the genetic gain of the selected species in the breeding program, the NZDFI aims to capture as much genetic diversity as possible for each species (NZDFI 2017a). For that reason, a large pool of genetic material was introduced to the programme by extensive seed collecting from a broad geographical range in Australia. These seeds were imported to New Zealand. According to the NZDFI Research Plans in 2017, the performance of eucalypts is more site-specific than that of radiata pine. Determining their performance therefore required relatively many trial sites in a large number of localities.

Prior to selecting the most promising naturally durable eucalypt species, comprehensive tests were conducted in New Zealand. Based on a previous evaluation of species performance and site requirements conducted by the FRI in 1991 and subsequent trial tests from 2003 and 2006 by both FRI and the New Zealand Farm Forestry Association (NZFFA), NZDFI identified the best performing species from those available (Millen et al. 2018). The wood durability of the candidate species was determined using the Australasian natural durability classification system (AS 5606-2005) as described in Table 1. The five species selected for further trials (*E. argophloia, E. bosistoana, E. globoidea, E. quadrangulata* and *E. tricarpa*) not only have good durability, but are also well adapted to the New Zealand environments, fast growing, have straight stems, are pest tolerant, and have potential as wood products in the market (e.g. aesthetic value) (NZDFI 2017a; Millen et al. 2018). A study of *E. bosistoana* at three sites showed a low degree of genotype by environment interaction (GxE), a differential response of genotypes to varying environmental conditions, which suggests that the New Zealand environment is suitable for the species (Apiolaza *et al.*, 2011). In addition to the five species selected for the NZDFI trials, six other species (*E. camaldulensis, E. cladocalyx, E. eugenioides, E. longifolia, E. macrorhyncha* and *E. notabilis*) that were considered to be of interest were planted together in small demonstration trials in several dry and warm areas of New Zealand (Ballekom and Millen 2017). All the seed collection from Australia and propagation by grafting for early selection was undertaken by Proseed NZ Ltd, a tree seed company and NZDFI partner (Schroeder 2017).

Table 1. Australasian natural durability classification system (AS 5606-2005) and NZDFI durable eucalypt selection.

Australasian classification	**Description**	**NZDFI naturally durable eucalypt selection (scientific name, common name)**
Class 1	Very durable	*E. argophloia* (Western white gum)*, E. bosistoana* (Coast grey box)*, E. tricarpa* (Red iron bark)
Class 2	Durable	*E. globoidea* (White stringybark)*, E. quadrangulata* (White-topped box gum)
Class 3	Moderately durable	
Class 4	Non-durable	

All seeds collected from a single eucalypt tree that was sampled in Australia are referred to as a 'family'. Whereas the mother tree of these seeds is known, the father is unknown. The collected seeds in a family are possibly half-siblings (i.e. same mother, but different father), but the precise family relationship is not known, i.e. whether they are 'identical twins' due to selfing, full siblings (i.e. same mom and dad) or half-siblings. Information about the family identity of each seed was maintained during the various stages of the trials, including seed collection and transportation, nursery of seeds for germination, planting germinated seedlings out in the trial plots, adaptation assessment (e.g. growth and wood qualities), etc. Trials of plants from each family were established at multiple sites in various New Zealand environments to be able to determine environmental effects on adaptation. At each site, plants of each species are planted together in separate blocks.

Within these blocks, trees from different families are planted in a randomized design. Trees are typically planted at 2315 stems per hectare (1.8m x 2.4m per stem). Each species is replicated at three or more different sites. This design ensures control of environmental variation at site level and enables between- and within-family selection.

Three key species (*E. bosistoana, E. globoidea* and *E. quadrangulata*) were selected as the focus for genetic tree improvement and *E. argophloia* and *E. tricarpa* were also selected due to their potential ability to hybridize with *E. bosistoana* (Ballekom and Millen 2017; Millen et al. 2018), with the aim to be able to introduce desirable traits of *E. argophloia* and *E. tricarpa* into *E. bosistoana* lineages. Since 2017, 23 breeding trials with more than 150,000 individual trees from the five species at 10 properties in four regions (Marlborough, Wairarapa, Hawkes Bay and Gisborne) were established. The key species are represented by numerous family groups: 192 groups for *E. bosistoana*, 161 for *E. globoidea* and 104 for *E. quadrangulata*. More families are expected to be included in the program as additional seed becomes available (Millen et al. 2018). *Eucalyptus argophloia* and *E. tricarpa* are represented by a relatively small number of family groups in New Zealand and their breeding programs in Australia are also small.

As part of the NZDFI, various research projects are carried out to support the breeding programme. Examples are projects on identifying trees with high levels of extractives, understanding and mitigating growth-strain, and exploring hybridization as a breeding method.

Extractives like secondary metabolites, organic compounds produced by plants, are playing an important role in making trees durable and accumulate in the heartwood of trees (Li and Altaner 2018). The extractives provide resistance to decay. Thus, the natural durability of timber is widely determined by the amount of extractives in heartwood (Hart and Hillis 1972; Hart 1981; Haupt et al. 2003; Aloui et al. 2004). A core extraction method is used by NZDFI researchers to identify trees with the highest levels of extractives at a young age. The amount of extractives and accordingly the amount of heartwood in trees is highly variable (NZDFI, 2017b; Mishra, 2018; Mishra et al., 2018).

Growth-strain is a major challenge for breeding eucalypt species for the purpose of providing a stable source of solid wood products as it results in splitting and deforming the log during wood processing (Malan 1995; Yang et al. 2002). To better understand this problem, the NZDFI research team developed a new method for assessing growth-strain more quickly and reliably in young *Eucalyptus* trees (Schroeder and Altaner 2016; NZDFI 2017b).

Hybridization of *Eucalyptus* species is also explored by the NZDFI. Artificial *Eucalyptus* hybrids are significant in plantation forestry in sub-tropical and tropical regions of countries like China, Congo, Brazil and South Africa (Potts and Dungey 2004; NZDFI 2017a). They are important because interspecific hybrids might capture complementary traits of their parental species that can improve breeding value, such as improving resistance to droughtiness (NZDFI 2017a), through additivity. However, hybrids might be more susceptible to pests than parental species because of 'hybrid susceptibility': hybrids sometimes have greater parasite loads than their parental species (Morrow et al. 1994; Whitham et al. 1994; Leboldus et al. 2013). Natural hybridization between *Eucalyptus* species and 'hybrid superiority' (e.g. heterosis) does occur in nature, although only a few cases have been reported (Stokoe et al. 2001). To date, only a few commercially affordable hybrids are used in plantations due to problems related to hybrid susceptibility, most notably *E. grandis* x *E. urophylla* in Brazil (Potts and Dungey 2004). Nevertheless, a small number of trials of *E. argophloia* and *E. tricarpa* have been established in the NZDFI progeny tests for their potential

to hybridize with *E. bosistoana* to introduce red timber coloring in the latter species, because this is a quality that customers prefer.

An introduction to *E. bosistoana* and *E. argophloia*

NZDFI selected *E. bosistoana* as one of its key species, because of its high durability (Class 1), its apparent wide site tolerance throughout New Zealand and its success in early and more recent Marlborough trials (Nicholas and Millen 2012). Although *E. argophloia* did not perform as well as *E. bosistoana*, it was selected for potential artificial hybridization with *E. bosistoana*.

Both *E. bosistoana* and *E. argophloia* are currently classified in *Eucalyptus* subgenus *Symphyomyrtus* section *Adnataria* (the boxes) (Brooker 2000). Both species have fruits with 5 or 6 valves, a moderate leaf reticulation, oil glands that are not noticeably irregular, an outer operculum that is united to the inner operculum, and have stamens that are all fertile (Brooker 2000). Amongst others, *E. bosistoana* differs from *E. argophloia* in the finely fibrous or smooth white bark (vs. smooth and usually white, but seasonally brown, yellow or pink-grey), a longer conical (vs. hemispherical) operculum and longer fruit (Chippendale 1988). The morphological characteristics of *E. bosistoana* and *E. argophloia* are compared in more detail in Table 2. The natural and cultivated occurrence records of *E. bosistoana* and *E. argophloia* in Australia are shown in Figure 2.

Also known as “Coast Grey box” or “Bosisto’s box”, *E. bosistoana* was first formally described by Ferdinand von Mueller in 1895. *Eucalyptus bosistoana* naturally occurs in New South Wales and Victoria in Australia along the eastern coast. The conservation status of *E. bosistoana* is rare in Victoria but not considered otherwise threatened. The wood of *E. bosistoana* is hard, strong and durable, although sometimes susceptible to borers, and has been used for heavy construction, poles, railway sleepers and fences (Chippendale 1988).

Eucalyptus argophloia, commonly known as the “Queensland western white gum”, “Queensland white gum”, “Scrub gum”, “Lapunyah”, “Burncluith gum” or “Chinchilla white gum” was first formally described by William Faris Blakely in 1934. It is endemic to a very small area in Chinchilla in Queensland and only a single wild population is known. This species is classified as 'vulnerable' under the Queensland Nature Conservation Act (1992). Around 1000 mature wild trees remain in the wild with little natural regeneration as most of the species’ habitat has been cleared for agriculture (Lee et al., 2011). The species is of high value for its potential value in wood production. The wood is hard, strong and durable, and has been used for fencing and general construction (Chippendale 1988).

	E. bosistoana	*E. argophloia*
Tree	to 60 m tall. Forming a lignotuber.	to 30 m tall. Forming a lignotuber.
Bark	partly or wholly rough on trunk but sometimes almost smooth, branches always smooth; rough bark box-type, thin and flaky, mottled grey and white patches or grey-brown, smooth bark white, cream, yellow or grey, sometimes with ribbons of decorticated bark in the upper branches.	smooth throughout, sometimes with thin flakes or strips of decorticating bark persisting on the lower trunk. Smooth bark grey or reddish grey or brown over white to creamy-white to yellow.
Juvenile growth	stem rounded in cross-section; juvenile leaves opposite for a few pairs then alternate, petiolate, oblong to elliptical to ovate, 3–7 cm long, 1.8–3.3 cm wide, margin entire, rarely crenulate, pale green.	stems square to round in cross-section; juvenile leaves shortly petiolate, opposite for up to ca 6 nodes then alternate, narrowly lanceolate to linear, 4.5–9 cm long, 0.4–1.4 cm wide, green.
Adult leaves	alternate, petiole 1–1.8 cm long; blade lanceolate to falcate, 5.8–20 cm long, 0.7–2.7 cm wide, base tapering to petiole, concolorous, glossy or dull, green, side-veins acute or sometimes at an angle greater than 45°, densely to very densely reticulate, intramarginal vein well removed from margin, oil glands island and intersectional.	alternate, petioles 0.5–1.5 cm long; blade narrowly lanceolate, 6.5–14 cm long, 0.7–1.5(2) cm wide, base tapering to petiole, margin entire, apex pointed, concolorous, glossy green, rarely dull grey-green to slightly glaucous, side-veins acute, somewhat irregularly spaced, reticulation moderate to dense, intramarginal vein remote from margin, oil glands irregular in shape, mostly island.
Inflorescence	usually axillary unbranched, less commonly terminal compound, peduncles 0.7–1 cm long, buds 7 per umbel, pedicels 0.3–0.8 cm long.	axillary single, peduncles 0.5–1 cm long, buds 7 per umbel, pedicels 0.1–0.4 cm long.
Mature buds	obovoid to ovoid, 0.6–0.8 cm long, 0.3–0.5 cm wide, green to yellow, smooth, scar absent, operculum conical to rounded rarely shortly beaked, stamens irregularly flexed, all fertile, anthers adnate to filaments, cuboid to globoid, dehiscing by broad lateral pores, style long, stigma pin-head shaped, locules mostly 5 or 6, the placentae each with 4 vertical ovule rows. Flowers white.	globular to obovoid to ovoid, 0.4–0.6 cm long, 0.3–0.4 cm wide, scar absent (both opercula shed together at flowering), operculum rounded, stamens inflexed, staminodes absent, anthers adnate, basifixed, cuboid to globoid, dehiscing by short lateral slits, style long and straight, stigma pin-head, locules regularly five, rarely 4, the placentae each with 4 vertical ovule rows. Flowers white.
Fruit	pedicellate (pedicels 0.4–0.7 cm long), cup-shaped, barrel-shaped or hemispherical, 0.4–0.8 cm long, 0.4–0.8 cm wide, disc descending, valves 5 or 6, near rim level or enclosed.	pedicellate or subsessile (pedicels to 0.6 cm long), hemispherical to cup-shaped, 0.3–0.5 cm long, 0.5–0.7 cm wide, disc descending vertically, valves regularly five, rarely 4, near the rim.
Seeds	black, brown or grey, 0.9–2 mm long, ovoid or flattened-ovoid, dorsal surface shallowly pitted or almost smooth, hilum ventral.	brown, 0.8–1 mm long, flattened-ovoid, dorsal surface shallowly reticulate, hilum ventral.
Cultivated seedlings	cotyledons bilobed to oblong; stems square in cross-section; leaves always petiolate, opposite for 5 to 10 nodes then alternate, ovate-orbicular, 4–8.5 cm long, 3–6.5 cm wide, base usually tapering, discolorous, mid-green above, paler beneath.	cotyledons reniform; stems square in cross-section; leaves always shortly petiolate, opposite for 6 to 8 nodes then becoming alternate, linear to narrowly lanceolate, 4.5–10.5 cm long, 0.3–1.2(1.8) cm wide, intramarginal vein prominent and well removed from the margin, green to grey-green, dull, discolorous. Seedlings highly branched
Recorded flowering time	January, February, March, April and July in Australia	May and June in Australia

Table 2. Morphological and flowering time comparison between E. bosistoana and E. argophloia (EUCLID, 2015)

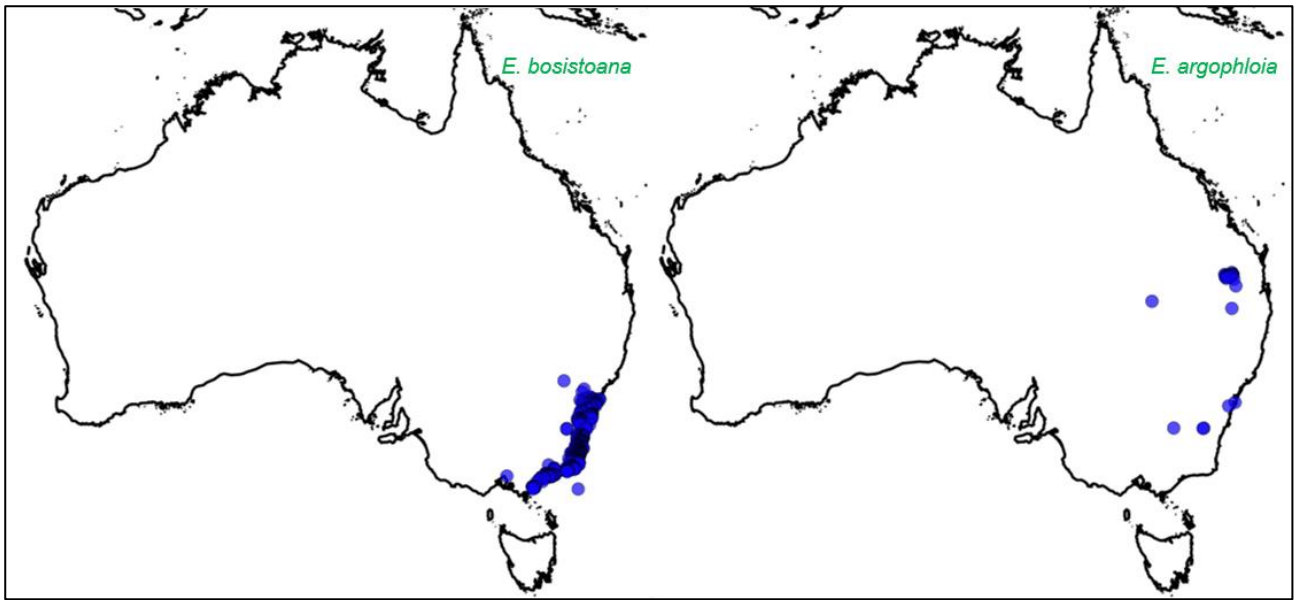

Figure 2. The natural and cultivated occurrence records of *E. bosistoana* (left) and *E. argophloia* (right) in Australia. (Australasian Virtual Herbarium)

Taxonomic confusion between *E. bosistoana* and *E. argophloia* in the NZDFI common garden experiments

Among the *E. bosistoana* plants grown in the NZDFI trials, some individuals are notably different in their morphology from most other plants. For example, all juvenile plants in Figure 3 are labelled as *E. bosistoana*, but they vary in leaf morphology. *Eucalyptus bosistoana* is recorded as having orbicular leaves when juvenile (Mueller 1895), but the young leaves of one of the two plants in the photo are lanceolate and resemble *E. argophloia* more closely. At present, the scale of this taxonomic problem in the NZDFI trials is not clear. It is presently unknown 1) approximately how many '*E. bosistoana*' plants show a deviating morphology, 2) if this always presents the same pattern (i.e. lanceolate instead of orbicular leaves) or there are plants that deviate in other characteristics, 3) how many families have deviating plants, 4) if some families are exclusively composed of deviating plants, 5) if some show a mixture of 'normal' and deviating plants, and 6) if the deviating plants come from one location in Australia or from all over the country.

The taxonomic confusion from the NZDFI trial plants might be a result of human error such as mislabeling during the collection of seeds and propagation or misidentification during seed collection. Also, this can potentially be explained the high adaptability and phenotypic plasticity that certain *Eucalyptus* species display in some environments (Grattapaglia et al. 2012). These hypotheses can be tested by determining if *E. bosistoana* plants with a deviating morphology genetically conform to non-deviating *E. bosistoana* plants or to a different *Eucalyptus* species.

It is also possible that the collected seeds from Australia are of hybrid origin. A recent study showed that *E. argophloia* not only hybridizes with intra-sectional relatives (*E. crebra*, *E. microcarpa* and *E. moluccana*) within section *Adnataria* but also with species in different sections (*E. pellita* and *E. resinifera*) from other section (Randall et al. 2016). Because the morphologically-deviating trees are labelled as *E. bosistoana* by experts, but their leaves look like *E. argophloia*, they might be hybrids between these two species. *Eucalyptus argophloia* is highly likely to hybridize with *E. bosistoana*, a potential close relative in the same section, but the relationship

between both species has not been critically studied (Lee et al. 2011; Thornhill et al. 2019). However, the natural distribution of *E. bosistoana* which is mostly in eastern New South Wales and Victoria in Australia is geographically distant from the small area of eastern Queensland where *E. argophloia* naturally occurs. Because of the allopatry, if the deviating plants are indeed hybrids between these species, they would most likely belong to families collected from the northern populations of *E. bosistoana*.

In this study, the taxonomic confusion between *E. bosistoana* and *E. argophloia* in the NZDFI trials will be examined by conducting morphometric and genomic taxonomic analysis in order to contribute to the efficient management of breeding populations.

Figure 3. Morphologically deviating *E. bosistoana* trees.

Genetic diversity and structure of *E. bosistoana* and *E. argophloia*

In breeding programmes, increasing genetic gain is a primary objective for breeding trees with desired properties. Genetic gain (ΔG) can be increased in the short term by increasing selection intensity (i), as per the breeders' equation ($\Delta G = (\sigma_a)(i)(r) / L$; as defined above). For example, as selection is continued from gene resource population (mother trees) to breeding population and eventually to production population, ΔG increases as a result of repeatedly selecting individuals as breeding stock that most strongly exhibit desirable traits (Figure 4). However, this eventually decreases the maximum ΔG because genetic diversity (σ_a, additive genetic variation) decreases through generations due to genetic drift, or random loss of alleles (gene variants) from the breeding population (Namkoong et al. 1988; Johnson and Lipow 2002).

Maintaining genetic diversity in gene resource population (breeding stock) of the breeding programme is important, because this provides the ability of addressing an unforeseen future need. To conserve genetic resources for future use, many breeding programs are using their first-generation selections (mother trees) as a gene resource population (Byram et al. 1999; Vallejo 1999; Lipow et al. 2002). Some methods for maintaining a gene resource population include maintaining a collection in seed banks, or seed orchards, or implementing a progeny or provenance test. A less costly and potentially more simple method is to conserve native habitat for wild populations. However, it is difficult for breeders to utilize a particular gene resource population if patterns of genetic diversity and structure among natural populations are unknown (Johnson et al. 2001). To aid geneticists and plant breeders in the efficient management and use of genetic resources for tree improvement, it is therefore important to understand patterns of genetic diversity and structure in natural populations from which breeding stock is obtained (Shi et al. 2017; Bernard et al. 2018).

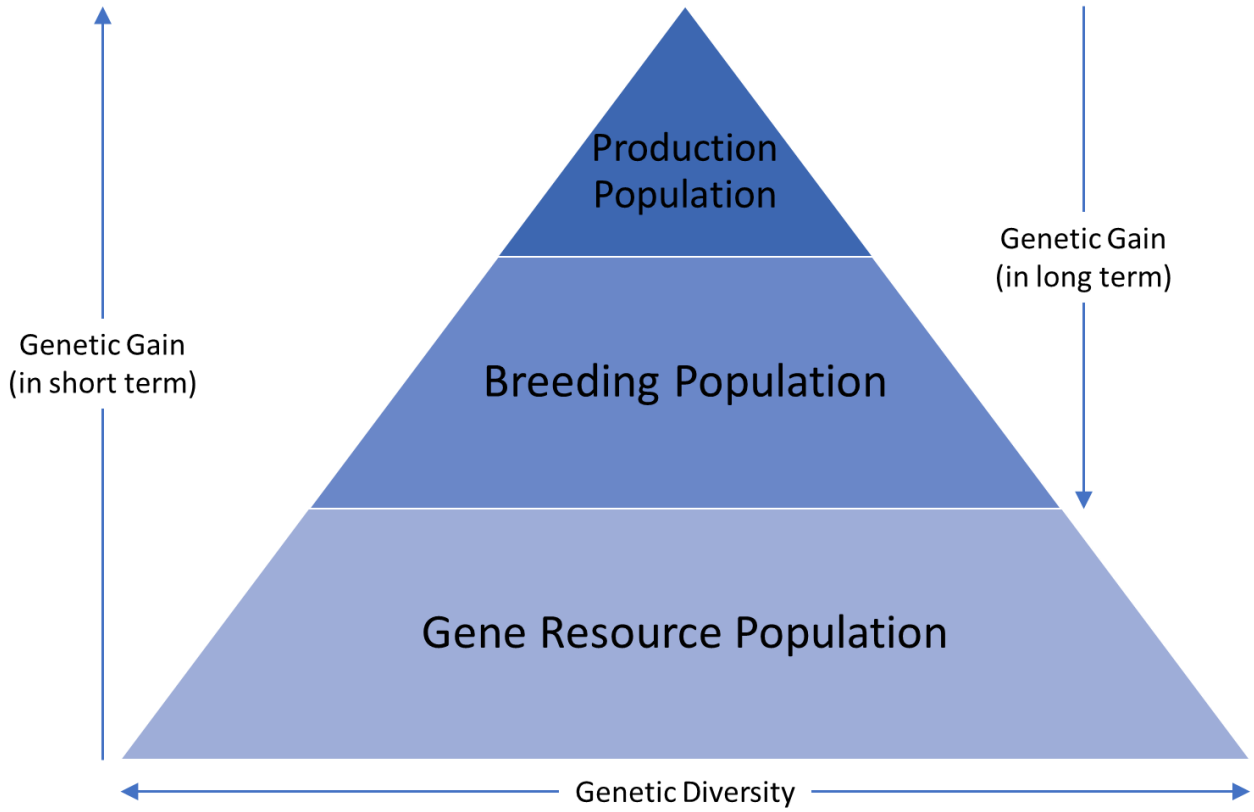

Figure 4. Conceptualization of genetic gain in the short and long term versus genetic diversity for a gene resource management program (Burdon 1995).

Prior to the start of common garden experiments, seeds of 192 *E. bosistoana* trees (mother trees) were selected from a broad geographic range in Australia with the aim of establishing a genetically diverse breeding stock for this naturally durable hardwood species in New Zealand (Figure 5). However, the patterns of genetic diversity and structure of the original natural populations are currently unknown. As a result, there is currently no informed strategy for augmenting the existing breeding stock with the aim of adding alleles of interest or increasing genetic diversity. Genetic diversity data are also important for informing the conservation management of *E. argophloia,* which is classified as a 'vulnerable' species and only has a single remaining natural population.

Because small eucalypt populations are often at risk of increased selfing and inbreeding (Butcher et al. 2005; Randall et al. 2016), knowing the genetic diversity of the *E. argophloia* population is important to inform conservation strategies. The goal of this study is therefore to determine patterns of genetic diversity and structure of *E. bosistoana* and *E. argophloia* using genomic analysis in order to contribute the NZDFI breeding programme and the conservation management *E. argophloia*.

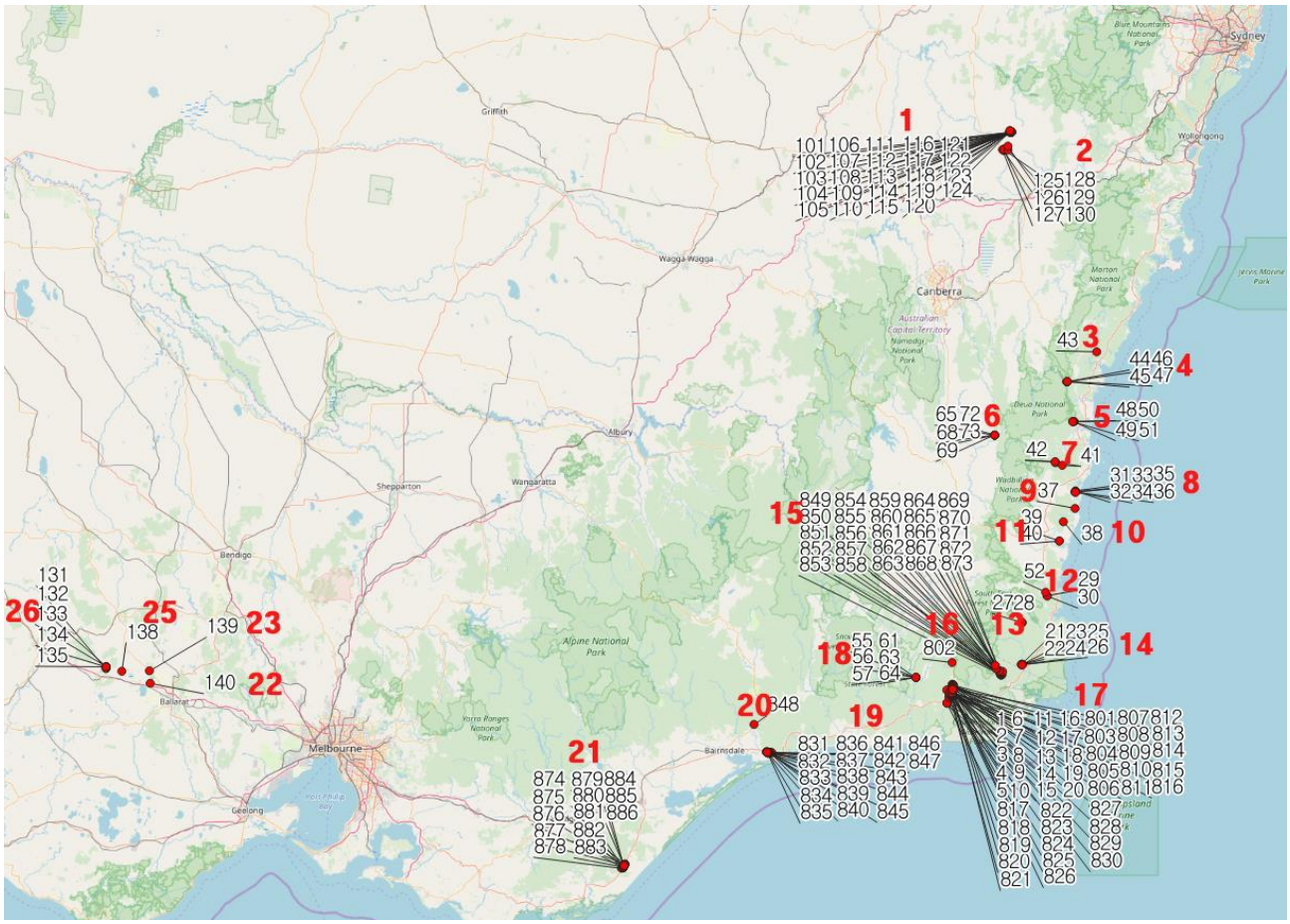

Figure 5. Natural population (mother trees) of *E. bosistoana* in Australia that selected for the NZDFI breeding programme. Family numbers are in black colour and population numbers in red.

Mating system within families of *E. bosistoana*

In breeding programmes, precise estimation of quantitative genetic parameters such as heritability (h^2) and genetic gain (ΔG) is important to ensure efficient selection (Bertoncini et al. 2017; Tambarussi et al. 2018). The calculated values of these parameters are dependent on assumptions about relatedness between individuals within the breeding population and this is in turn underpinned by knowledge of the mating system, as for instance measured by relationship coefficients (ρ, also called relatedness coefficient or correlation coefficient) (Weir et al. 2006; Gauzere et al. 2013; Tambarussi et al. 2018). For instance, when heritability in the narrow sense (realized heritability) is defined as $h^2=\sigma^2_{Family}/\rho \cdot \sigma^2_{Phenotypic}$ (where σ^2_{Family} is among-family genetic variance and $\sigma^2_{Phenotypic}$ is estimated phenotypic variance), the value of ρ that is used varies depending on mating system (i.e. selfing or outcrossing) and family-relatedness (e.g. when offspring is from the same mother, ρ is 1 for selfing, 0.5 for full-siblings, and 0.25 for half-siblings).

If members of the same family are considered to be half-sibs, the estimated value of the relationship coefficients (ρ=0.25) is based on assumptions that: (1) the female trees selected as a source of progeny collection are unrelated to each other, (2) the trees are outcrossing (no self-fertilization), (3) that the pollen mix arriving to the mother trees is from a high number of unrelated trees and (4) no assortative mating occurs (e.g. no preferential mating between males and females) (Gauzere et al. 2013).

Outcrossing rate (t) is a mating system parameter often used to obtain a rough estimation of relationship coefficients (Whitehead et al. 2018; Griffin et al. 2019). Studies on many plant species have been carried out under the assumption that they are either predominantly autogamous (i.e. self-fertilization) whereby its t ranges from 0.00 to 0.05 (ρ=1) or allogamous (i.e. outcrossing) with t between 0.95 and 1.00 (ρ=0.25) (Fuchs et al. 2015). When it comes to *Eucalyptus* species, however, t may range widely because of their mixed-mating system and, indeed, Eldridge (1993) recorded a range between 0.45 and 0.96 (Tambarussi et al. 2018). Thus, assuming that *Eucalyptus* species are allogamous and therefore using ρ=0.25 for calculating heritability will often lead to errors in calculating heritability. However, many foresters still use the half-sibs model as the mating system of *Eucalyptus* species (Apiolaza *et al.*, 2011; Denis *et al.*, 2013; Tambarussi *et al.*, 2018), because determining the precise mating system is difficult without genetic data (e.g., because it might require tracing the pedigree of all the individuals).

The use of molecular markers for determining the mating system of forestry trees is now becoming more prevalent, and these provide better-informed estimates of the degree of selfing or outcrossing for breeding populations (Tambarussi et al. 2018). In this study, I aim to understand the mating systems of families of *E. bosistoana* as measured by relationship coefficients obtained from SNP data to better inform the NZDFI breeding program by enabling more accurate calculations of heritability.

Eucalyptus genomics

Genomic markers are heritable polymorphisms that can be measured in populations of individuals (Davey et al. 2011). Luikart *et al.* (2003) stated that the ideal approach for population genomics uses hundreds of polymorphic markers that cover the entire genome in a single, simple and reliable experiment. Thanks to advances in high-throughput sequencing (HTS) technologies, there are several such methods currently available, even for populations for which little or no genetic information is available, enabling sequencing and genotyping thousands of markers of interest in a single step (Davey et al. 2011). Among the various HTS methods, high-density Single Nucleotide Polymorphisms (SNPs) genotyping has become an important tool for gene discovery, association genetics, germplasm characterization, QTL mapping, molecular breeding and population genomics studies in many economically important plant species (Neale and Kremer 2011; Paiva et al. 2011). Plant genomes have many SNPs, for example, maize has 1 SNP per 60-120 bp, whereas humans have an estimated 1 SNP per 1,000 bp (Agarwal et al. 2008). The abundance of SNPs in plants has been exploited by researchers for genetic analysis for selection purposes. Development of SNP markers also helped to enhance the resolution and capacity of genomic analysis in less commonly domesticated plant species with less or no represented genomes such as those of certain crops, fruit and forest trees (Grattapaglia et al. 2011).

There are various ways to generate SNPs. One of the most powerful methods is whole-genome re-sequencing (WGRS), as this method provides the most markers, widely distributed throughout the genome. WGRS using long-read sequencing technology usually has a high initial cost to develop a reference genome and requires substantial bioinformatics computation power and effort to gain high DNA quality and quantity (de Villemereuil et al. 2016). However, recently, it is possible to generate a 'good enough' reference genome using short-read sequencing technologies that do not require large amounts of high-quality DNA. Despite of this, the costs are still higher than those of other approaches for generating SNPs (Galla et al. 2019). Still, WGRS is the ultimate high-throughput genotyping method that generates up to millions of SNP markers across the whole genome.

Genome representation sequencing like genotyping-by-sequencing (GBS) is a different approach for generating SNPs. The general principle of this method is to sequence only limited, but random parts of the genome to save the sequencing effort, because genotyping the whole genome of every individual in a population is often unnecessary and costly (Davey et al. 2011). GBS may be cost-effective for genotyping SNPs in a genome, but a disadvantage of this approach is that HTS data yielded by GBS often has high error rates caused by variety of factors, including alignment and base-calling errors (Qi et al. 2017). For example, when GBS uses a low-coverage approach (that is a low number of unique reads for reconstructing sequences) for sequencing and if the available number of individuals is large, the frequency of false positive SNP calls and missing data gets higher (Wong et al. 2015). Although the error rate can be decreased using a high-coverage approach, the increased cost cannot be ignored.

A SNP chip, which is a kind of DNA microarray, is useful for detecting polymorphisms within a population and to study slight variations between whole genomes. SNP chip methods generally have a higher reproducibility between biological and technical replicates for independent experiments and laboratories than GBS (Elshire et al. 2011; Silva-Junior et al. 2015) and have a very high efficiency in identifying off-type (i.e. outlier) samples within clones (Durán et al. 2018). However, the existing SNP chips do not work effectively for capturing rare variants in genetically diverse taxa because of ascertainment bias and this ultimately disturbs the identification of introduced segments from distantly related taxa (Rasheed et al. 2017). To develop less biased SNP chips, customization of SNP marker development methods is required. This may involve adding or changing customized SNPs on the chips, and combining markers from several chips for applying to broader genetic resources (Rasheed et al. 2017). Overcoming these shortfalls while taking advantage of the benefits of array based genotyping was accomplished by the development of customized SNP chips for several major grain (Ganal et al. 2011; Bekele et al. 2013; Song et al. 2013), vegetables (Felcher et al. 2012; Sim et al. 2012), fruit (Chagné et al. 2012; Verde et al. 2012), and forestry species (Chancerel et al. 2013; Geraldes et al. 2013; Pavy et al. 2013; Silva-Junior et al. 2015).

Despite their dominance in hardwood plantations worldwide, eucalypt breeding programmes are still in their early stages, and major genetic improvements for domestication have not yet been made (Grattapaglia 2004). However, there is a continuing effort to understand the genetic control of economically important traits of eucalypts. Among all Myrtaceae, *Eucalyptus* is the focal genus as indicated by the large volume of genomic literature about this taxon (Grattapaglia et al. 2012). The relatively small size of the *Eucalyptus* genome, varying between 600 and 700 Mbp (Grattapaglia and Bradshaw Jr. 1994), made whole-genome sequencing an accessible option. *Eucalyptus grandis*

was the first species in the Myrtales for which the whole genome was sequenced (Myburg et al. 2014). This enabled the creation of resources for genome-wide genetic analysis underpinning further genomic research in eucalypts. In addition, the SNP genotyping system of EuCHIP60K with 60,904 unique SNPs present on the chip was developed (Grattapaglia et al. 2011). This enabled a number of eucalyptus studies into genomic selection for breeding (Costa and Santos 2017; Resende et al. 2017; Ballesta et al. 2018; de Moraes et al. 2018; Durán et al. 2018). In New Zealand, Scion employed the EuCHIP60K to study the genetic architecture and relatedness of *E. nitens* as a potential pulp resource (Klápště et al. 2017; Klápšte et al. 2018; Suontama et al. 2018).

Following the development and production of the first Eucalyptus SNP chip (EuCHIP60K) in 2013 (Silva-Junior et al. 2015), a team of researchers established the Eucalyptus 65kSNP Axiom array production and deployment initiative (ESAI) to develop a second, improved version of a high-throughput SNP genotyping system. The University of Canterbury joined the ESAI to develop a customized SNP chip for our target species: *E. argophloia* and *E. bosistoana*. In this study, data generated by the 65kSNP SNP chip will be used to answer the three main research questions which are: 1) what is the taxonomic identity of a morphologically deviating population of *E. bosistoana,* 2) what is the patterns of genetic diversity and structure of *E. argophloia* and *E. bosistoana*, and 3) what is the mating system of within families of *E. bosistoana*.

4. Research approach or methodology

Plants of the NZDFI's breeding populations of *E. bosistoana* and *E. argophloia* have been planted in replicates of common garden trials at multiple locations in New Zealand (7 different trial sites in Avery, Cravens, Dillon, Ngaumu, Lawson, Martin and McNeil). These trials contain thousands of individuals of 192 and 33 family groups from each species, respectively. DNA of selected individuals from these trials will be used to generate SNP data using the ESAI SNP chip. These SNPs will be the main data that I will use for addressing my three research questions.

For the development of the 65K SNP Axiom array, we, as a participating member of ESAI, provided a set of 24 DNA samples (12 of each species) to be included in the screening of up to 420 thousand SNPs, which include SNPs from the previously developed EuCHIP60K array. Other participants contributed DNA from additional eucalypt species for the development of this chip. For this purpose, leaf samples of *E. bosistoana* and *E. argophloia* were collected from the NZDFI trial in Harewood, Christchurch in New Zealand. The DNeasy® Plant Mini Kit from Qiagen was used for DNA extraction. The general DNA extraction protocol recommended by the supplier was used. Each sample submitted for screening consisted of 60 µl of DNA at a concentration of around 30 µg/ul, as recommended in the Axiom service guide. Each sample was at the conventional absorbance ratios: 260/280 ~1.8 and 260/230 ~2.0, as measured with a Nanodrop ® ND-1000 Spectrophotometer. The prepared samples were dried using a SpeedVac and sent to Thermo Fisher Scientific (TF) in the USA for the development of the SNP array, which is coordinated by EMBRAPA Genetic Resources and Biotechnology in Brazil and TF.

In our agreement with ESAI and TF, we committed to submit a total of 1,536 samples for SNP genotyping in the next two years. This number is appropriate for achieving my research objectives. Below, I outline the sampling strategy for each chapter. For DNA extraction of those samples, the

PDQeX phytoGEM method from ZyGEM will be used. All the prepared samples will be dried using a SpeedVac before they will be sent to TF for genotyping.

To resolve the taxonomic confusion between *E. bosistoana* and *E. argophloia*, I aim to use morphometrics, a quantitative analysis of the morphology of organisms, to find patterns of morphological variation among 'pure' *E. bosistoana*, 'pure' *E. argophloia* and the group of *E. bosistoana* plants with a deviating morphology. I will contrast these patterns with patterns of genomic data obtained from these three groups. Around 50 individual trees of each group will be sampled (i.e. 50*3=150 samples). I will record characters of the leaves and fruit, such as fruit width at rim, adult leaf length and width, and leaf midrib thickness, and other characters that are listed in the literature as being potentially taxonomically informative (Collins et al. 2019). These data will be obtained from herbarium specimens, which will be prepared for each collected tissue sample, so that both morphometric and genomic data will be available for the same specimens. I will use the method of Collins et al. (2019) to analyze the morphological data. This involves analyzing the morphometric data in PATN (ver. 3.12, Blatant Fabrications, Belbin (1990)). All morphological characters will be weighted equally for cluster and ordination analyses aimed at visualizing patterns of morphological similarity and difference among individuals and groups of individuals. For this, an association matrix will be generated from these data using the Gower metric (Gower 1971), which includes range-standardization of each character. Phenograms will be produced using the flexible unweighted pair-group method with arithmetic mean (UPGMA). The semi-strong hybrid multidimensional scaling (SSH MDS) method will be used for the ordination analyses, and the results will be plotted in three dimensions using R (ver. 0.99.896, R Foundation for Statistical Computing, Vienna, Austria, see http://www.R-project.org/). To identify genomic patterns, genotyped SNPs data will be analyzed using STRUCTURE v. 2.3.4 (Pritchard et al. 2000) as described in the next paragraph.

For the study of patterns of genetic diversity and structure, the 192 family groups of *E. bosistoana* trees were each assigned to one of 26 putative populations. This was done on the basis of their geographical proximity and distribution in Australia (Figure 5). Around 35 individual trees for each of these 26 populations in the NZDFI trials will be sampled (i.e. 26*35=910 samples), which is a little more than the recommended number (25~30 individuals per population) of samples for population genetic studies (Hale et al. 2012). I aim to maximize the number of families that will be sampled in each population to get the best approximation of their genetic diversity. Because *E. argophloia* has only one geographical population, although it has 33 family groups, only 50 plants will be sampled. The acquired SNP data will be computationally analyzed to understand the patterns genetic diversity and structure among the populations. For the analysis of genetic structure, STRUCTURE v. 2.3.4 (Pritchard et al. 2000) will be used to study the genomic structure among populations. To determine the appropriate value of K (number of populations), STRUCTURE HARVESTER will be employed, using the Evanno method (Evanno et al. 2005; Earl and vonHoldt 2012). The results will be summarized using CLUMPAK v. 1.1 (Kopelman et al. 2015). Analysis of Molecular Variance (AMOVA) and Principal Coordinates Analysis (PCoA) will be conducted using GenAlEx v. 6.5 (Peakall and Smouse 2012). For the quantification of genetic diversity, allelic richness (AR), proportion of polymorphic markers (PN), observed heterozygosity (HO), and expected heterozygosity (HE) will be calculated using PLINK v1.9 software (Purcell et al. 2007).

For the study of the mating system, 30 plants from 16 families from 8 populations (two families per population) will be sampled and genotyped (i.e. 30*16=480 samples). One of the two families

per each population will be a poorly performing family (i.e. showing suboptimal values for important breeding traits) whereas the other plant is from a family displaying desirable traits in order to be able to identify possible cases of inbreeding depression or heterosis using pairwise relatedness analysis. For the statistical analysis of mating system and subsequent estimation of genetic parameters, the method used in Cappa et al. (2016) will be employed. The realized relationship matrices based on the SNPs data will be calculated using SPAGeDi software (Hardy and Vekemans 2002). Because marker-based relationship pairwise matrices are often not positive definite due to technical reasons, the ‘‘nearPD’’ function implemented in R package ‘‘Matrix’’ will be used to compute the nearest positive definite matrix from the original matrix. Finally, the variance components and derived genetic parameters such as relationship coefficient and heritability will be estimated by restricted maximum likelihood (REML, Patterson and Thompson (1971)) implemented in the ASReml statistical program (Gilmour et al. 2007).

5. Limitations and key assumptions

Because the total number of samples for genotyping is 1,536, which is immense for a single person, support for, or outsourcing of lab work might be needed.

In case the 65K SNP chip does not work, we are going to use GBS. GBS is widely used for population genomics and we can overcome the aforementioned drawbacks by using *de novo* genome assembly.

6. Proposed thesis chapters

1. Introduction and in-depth literature review
2. Taxonomic confusion between *E. bosistoana* and *E. argophloia* in the NZDFI common garden experiments
3. Population genetic structure of *E. argophloia* and *E. bosistoana* (and conservation management of *E. argophloia*?)
4. Population structure within families of *E. bosistoana* in NZDFI field site
5. Conclusions

7. Research plan

	2018 Q4			2019 Q1			2019 Q2			2019 Q3			2019 Q4			2020 Q1		
Enrolment																		
Supervisors meeting																		
Write research proposal																		
Submit research proposal & supervisor agreement																		

H&S induction																		
Conduct field work (sampling)																		
Conduct lab work (DNA extraction)																		
Data analysis																		
Write first draft																		
PhD confirmation report & oral presentation																		
Progress report																		
	2020 Q2			2020 Q3			2020 Q4			2021 Q1			2021 Q2			2021 Q3		
Progress report																		
Write second draft																		
Data analysis																		
Write final draft																		
PhD submission																		

8. Draft budget and resource requirement

1. 45,000 NZD (Approximately 30,000 USD) for TF SNPs genotyping
2. 14,000 NZD for ZyGEM DNA extraction kit and reagents
3. 1,000 NZD for field work (500 per trip, South and North Island in New Zealand)
4. 1,820 NZD for Eucalypt Genetics 2019 conference at University of Tasmania (including flights and accommodation)

These expenses can be covered from external funding through the Specialty Wood Products Partnership (SWP) contract.

9. Other considerations

Biosecurity risk management during field work

Risk Assessment

Preparation:

- Become familiar with the symptoms of myrtle rust and insect pests

- Wear disposable overalls and gloves and washable footwear (if Myrtle rust is a risk)

- Carry mobile phone (to contact MPI (0800 80 99 66) if required) and camera

- Carry several large plastic bags and several specimen containers

Inspection:

- Spend an hour walking up and down rows of eucalypts prior to collecting any material

- Closely inspect 20 – 30 trees across the site

- Conduct an overall assessment of each tree from a distance before touching

- Inspect tops and bottoms of foliage throughout the crown as well as stems, buds or flowers for myrtle rust symptoms

- Look for signs of insect feeding damage, eggs, larvae and adult beetles, particularly in association with flush foliage

Packing & unpacking protocols – Foliage

- Be vigilant through-out sampling and remove any insects or eggs detected (laying cut foliage on a white sheet can help in the detection of insects)

- Run fingers over foliage tips to detect paropsine eggs and remove

- Apply fungicide (Appendix 1) and insecticide (e.g. floragas) spray to packed foliage in chiller boxes before closing and tape shut https://www.boc.co.nz/shop/en/nz/gases/more-

gases/floragas

- Upon return to UC open each box in the 5th floor PC2 lab of Biological Sciences for visual inspection. Call MPI if any biosecurity hazards are detected. Plant material that enters the PC2 lab cannot be removed from the lab unless it is double bagged and destined to be autoclaved.

- Check the full protocol of 'NZDFI Biosecurity Risk Management Plan' when visiting the North Island.

Detection protocols

- If myrtle rust symptoms are detected in the field or during sample collection follow the NZPPI

protocol:

1) Do not move the plants from the site / vehicle / glasshouse

2) Take photos of the suspected myrtle rust and the whole plant.

3) Do not attempt to touch or collect samples as this may increase the spread of this disease.

4) If possible, isolate the plants with an igloo-hoop-like plastic cover.

5) Call MPI's exotic pests and diseases hotline on 0800 80 99 66

- If myrtle rust symptoms are detected when handling material in the UC PC2 lab:

1) Prevent anyone entering / leaving the room.

2) Photograph symptoms and call MPI on 0800 80 99 66 and follow instructions.

3) Cover the infected material if possible (e.g. replace lid on bins) and stop work on any other material to prevent spread.

4) Phone another staff member to ensure any material from the same consignment stored elsewhere (e.g. stored in warehouse / coolstore) is secured and pass on any other instructions from MPI.

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