2023

Re-establishment of pollen-mediated connectivity is key to successful restoration of fragmented populations of Eucalyptus albens

Ella R. Dunn
University of Wollongong

Re-establishment of pollen-mediated connectivity is key to successful restoration of fragmented populations of Eucalyptus albens

Abstract

An essential factor in assessing the success of woodland restorations is understanding whether gene flow and connectivity between restored and remnant populations has been re-established. Without pollinator services, isolated populations can further subdivide and face concerns of inbreeding depression, which is not the target of restoration projects. Within the 'Central Valley' of the Warrumbungle National Park, a series of restoration plantings were performed between the 1980s and 1990s to restore the previously abundant Box-Gum Grassy Woodlands, in particular White Box Gum trees (Eucalyptus albens). Extensive land clearing meant that these populations became extremely fragmented within the agricultural matrix, with only a few remaining extant remnant trees. This restoration was discovered to use locally sourced genetic material for plantations within the park, which is known to cause issues with inbreeding depression and lower genetic variability. Extending upon previous studies, I analysed relictual (historic scattered trees), natural (leftover extant populations), planted (restored trees) and sapling/ seedling populations (juveniles grown in situ and ex situ) of E. albens trees for the genetic diversity and population structure by extracting genomic DNA and genotyping of SNP presence and absence conducted using DArTseq microarray developed for Eucalypt species. For the first time for this species, a high-confidence paternity analysis of seedlings and a parent pair analysis of saplings were conducted from a range of populations and were used to quantify pollen-mediated gene flow respectively to analyse connectivity between populations. By combining all analyses, I assessed the genetic success of this mature restoration project, with a focus on determining whether planted populations of E. albens displayed comparable genetic diversity levels and population structure to those of their remnant cohorts and whether there was evidence of gene flow between these groups. Analysis of genetic diversity and differentiation in dartR yielded no significant difference in genetic diversity between all groups, and most populations were relatively homogenous (especially natural stands) in structure, except for two planted populations, that were sourced externally from the valley. Seedlings planted in situ had lower inbreeding levels, suggesting that there was further outcrossing between stands between generations. Parentage analysis revealed that planted and natural populations were outcrossing, suggesting successful gene flow and genetic compatibility. Overall, there was little negative effect of local provenance sourcing, and the restoration was actively producing many viable saplings ameliorating inbreeding issues.

Degree Type

Thesis

Degree Name

Bachelor of Conservation Biology (Honours)

Department

School of Earth, Atmospheric and Life Sciences

Advisor(s)

Natalie Rosser

Keywords

Restoration Ecology, Eucalyptus, gene flow, polinator services, genetic assessment

Re-establishment of pollen-mediated connectivity is key to successful restoration of fragmented populations of *Eucalyptus albens*

Ella R. Dunn

Bachelor of Conservation Biology (Honours)

University of Wollongong, Australia

School of School of Earth, Atmospheric and Life Sciences,

Faculty of Science, Medicine, and Health

October 2023

Supervisors: Andrew Denham DPE, Natalie Rosser PhD, Johanna Turnbull PhD

This thesis is submitted in accordance with the regulations of the University of Wollongong in partial fulfilment of the degree of BCons Biol Hons. It does not include any material published by another person without due reference within the text. The field and laboratory work presented in this thesis was performed by the author, expect where acknowledged. This thesis has not been submitted for a degree at any other university.

_____

ELLA R. DUNN

Dated: 05/10/2023

Thesis Abstract

An essential factor in assessing the success of woodland restorations is understanding whether gene flow and connectivity between restored and remnant populations has been re-established. Without pollinator services, isolated populations can further subdivide and face concerns of inbreeding depression, which is not the target of restoration projects. Within the 'Central Valley' of the Warrumbungle National Park, a series of restoration plantings were performed between the 1980s and 1990s to restore the previously abundant Box-Gum Grassy Woodlands, in particular White Box Gum trees (*Eucalyptus albens*). Extensive land clearing meant that these populations became extremely fragmented within the agricultural matrix, with only a few remaining extant remnant trees. This restoration was discovered to use locally sourced genetic material for plantations within the park, which is known to cause issues with inbreeding depression and lower genetic variability. Extending upon previous studies, I analysed relictual (historic scattered trees), natural (leftover extant populations), planted (restored trees) and sapling/seedling populations (juveniles grown in situ and ex situ) of *E. albens* trees for the genetic diversity and population structure by extracting genomic DNA and genotyping of SNP presence and absence conducted using DArTseq microarray developed for Eucalypt species. For the first time for this species, a high-confidence paternity analysis of seedlings and a parent pair analysis of saplings were conducted from a range of populations and were used to quantify pollen-mediated gene flow respectively to analyse connectivity between populations. By combining all analyses, I assessed the genetic success of this mature restoration project, with a focus on determining whether planted populations of *E. albens* displayed comparable genetic diversity levels and population structure to those of their remnant cohorts and whether there was evidence of gene flow between these groups. Analysis of genetic diversity and differentiation in dartR yielded no significant difference in genetic diversity between all groups, and most populations were relatively homogenous (especially natural stands) in structure, except for two planted populations, that were sourced externally from the valley. Seedlings planted in situ had lower inbreeding levels, suggesting that there was further outcrossing between stands between generations. Parentage analysis revealed that planted and natural populations were outcrossing, suggesting successful gene flow and genetic compatibility. Overall, there was little negative effect of local provenance sourcing, and the restoration was actively producing many viable saplings ameliorating inbreeding issues.

Table of Contents

Acknowledgements

I would like to acknowledge firstly all my supervisors; Andrew Denham, Natalie Rosser and Johanna Turnbull who guided me through this project and helped me with planning, field and lab work, data analysis, writing and editing. This project would not be possible without continuous assistance from my supervisors, and I thank them for this opportunity. Further, I would like to thank my friends and family, Michael Dunn, Karen Dunn, and Dallys Baker for volunteering their time to help me with lab preparation. Finally, I would like to acknowledge the contribution of the late Prof. David Ayre to my previous work, the use of samples within this study and the field of population genetics, he will be dearly missed. This study was funded by the NSW Department of Planning and Environment Saving our Species Science and Research grant to Andrew Denham and David Ayre.

List of Figures

List of Tables

1.0 Introduction

1.1 History of ecological restoration

Vegetation restoration has occurred across Australia for more than 50 years in hopes of returning more of the landscape to the previous, more forested environment. However, there is little guidance regarding the appropriate selection of seed sources to use in restoration efforts. Outbreeding depression can result when genetically distinct parents produce offspring of intermediate phenotype with reduced fitness. Since the 1990s these concerns have led to an increase in the use of locally adapted provenance sourcing where seeds are collected close to the restoration site (Broadhurst *et al*. 2008). Earlier in the efforts of restoration, the seed was often collected from very few, highly fragmented and fecund trees (sometimes even one) and these seeds were deployed across the environment for repopulation (Broadhurst 2013). Seeds were often collected from fragmented, small, inbred populations again resulting in low diversity and poor-quality planted populations. It is therefore highly likely that older restoration sites used poor-quality seeds leading to the establishment of populations with low genetic diversity, which has the potential to limit the long-term persistence of the restoration project and its ability to adapt to environmental change.

1.2 Costs and benefits of restorations

To restore a landscape to its previous healthy state, environmental managers must target the most effective way to conduct this process. The health and diversity of Australia's ecosystems and species are continuing to decline, as more than 100 extinctions of plants and animals have been recorded since European settlement (Woinarski *et al*. 2019). Eastern Australia is a hotspot for deforestation (WWF 2015) and Australia has been identified as the most vulnerable developed country to climate change impacts (IPCC 2014) as altered rainfall and temperature regimes accelerate ecosystem changes and plant and animal declines (Hughes *et al*. 2019). Australia has an urgent need to prevent the mass decline and extinction of native species, however, this process requires support from legislation and the Commonwealth government (for example, funding, policies, and legislation).

Eucalypt woodlands can be restored on non-prime agricultural land (5,543,942ha [45%]) which is estimated to cost approximately $10.3 million (Mappin *et al*. 2022). While this may seem unreachable in the near future, (Mappin *et al*. 2022) calculated the carbon revenue is estimated to be 53-219% of the total restoration cost. The cost of inaction on landscape degradation, biodiversity loss and climate change, especially for eucalypt woodlands, is high. The potential to earn up to 219% of carbon revenue after restoring eucalypts means the federal government is missing this opportunity. Large-scale landscape restoration is a win-win solution for combating future climate change and reducing Australia's valuable biodiversity loss. It must be utilised to our advantage to return Australia's lost vegetation and reduce the cascading trophic effects of loss of trees on biodiversity and ecosystems.

1.3 How to measure restoration success

The goal of restoration is to create a self-sustaining, healthy environment, that is resilient to stress and environmental changes (Jordan *et al*. 2019; Ruiz-Jaen and Mitchell Aide 2005; Suding *et al*. 2015). However, multiple factors influence the long-term survivability and viability of restored populations. Fundamental to this is seed-sourcing techniques which influence a suite of related population genetic issues. (Mijangos *et al*. 2015; Breed *et al*. 2019, Millar *et al*. 2021). Restoration programs in Australia aim to species extinctions due to extensive land clearing (Broadhurst 2013; Broadhurst *et al*. 2008). Modern and emerging genetic technologies provide comprehensive measures of restoration success including inbreeding or outbreeding depression, lack of genetic diversity, lack of gene flow and population subdivision (Rice and Emery 2003; Broadhurst 2013; Millar *et al*. 2021).

Successful restoration involves the re-establishment of connectivity through important species interactions, such as pollination between natural and restored populations (Millar *et al*. 2021). But how do we know when we have reached that goal? It is important to critically analyse whether restorations are successful and continue to be a viable, resilient population in the future. Restoration success can be measured in several different ways (Wortley *et al.* 2013). These measures can be based on vegetation characteristics (vegetation cover; biomass density [Walters 2000; Wilkins *et al*. 2003]), ecosystem processes (biological interactions e.g. pollination, or nutrient cycling [Rhoades *et al*. 1998]) or species diversity (richness and abundance [Passell 2000]). In combination, these measures can provide a richer understanding

of restoration success (Hobbs and Norton 1996; Neckles *et al*. 2002). Genetics is a powerful tool to measure restoration success in terms of genetic diversity and differentiation of populations (e.g. Broadhurst 2013; Rosser *et al*. 2023; Quinton 2019; Zucchi 2018). Genetic inquiry can measure connectivity between populations (Lowe and Allendorf 2010), determine the parentage of offspring (e.g. Liu *et al*. 2016; Quinton 2019) and determine if outcrossing and significant gene flow is occurring across the landscape. These genetic measures of gene flow provide insight into the maintenance of ecological processes such as pollination and seed dispersal. Restored areas should be compared to natural sites to adequately measure success (Passell 2000; SER 2004).

The Society of Ecological Restoration ('SER') (2004) suggest nine characteristics of a successfully restored population. The restoration should be self-sustaining and include similar diversity and community structure to corresponding sites, presence of indigenous species, presence of functional groups for long-term stability, capacity to sustain reproducing populations, normal functioning, elimination of potential threats, integration within the landscape and resilience to natural disturbances. Financial restrictions do not allow a thorough assessment of restoration success and there are no studies within the literature that have measured all SER attributes (Ruiz-Jaen and Aide 2005). Ecological processes are not measured frequently due to their slower recovery after restorations. Identifying cheap and effective ways to evaluate restoration is important. Ideally, the most effective way to measure restoration success is to acknowledge all attributes provided by SER guidelines.

Few studies measure the reproductive rate of populations or evaluate the fitness and self-sustainability of species long term (Ruiz-Jaen and Aide 2005) as more focus is on easier, less time-consuming measures such as diversity and vegetation structure. An important measure of restorations is evidence of natural recruitment to replace losses through senescence. Without the ability for restored populations to reproduce, there will be no population growth or contribution to future generations. However, there is limited evidence for natural recruitment occurring as a result of reproductive connectivity between restored and remnant populations (Gibbons *et al*. 2008; Ottewell *et al*. 2010). Assessments should therefore focus on measuring reproductive rate, population growth and fitness to determine the level of success. Additionally, the maintenance of the assemblage of indigenous species and species richness within restored

populations should be considered (SER 2004). Without considering the richness of species used in active restoration, the restored population will not be able to maintain itself if community structure and composition are compromised. Finally, new and emerging genetic technologies such as genomics can evaluate the viability of populations. This will allow us to close significant gaps in knowledge and understand why restorations may fail, and what practitioners and ecologists must do to maximise successful outcomes. Genetic technologies can reveal whether genetic diversity, gene flow and homogeneity are restored into the landscape.

1.4 Major questions in ecological restoration

The field of ecological restoration is young, with an imperative to understand how to maintain gene flow, connectivity and genetic diversity within diverse populations, communities, and regions. Research avenues should focus on establishing long-term knowledge and persistence of restoration projects (Broadhurst *et al*. 2017) so that restoration is future-focused while acknowledging the importance of the heritage of flora in landscapes (Broadhurst *et al*. 2008). Due to the young age of most restoration projects, long-term success is unknown (Broadhurst *et al*. 2017). The ability of restored cohorts to persist compared to natural and relictual trees is also unclear and further research is needed to evaluate fitness and viability differences between restored and remnant stands (Broadhurst 2013; Rosser *et al*. 2023). There is also a lack of studies involving the relative connectivity and gene flow existing between restored and remnant populations and whether they are producing viable outcrossed recruits. Finally, genetic issues relating to seed sourcing and the most effective seed sourcing technique to utilise is not yet well understood. Therefore, there are three outstanding questions highlighted within the literature.

1) What is the most effective way to source suitable genetic material to use in restorations to increase long-term success?

2) Are restored populations sufficiently genetically diverse to be able to produce genetically viable offspring?

3) Can the connectivity and gene flow between remnant and restored populations be re-established and persist long-term?

Careful selection of gene stock used in restorations can lead to the production of viable populations that can withstand environmental stressors. However, genetic diversity and differentiation should be assessed in populations used for sourcing seeds to support self-sustaining planted populations. Existing and emerging genetic tools offer the potential to improve our understanding of restoration ecology which can assist with filling these knowledge gaps and improve ex-situ conservation management long-term in the face of climate change (Breed *et al*. 2019; Jordan *et al*. 2019; Mijangos *et al*. 2015).

Some studies suggest that genetic connectivity and gene flow are restored between natural and restored populations (Liu *et al*. 2008; Reynolds *et al*. 2012; Ritchie and Krauss 2012) but in reality, too few studies confirm the existence of gene flow and genetic variation in restoration programs. Restored and natural populations must interact to re-establish linkages to sustain reproductive potential and genetic variation for resilience long-term. However, few examples demonstrate whether Australia's restoration efforts are provisioning genetically viable new populations to ensure a long-term future in the face of climate change (Broadhurst 2013). Additionally, it is also valuable knowledge to examine connectivity between populations by focusing on offspring: natural saplings and within collected seeds. This next generation can allow us to understand whether outcrossed individuals are prevalent in restorations, indicating the restoration of pollination services.

1.5 Genetic issues in restorations

Ecological restoration can be a powerful tool for the sustainable conservation of species and communities, and population genetics can be used to increase the likelihood of success (Zucchi *et al*. 2018). This section reviews genetic measures that can indicate long-term population viability. As these become cheaper and more accessible (Gellie *et al*. 2018; Perring *et al*. 2015), they can inform appropriate seed sourcing to maintain future genetic capacity and persistence in changing environments. Little data exists to give guidance on a successful restoration with sufficient genetic background to ensure their long-term future (Broadhurst 2013). The goal of restorations is to first, establish source populations with sufficient genetic diversity to maintain the ability to adapt to environmental change and altering habitats (Broadhurst *et al*. 2008), to establish whether inbreeding is likely to occur or whether genetic differentiation can cause

outbreeding depression (Edmands 2007). Further, it is important to measure genetic issues likely to arise concerning population connectivity (Lowe and Allendorf 2010) and finally avoid factors likely to critically affect fragmented populations (inbreeding, genetic drift and genetic contamination). These genetic issues are of primary concern within restoration programs because they are likely to affect or reduce population fitness and survivability. Genetic issues may impact the success of restoration projects over the long term through processes such as reduced genetic diversity, outbreeding depression, inbreeding depression, hybridisation, and genetic contamination (Rice and Emery 2003).

1.5.1 *Genetic diversity*

Genetic diversity underlies ecological viability and long-term sustainability and the capacity for restored populations to persist without human support. Restored/revegetated populations often lack sufficient or appropriate genetic diversity due to genetic drift, founder effects, inbreeding or inappropriate sourcing of seeds (Rosser *et al*. 2023; Broadhurst 2013; Jordan *et al*. 2019; Zucchi *et al*. 2018). Fitness can be reduced in restored stands and their offspring if they are lacking in genetic diversity and express recessive deleterious traits, as they may be more vulnerable to environmental stressors and suffer reduced seed crops/fecundity, poor seedling survival and germination, smaller seeds, and poorer fitness (Broadhurst 2013; Zucchi *et al*. 2018; Broadhurst *et al*. 2006; Aguilar *et al*. 2006).

Genetic diversity of restored stands is closely tied to adaptive capacity and should be compared between natural and restored stands (Jordan *et al*. 2019; Reusch *et al*. 2005). For example, Broadhurst (2013) reports overall genetic diversity was significantly lower in restored trees in comparison to remnant trees within Australian *Eucalyptus melliodora* restoration sites. However, Zucchi *et al*. (2018) found similar levels of genetic diversity, in restored and remnant populations of species from the Brazilian Atlantic Forest indicating that restoring genetic diversity may vary between species and ecosystems. In Germany, some restored trees had higher levels of genetic diversity than remnant populations (Kaulfuß and Reisch 2019). If seed sources for restoration plantings have low genetic quality the plantings may lack genetic diversity, and gene flow and have an increased risk of inbreeding and outbreeding depression. The genetic quality of seed sources should be assessed before, the restoration has taken place.

1.5.2 *Inbreeding depression*

Inbreeding depression is the decrease in fitness because of increased homozygosity and may occur because the likelihood of mating between closely related individuals increases if seeds are locally sourced from a few individuals (Vander Mijnsbrugge *et al*. 2010). Therefore, the risk of restoration failure can be increased if sourced populations are genetically similar to remnant populations and lack genetic differentiation (Steane *et al*. 2017; Zucchi *et al*. 2018). Thus, restorations should aim to reduce the risk of inbreeding depression by increasing the source of seed from multiple trees and avoiding seed collection from small, fragmented populations (Breed *et al*. 2015). Restored cohorts often have increased levels of inbreeding (Broadhurst 2011; Dolan *et al*. 2008) due to sourcing from local, fragmented populations which may have a severe effect on the long-term validity of the restoration program. This is particularly pertinent to the restoration of long-lived species, like trees, where impacts of inbreeding depression may not be apparent for several decades after planting due to a lag between generations in expressing deleterious alleles associated with inbreeding. Additionally, a recent study of *Carniana legalis* from the South American Atlantic Forest discovered that selfed seedlings experienced moderate levels of inbreeding depression and reduction in fitness (Tambarussi *et al*. 2017). In comparison to outcrossed seedlings, selfed *Banksia marginata* seedlings were only 62% as fit as open-pollinated progeny and produced smaller seedlings less likely to survive (Vaughton and Ramsey 2006). Therefore, restoration programs must limit the level of inbreeding within populations and attempt to promote outcrossing to reduce the risk of restored cohorts producing less fit individuals.

1.5.3 *Genetic differentiation*

The level of population differentiation among remnant and restored populations can have implications for genetic diversity and the likelihood that the restoration will avoid the risk of inbreeding and outbreeding depression (Frankham *et al*. 2011; Hufford *et al*. 2012; Wilkinson 2008; Zucchi *et al*. 2018). Over time, genetic subdivision between remnant and restored cohorts may increase if pollen dispersal has not been re-established between populations (Broadhurst *et al*. 2015). If gene flow and pollen dispersal are not established between remnant and restored stands the populations may continue to genetically differentiate from each other due to atypical gene flow. For example, Zucchi *et al*. (2018) found that planted populations were composed of

entirely different gene pools due to large physical geographical distances separating them due to fragmentation. These heterogeneous genetic differences can be impactful as they risk further subdivision in the future unless gene flow is restored. This fragmentation or population subdivision can lead to a small effective population size which can also reduce genetic diversity and increase inbreeding. Consequently, restorations must aim to reduce the pressures of fragmentation and increase effective population size to reduce further genetic subdivisions between populations.

1.5.4 *Outbreeding depression*

The risk of hybridisation between species is often raised but the lack of maturity of many restoration programs hampers investigation of this issue. Many species targeted for woodland restorations can hybridise with both native and exotic taxa (Field *et al*. 2008; Field *et al*. 2011; Goto *et al*. 2011; Bradbury *et al*. 2021). This can lead to many genetic issues as recent or later generations of hybrids can have lower fitness and viability than purebred individuals. Furthermore, hybrids may limit the value of the restoration as pure gene pools diminish (von Takach Dukai *et al*. 2019). Mixed provenance sourcing of seed can increase the risk of outbreeding depression and hybridisation between subpopulations and lead to a decrease in fitness (Edmands 2007; Hufford *et al*. 2012). Field *et al*. (2008) report presence of hybridisation within restoration programs and small populations in particular had reduced viability through genetic and demographic swamping. Hybridisation can lead to local extinction and dilution of the gene pool through introgression (Field *et al*. 2008; Field *et al*. 2011). While nonlocal seed sources can increase genetic diversity this external genetic material can increase the risk of outbreeding depression and population subdivision within the restoration site. A study in 2018 found that outcrossing between multiple populations of *Primula vulgaris* ranging throughout regions of the Netherlands has resulted in outbreeding depression developing in the next generations (Barmentlo *et al*. 2018). Consequently, managers should consider the risk of outbreeding depression and hybridisation when utilising mixed seed sourcing strategies for use within restorations.

1.5.5 *Genetic contamination*

Genetic contamination is when seed sourced externally to the site introduces genes that are maladapted to the local environment and small, fragmented populations in particular are very vulnerable to this (Hufford and Mazer 2003; Rice and Emery 2003). A previous study in 2010 discovered substantial amounts of genetic contamination of *Acacia saligna* subspecies because of previous large-scale use of a nonlocal seed source to restore native taxa within a highly fragmented landscape (Millar *et al*. 2012). Genetic contamination with nonlocal genotypes was found to cause reductions in height, diameter at breast height, survival, and overall fitness (Goto *et al*. 2011). To alleviate these concerns, it is important to implement provenance sourcing and genetic assessment into the decision-making process. Survival and reproduction should be monitored for some time after the restoration to determine if the provenance of restored cohorts is optimally adapted to the local habitat (McKay *et al*. 2005).

1.5.6 *Climate adjustment*

Land availability and habitat suitability are serious concerns for restoration programs in the future as plants have limited capacity to move to new habitats in response to climate change (Broadhurst *et al*. 2018). To persist, species must respond to a changing climate by adjusting through range shifts and in situ adaptation. However, *Eucalyptus* longevity and poor seed dispersal suggest that these species may not be able to keep up the pace to track climate change and must shift up to >1km per year (Corlett and Westcott 2013). This issue is also present in many flora and fauna species globally, as recent studies raise concern for the ability of less fit populations to adapt to rapid environmental changes (For example, *Chamaecrista fasciculata* in America; Etterson (2004) and climate-smart restorations of tropical forests in Columbia; Fremout *et al*. (2021). Climate change adjustment adds another dimension to restoration programs in a fragmented landscape. Poor progeny fitness is likely to limit the successful movement of fragmented landscapes, particularly ones that have been restored.

Additionally, there is an increasing lack of habitat suitable for species to migrate into (Broadhurst *et al*. 2018). Thus, the ability of restored cohorts to adjust to changing climates should be considered. Linking adaptive genomic data to current and future environmental

change predictions can help facilitate decisions regarding provenance choices for restored plantings under climate change (Breed *et al*. 2019). As a result, restoration programs can focus on a future climate-adapted population to help improve range shifts and migration rates of restored species. Many concerns arise when making decisions regarding restorations, and decision-makers need to consider policies, and recommendations from genetic research and employ the use of genomic tools for the increase in success.

1.6 Seed sourcing considerations

The choice of seed source should ensure maximum genetic variation and evolutionary adaptive potential (Broadhurst *et al*. 2008; Vander Mijnsbrugge *et al*. 2009; McKay *et al*. 2005). Debate exists as to whether local provenance or mixed provenance sourcing will reduce the risks highlighted above, and allow for the restoration to become a self-sustaining, biodiverse population (Broadhurst *et al*. 2008; McKay *et al*. 2005; Vander Mijnsbrugge *et al.* 2009). The main goal of seed sourcing is to reduce the risks of inbreeding depression and limit the deterioration of gene complexes via outbreeding depression. Conversely, there is little empirical understanding of how to select seeds for the best restoration outcomes (Bower and Aitken 2008). Key questions surrounding the idea of appropriate genetic sourcing include:

1) *What level of starting genetic diversity is important to maintain a diverse restored population?*
2) *How local is local? – how far can genetic material be sourced to maintain local adaptation advantages?*

It can be difficult to balance the need for genetic diversity and the requirements for local adaptation especially when local remnant populations are small. Externally sourced seed may introduce a distinct population and create a barrier to restoration growth and connectivity. Other impacts of poor seed selection include further subdivision of populations, founder effects, outbreeding and inbreeding depression, and in turn a reduction in fitness (Hufford and Mazer 2003). These genetic limitations can limit the survival and reproduction of restored cohorts (McKay *et al*. 2005). Therefore, understanding the genetic issues surrounding the sourced material and managing the genetic viability of restored cohorts long-term.

1.6.1 *Local provenance sourcing*

Local seed is widely advocated for restoration projects as it is adapted to the location of the restoration site (McKay *et al*. 2005; Vander Mijinsbrugge *et al*. 2009). Local provenance sourcing provides many advantages (Table 1) and can lead to better survival and growth, due to the reduction of maladapted genotypes to local conditions (McKay *et al*. 2005). It alleviates the risk of 'genetically polluting' local gene pools with novel genotypes sourced from outside the restoration site, despite the advantages of such material having extensive genetic variation. Sourcing local genetic material can employ a homesite advantage and increase the fitness of restored plants and second-generation offspring (Hufford and Mazer 2003). Local seed can also improve resistance to abiotic conditions and maintain biotic interactions such as pollination services, pathogen resistance and drought resistance (Cunningham *et al*. 2005; Jones *et al*. 2001) and reduce the likelihood of outbreeding depression.

Table 1. Comparison of the advantages and disadvantages associated with local and mixed seed sourcing strategies for the use in restoration plantings

	Advantages	**Disadvantages**
Local Provenance Sourcing - The use of locally collected seeds/genetic material from native plant populations occurring within the restoration site	• Reduced risk of failure due to maladaptation to local conditions • Limiting the risk of 'genetic pollution' • Limiting the risk of outcrossing/outbreeding depression • Maintains 'home-side advantage as it conserves biotic and abiotic interactions (pollinators, pathogen resistance; Broadhurst *et al*. 2008) • Maintains local adaptation (Hufford and Mazer 2013)	• Increases the risk of inbreeding depression (Broadhurst *et al*. 2013) • Decreased genetic diversity, fitness and evolutionary potential due to diminishing the local gene pool (Vander Mijnsbrugge *et al.* 2010; Hufford and Mazer 2003; Charlesworth and Willis 2009) • Decreases genetic variation and genetic availability (Broadhurst 2011) • Not particularly useful for small, fragmented populations and rare species as the gene pool is small • Little information/research to support this (Bower and Aiken 2008) • Risk of using low-quality seeds as a source • Local overharvesting risks and mistakes in local collecting (Broadhurst *et al.* 2008; Peres *et al*. 2003)

Mixed Provenance Sourcing - The use of local (native) and external genetic material for restoration efforts	• Maintains and increases genetic variation (Broadhurst 2013) • Reduces the risk of inbreeding and decreased fitness (Prober *et al*. 2016; Bucharova *et al*. 2019) • Increases species gene pool (Aitken *et al*. 2013) • Facilitates adaptation and increases resilience to future environments by introducing novel genotypes (Broadhurst *et al*. 2008; Harrison *et al*. 2017) • Reduces the risk of using low-quality seeds for sourcing (Prober *et al.* 2016) • Useful for restoring small fragmented populations • Increases genetic diversity and fitness (Jordan *et al*. 2019) • Provides a bet-hedging strategy so that even if local or external sources are maladaptive, it still has room for success (Sampson and Byrne 2008) • Can choose provenance for a 'future climate for a widely distributed species (for example*, Eucalyptus*)	• May suffer from maladaptation to the local environment and lower fitness (McKay *et al*. 2005) • Risk of intraspecific hybridisation of local and introduced individuals and disruption of co-adapted gene complexes (McKay *et al*. 2005; Edmands 2007) • Risk of outcrossing depression (Broadhurst 2013; McKay *et al*. 2005) • Introducing superior or invasive genotypes (Hufford and Maxer 2003) • May cause genetic pollution of the gene pool (McKay *et al*. 2005; Hufford and Mazer, 2003) • Little research to support mixed-provenance sourcing • Species 'may never be the same' (McKay *et al*. 2005) • Negative effects associated with species interactions (Vander Mijnsbrugge *et al*. 2010)

Local adaptation of taxa to regional climatic and ecological factors is poorly understood. This makes it difficult to assess the appropriate ranges (distances) to apply for local seed sourcing. However, questions arise regarding the distances involved in local adaptation and whether sourcing material externally from the site can affect local adaptation. The scale of local adaptation also varies among species, especially within *Eucalyptus* species (Supple *et al*. 2018). Too little information is available to solely support local provenance sourcing. Restricting restoration sourcing to local ignores the reality that populations exist along a continuum, with some populations and species being more locally adapted than others (Raabová *et al*. 2007). Consequently, it is proposed that decisions on the use of local provenance sourcing should be species-specific and only be employed if the species has sensitive local adaptation scales.

Locally sourced seeds are readily accessible. However, unless local adaptation of a particular site is shown to be important, the limitations that arise (Table 1) when utilising the technique may outweigh the benefits. Sourcing local genetic material, especially from small, fragmented populations can lead to an increased risk of inbreeding depression and expression of deleterious recessive alleles due to low genetic diversity levels and may result in a decrease in fitness

(Broadhurst *et al*. 2008; Hufford and Mazer 2003; Vander Mijinsbrugge *et al*. 2009; Charlesworth and Willis 2009). Therefore, the use of mixed provenance sourcing is becoming more widely accepted, since it can actively increase genetic diversity, and reduce inbreeding.

1.6.2 *Mixed provenance sourcing*

Mixed provenance sourcing uses local and external seeds. This can combat the risks associated with local provenance sourcing and can produce genetically variant offspring. There are many advantages and disadvantages of this technique highlighted throughout the literature (Table 1). A study from 2019 (Bucharova *et al*. 2019) suggests that mixed sourcing is a useful technique to best compromise the risk of inbreeding and outbreeding effects. However, it may do more harm than good due to the risks of inducing genetic contamination and outbreeding depression (Keller *et al*. 2000 [for example, commercial seed mixtures of weed species *Agrostemma githago*, *Papaver rhoeas* created hybrids with negative outbreeding effects]; Hufford and Mazer 2003). Nevertheless, genetic contamination from externally sourced seeds is a serious concern in fragmented and small populations that are vulnerable to the translocation of potentially maladapted genetic material (Hufford and Mazer 2003; Rice and Emery 2003). Nonlocal genotypes can be maladapted to the local environment which thus reduces the fitness of restored populations and offspring (McKay *et al*. 2005; Crémieux *et al*. 2010). Crossing among ecologically divergent populations (differences in local adaptation) can cause outbreeding depression where intermediate phenotypes are not successful within the local environment (McKay *et al*. 2005). This can increase the proportion of maladapted individuals and reduce overall population viability. Furthermore, certain loci across the genome can unnaturally interact (known as epistasis) and produce integrated phenotypes known as co-adapted gene complexes. This can increase the production of unfit hybrids due to the breakdown of these complexes within the genome forming deleterious alleles. This idea has been explored thoroughly within the literature (e.g., Keller *et al*. 2008; Galloway and Fenster 2000).

Mixed seed sourcing is postulated to maximise climatic adaptive potential through outcrossing, particularly in *Eucalyptus* species (Prober *et al*. 2016). This involves sourcing genetic material with a broad range of environmental adaptations to increase survival in future climates

including increasing drought and temperature tolerance (Rossetto *et al*. 2019; Bucharova *et al*. 2019). Putative genome regions in *Eucalyptus* species have recently been associated with climate adaptability. These regions can be identified in genetic sources and selected to be used in restoring populations to increase the capacity for adaptation to differential climatic conditions predicted for southern Australia (Prober *et al*. 2016). Mixed provenance sourcing may increase fitness and combat climate change. Additionally, mixed provenance sourcing can also reduce inbreeding depression (Bucharova *et al*. 2019) unlike local provenance sourcing. Mixed sourcing has also gained support globally, as this technique has also been developed commonly in non-woody plants in Germany (Prasse *et al*. 2010). Ultimately, the use of this technique has many advantages and disadvantages (Table 1) which should be considered when conducting restorations for each species.

1.7 Connectivity of populations

Altered and reduced gene flow patterns are outcomes of isolated and fragmented species (Lowe *et al*. 2005). Further research is needed to understand the intensity of gene flow required to mitigate the negative effects of fragmentation (Broadhurst *et al*. 2008). It is well understood that fragmentation can influence pollinator abundance and behaviour which can increase inbreeding levels and reduce connectivity between populations impacting outcrossing rates (Armbruster and Reed 2005; Coates *et al*. 2007). However, the probability of gene flow between populations decreases with higher genetic differentiation even in similar environments (Epperson 2003). As a result, retaining gene flow between populations of mixed provenance can be challenging. If gene flow and connectivity are not restored between natural and planted populations, there is an increased risk of genetic drift can contribute to further non-adaptive population differentiation (Galloway and Fenster 2000). Overall, low gene exchange from planted populations can reduce effective population size and genetic diversity. Additionally, this can increase inbreeding levels and low gene pools can result in a reduction of fitness (Proft *et al*. 2018; Aitken *et al*. 2016; Jordan *et al*. 2017). Increased selfing and decreased pollen diversity are common in fragmented populations, particularly when pollinators are isolated and less mobile (Breed *et al*. 2015).

The re-establishment of pollen services between natural and restored populations is crucial to maximise the reproductive potential and resilience of plantings (Dixon 2009). A study in 2013

(Broadhurst 2013) reported that active pollen movement between remnant and restored Yellow Box (*Eucalyptus melliodora*) trees occurred within a 250m collection zone. A similar result was also observed in other studies (Liu *et al*. 2008; Reynolds *et al*. 2012; Ritchie and Krauss 2012; Rosser *et al*. 2023 Quinton 2019). Connectivity between remnant and restored populations allows genetic diversity from remnant trees to carry forward to subsequent generations (Broadhurst 2013). However, as remnant trees age this may reduce pollen pools and impact (Broadhurst 2013) increase inbreeding levels with subsequent reduced reproductive output and progeny fitness (Mimura *et al*. 2009). Therefore, connectivity requires the establishment of sufficient genetic diversity and the presence of mature reproductively active remnant trees.

Pollinator movement and behaviour are affected by landscape changes such as land clearing. Pollen dispersal is important in re-establishing connectivity within a restoration. For example, the loss of remnant relictual trees can be detrimental to pollinator dispersal as they act as 'stepping stones' between remnant and restored sites. Pollinator success reduces with distance between sites; thus, restorations must aim to reduce isolation and fragmentation to restore connectivity to the landscape to increase pollinator success. More localised pollen dispersal can directly facilitate population differentiation which can explain the complex genetic structure of many Box-Gum seeds collected (Broadhurst *et al*. 2015; as seen in Rosser *et al*. 2023). Long-distance pollination (facilitated by honeybees and other insects) can travel up to 2km (Sampson and Byrne 2008), and more typical distances are around 200m (Broadhurst 2013; Byrne *et al*. 2008). This large theoretical range provides high potential for the re-introduction of connectivity, pollination, and dispersal services of eucalypt restoration sites provided that restoration projects are within pollen dispersal range with close distances between populations (fragmentation). Close restoration plantings allow for the increase of opportunities for pollen dispersal and abundance (Broadhurst 2013).

Genetic research to inform strategies to improve on and measure connectivity between planted and remnant populations is critical to successful restoration outcomes. Knowledge of successful genetic outcomes in restorations can lead to an increase in overall connectivity. Concentrating on connectivity can have positive cascading effects on other important genetic factors such as variation, adaptive potential reducing inbreeding and genetic drift on the fitness

of offspring (Proft *et al*. 2018). Additionally, genetic tools to model gene flow mechanisms can also identify genetic barriers (Raeymaekers *et al*. 2008) and determine where gene flow can be maximised for the highest benefit of the restoration project. Ultimately, genetic research into connectivity, pollination and gene flow modelling can allow for successful restoration outcomes.

High-throughput sequencing uses genetic markers such as single nucleotide polymorphisms (SNPs) to assign parentage. Parents have been successfully assigned with as little as 48 SNPs for 98% accuracy within rainbow trout using the program 'CERVUS' (Liu *et al*. 2016) and in 2019 for *Eucalyptus melliodora* (Quinton 2019). Further, using a significant amount of SNP markers, the research found success in assigning parentage of inbred soybean populations utilising the program 'ParentOffspring' alongside developing parentage maps within the 'R' coding program. (Abdel-Haleem *et al*. 2013). In application, these technologies can assist with developing models of connectivity between restored plant populations and remnant stands in mature restoration programs to determine whether adequate levels of gene flow were re-established.

1.8 Eucalypts in Australia

Eucalypts dominate the Australian landscape. *Eucalyptus* is a large genus in the family *Myrtaceae*, with a very wide distribution making it difficult to grasp broad patterns in composition (CSIRO 2007). About three-quarters of Australia's forests are *Eucalyptus* forests ranging in all areas of Australia (CSIRO 2006). There are more than 700 *Eucalyptus* species distributed across Australia in a broad environment including woodlands, forests, and arid areas which provide food, and shelter and determine the distribution of many vulnerable native species of Australian fauna including Musk Lorikeets, Koalas, and other arboreal mammals (Smith and Lill 2008; McGregor *et al*. 2013; Cork and Catling 1996). However, eucalypts are threatened by agriculture (land clearing and fragmentation), residential/commercial development and invasive species/diseases (IUCN 2022).

1.8.1 *Box-Gum Grassy Woodlands*

Eucalyptus forests and woodlands are key landscapes for many ecosystems around Australia (Ottewell 2010). However, due to land-clearing events, they are becoming less prevalent and, in some instances, endangered (Broadhurst 2013). *Eucalyptus* is a key target genus in restoration ecology across Australia. Box-Gum eucalypt woodlands (comprised of Yellow Box, White Box, and Blakely's Red Gum) are nationally important and were once widespread in south-eastern Australia. They thrive on moderate to highly fertile soils, which, unfortunately, are also the target for agriculture. These keystone woodlands are of ecological importance as they maintain soil nutrient cycling, plant richness, and landscape heterogeneity and provide food and shelter for local invertebrate and vertebrate communities (Gibbons and Boak 2002; Manning *et al*. 2006; Broadhurst *et al*. 2013). Currently, in eastern Australia, the White Box-Yellow Box- Blakely's Red Gum Grassy Woodland ecosystem is listed as critically endangered and has been a target for restoration programs, particularly within NSW. They are protected by Commonwealth and state (NSW and ACT) government legislation with a focus on intensive restoration and aims to increase research and stewardship programs into maintaining restored populations long term (Broadhurst 2013). Particularly, Blakely's Red Gum, Yellow Box and White Box *Eucalyptus* are all listed as vulnerable (under criteria A2c; population decline is stable) on the IUCN Red List of threatened species (last assessed March 2019; IUCN 2019a; IUCN 2019b; IUCN 2019c).

The Box-Gum community also provides a home for many endangered and vulnerable birds and animals including the Superb Parrot, Regent Honeyeater and Koalas that are listed under the Commonwealth Government Environmental Protection and Biodiversity Conservation Act 1999. The decline of these endangered birds can have a detrimental effect on levels of wide-spread pollination within Box-Gum Woodlands. However, land clearance and modification of environments after the European settlement have left Box-Gum Woodlands extremely fragmented and have reduced several million hectares to less than 10% today, causing historical remnant relictual trees to be scattered among the landscape. Up to 54% of these woodlands exist as patches and are highly isolated (Gibbons and Boak 2002; Prober *et al*. 2002) and are in extreme need of successful restoration programs to help restore the key ecosystem to its original state. This involves restoring populations of each species, White Box, Yellow Box and Blakely's Red Gum.

Long-term monitoring of these ecosystems and the success of Box-Gum restorations is necessary to preserve the habitats for these endangered native species. For example, it was observed in 2023 that Yellow Box and White Box have moderate levels of *in situ* recruitment within the Warrumbungle National Park ('WNP') Central Valley area, surrounding older relictual trees and larger planted trees. Previously, the success of the restoration that took place in the WNP was evaluated by comparing Yellow Box and White Box genetic diversity and variation of remnant and restored stands across the Central Valley (Rosser *et al*. 2023). It was discovered that there were similar levels of genetic diversity across all cohorts. However, further research is needed to determine the resilience and fitness of offspring to environmental changes. There is high value in comparing restored and remnant populations of species, as it gives insight into determining whether the restoration has created a homogenised population or has produced a further subdivided population creating a barrier for restorative growth of both the introduced and native populations, potentially inducing competition.

1.8.2 *Yellow Box and White Box*

Yellow Box (*E. melliodora*) and White Box (*E. albens*) are prominent *Eucalyptus* species occurring in New South Wales and Victoria in south-eastern Australia (Figure 1). *Eucalyptus melliodora* is more broadly distributed than *E. albens*, spanning further north into Queensland. These are highly valued as they support biodiversity, production, and species interaction benefits (Broadhurst 2013). Previous genetic research (using microsatellite data) suggests that restored populations of *E. melliodora* in regions surrounding Canberra and Yass, NSW, Australia are genetically poorer than relictual/natural trees (Broadhurst 2013) due to a lack of high-quality seed supply and extreme isolation. Conversely, a more recent study (Rosser *et al*. 2023) found similar levels of genetic diversity between restored and remnant stands. Relictual trees were reported to be historical reservoirs of genetic diversity and are key components that should be considered within restoration programs as they provide active recruitment and pass on valuable genotypes of natural and planted offspring providing local adaptation advantages. Consequently, this suggests that depending on the restoration of Box-Gum Grassy Woodlands may have variable success depending on the region.

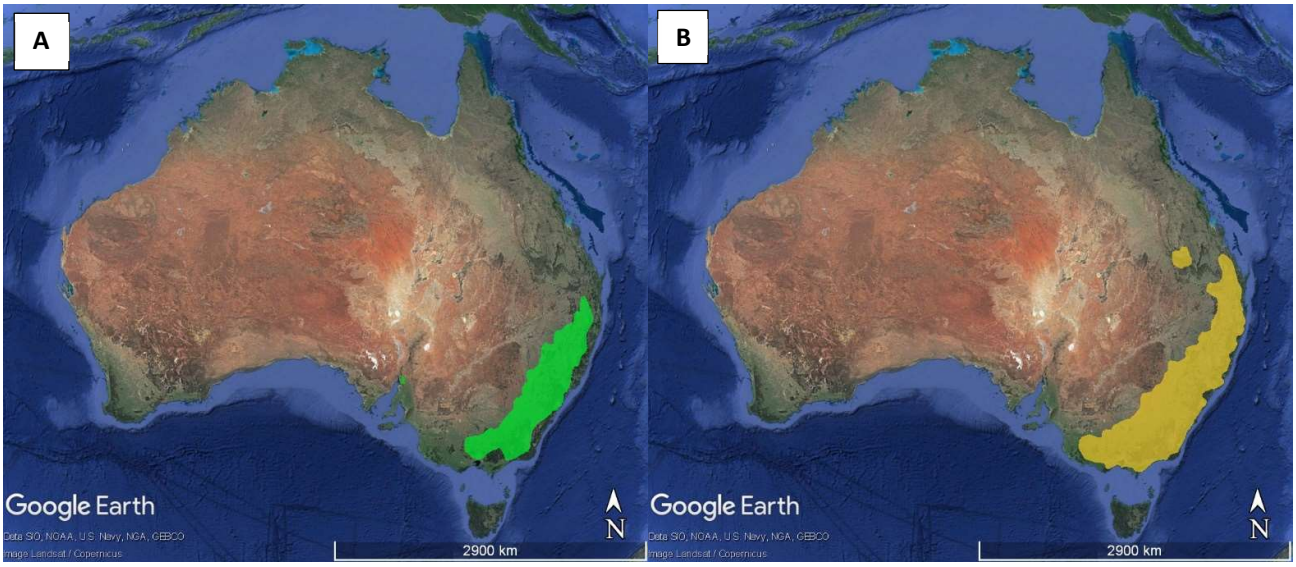


Figure 1. Atlas of living Australia (ALA 2023a; ALA 2023b) occurrence records map of *Eucalyptus albens* (Figure A; denoted by green) and *Eucalyptus melliodora* (Figure B; denoted by yellow). Scale and North direction are included. Map created using Google Earth Pro 7.3.4.8

Fitness testing can be applied to understand how well offspring can survive in the future of restorations. Restorations of *E. albens* have been understudied in comparison to *E. melliodora*, however, it can be expected that comparable results will arise since they are phylogenetically similar. *Eucalyptus albens* populations are impacted by tree clearing, herbivore grazing and weed invasion, especially in smaller fragmented populations (Prober and Thiele 1995). They are vulnerable to further fragmentation as isolation pressures and lack of recruitment continue, therefore successful restoration programs are critical and provide an opportunity to genetically evaluate restoration efforts to retain elevated levels of fitness and resilience in populations.

Yellow Box and White Box are both pollinated by invertebrates such as bees and some species of birds. Pollen dispersal and gene flow between populations of these species is often interrupted during fragmentation and restoration events. As a result, understanding pollen dispersal between natural and restored trees can help identify whether genotypes within the landscape are being passed on through offspring (Broadhurst 2013). *Eucalyptus* trees are monoecious, meaning they possess both male and female reproductive organs. It is unknown whether restored cohorts possess both male and female fertility structures. This may only

indicate immaturity, but it may indicate that they cannot produce viable seeds due to incompatibility or pollination failure. There are significant gaps within the literature assessing the re-establishment of pollen dispersal after restoration programs have taken place as it is difficult to assign parentage to compare remnant and restored pollination levels.

1.8.3 *Importance of relictual trees to restorations*

Land clearance and fragmentation have left old, large trees isolated and scattered agricultural matrixes. These scattered trees are relicts of a more continuous ecological Box-Gum community and have been shown to encompass rich genetic diversity (Broadhurst 2013). They play a significant role in local and regional biodiversity conservation (Manning *et al*. 2006). Relictual trees are keystone structures that provide landscape heterogeneity and other important services such as maintaining soil nutrients and plant species richness (Gibbons and Boak 2002; Manning *et al*. 2006). A recent review (Proft *et al*. 2018) suggests the focus should be placed on the genetic characterisation of these trees which can inform historical gene flow, dispersal, and elimination of dispersal barriers in specific populations. These trees have high value these trees and can provide valuable genetic resources to offspring (Gibbons and Boak 2002; Manning *et al*. 2006; Rosser *et al.* 2023). Overall, relictual trees can pass on high fitness and resilience to future generations and are imperative to restoration programs.

There is a global decline of relictual trees as they age which increases the imperative to conduct urgent restoration plantings. Relictual trees suffer from a chronic lack of recruitment and there are concerns that without sustained restoration efforts, these trees are set to disappear from landscapes within the next 90-180 years (Gibbons *et al*. 2008). Further research is required to understand the reproductive traits and output of these trees and whether their seed quality is suitable for restoration programs. Although relictual trees possess the ability to offer genetic diversity to their offspring, their isolation may reduce the quality of seeds to be used as sources for plantings. A previous study in 2010 (Ottewell *et al*. 2010) suggested that relictual trees represent a potential source of seeds due to their high genetic diversity though further trials are required to determine seedling survival and longevity. Thus, there is a more pressing need to compare relictual and restored offspring fitness.

1.9 Single nucleotide polymorphisms (SNPs)

Next-generation sequencing (NGS) to detect single nucleotide polymorphisms (SNPs) holds the potential to assist with increasing the success of the restoration of key species globally (e.g. American chestnut in North-western America; Wheeler and Sederoff 2008). NGS uses genome-wide information to generate measures of the diversity of individuals and populations. This involves using NGS to detect SNPs across the genome of each sample and compare it against a library developed for species (eucalypts, for example). An SNP is a germline substitution or genomic variant of a single base position at a specific position on a genome. These are biological markers and help locate genes or regions of alleles that can be used to understand population differentiation or locate genes that are associated with disease. This allows the detection of beneficial genotypes and phenotypes that can be used to increase the adaptive potential for use in restorations (Luikart *et al*. 2003). Utilising this genetic technology can enable researchers to better understand restoration ecology and increase the potential to assess restoration success long-term, a significant gap in our current knowledge within the field.

Prior to the development of NGS, genetic assessment involved genetic markers that target putatively inactive regions of the genome (for example microsatellites, mtDNA markers as used in Zucchi *et al*. 2018; Broadhurst 2013; Broadhurst *et al*. 2017; Ottewell *et al*. 2010). Although effective, genetic variation was measured utilising several gene fragments and locations on genomic regions that may have not been the most appropriate region for genetic differentiation. Neutral genome-wide markers often outperform traditional microsatellite markers as they can effectively map breaks in gene flow and migration routes as well as estimate effective population sizes (Dick *et al*. 2008; Hardy *et al*. 2006). NGS allows us to have a greater capacity to measure relevant genomes (including active regions and well as neutral e.g., DArTseq technologies; targeting areas that contain the most useful information) with higher clarity and accuracy due to an increase in marker volume. Box-Gum Grassy Woodlands have mostly been assessed using only five microsatellite markers (Broadhurst 2013). However, they have also been recently assessed using NGS targeting SNP markers (Rosser *et al.* 2023; Quinton 2019). This provides insight into how the genome is responding to the environment. Further validation of restoration success using NGS may reveal patterns of the negative effects found associated with local provenance sourcing, especially in the understudied *Eucalyptus albens*.

Additionally, linking adaptive genomic data to predicted environmental changes can help us improve provenance choices and resilience in future generations (Luikart *et al*. 2003). Modern-day population genomics offers a much more detailed picture of the distribution of genome-wide variation of local and nonlocal provenances and can allow researchers to favour areas of arid-adapted provenances to prepare sites for future environmental stress (Bohmann *et al*. 2014; Steane *et al*. 2014). Insight into neutral and adaptive genomic variation and its implementation in restoration plantings is limited. However, its value has been demonstrated recently in a landscape-wide analysis of genomic diversity assessed in *Eucalyptus macrocarpa* to capture patterns of diversity in revegetated sites; Jordan *et al*. 2016). Therefore, employing the field of population genomics in restoration projects will allow for increased success in long-term climate adjustment.

1.10 Thesis aims and hypotheses

There is little information within the literature revealing whether gene flow occurs between remnant and restored populations, even though this is crucial for long-term success. Connectivity between populations is an excellent indicator of success as it is evidence of a reduction in the negative effects of fragmentation. This study will assess restoration success in the WNP where tree planting occurred throughout the 1980s and 1990s with the use of local provenance sourcing. The maturity of planted stands in WNP provides an excellent model system to use modern genetic analysis to investigate the reproductive connectivity between the planted stands, naturally regenerated stands, and remnant solitary old trees (relictual: survivors of agricultural deforestation).

Ultimately, information harnessed from this study will determine whether the matured restoration of the WNP was a success, or whether it needs more attention in the future to become self-sustaining. A key aim of this study is to investigate whether the seed sources of particular stands of trees planted throughout the 1980s and 1990s have local provenance as there is a lack of detailed records. These planted stands will be compared to relictual trees and to mature naturally regenerated trees. Furthermore, the connectivity between planted and natural stands will be assessed by determining the level of gene flow and outcrossing occurring. Investigations of genetic diversity within three categories of mature trees (relictual, natural and

planted) can be compared to naturally occurring juveniles and seeds collected from within the mature stands. This will give insight into whether pollen services were re-established between restored and remnant populations. It also gives us insight into the usefulness of the local provenance sourcing for planted stands for the restoration of a highly valuable eucalypt species.

1.10.1 *Parentage-Connectivity and gene flow in offspring*

The primary aim was to (1) establish the parentage of collected seeds (maternal parentage known) and from natural saplings (parentage unknown) to determine connectivity between planted and remnant populations by exploring the level of differentiation. This determines pollination success occurring between mature planted and natural/relictual populations of *E. albens*. Furthermore, the population structure within collected seeds will be assessed to determine whether outcrossing/connectivity is producing a homogenous population of offspring. The genetic analysis of the collected seeds is likely to show most of the parents are produced from the larger relictual trees fragmented within deforested lands, with some outcrossing between mature natural and planted populations, particularly for collected seeds with the confirmed assignment of the maternal tree.

1.10.2 *Genetic diversity, inbreeding and population structure in mature stands and juveniles*

The second aim of this study was to (2) determine whether there is a difference in genetic diversity, inbreeding levels, and population structure between all groups of mature trees (relictual, natural and planted) and juveniles (natural saplings and collected seeds) using an extensive dataset of samples. Genetic diversity will be measured by calculating heterozygosity and inbreeding levels of populations. Population structure will be explored using measures of genetic differentiation and clustering of populations. The inclusion of juveniles provides a second-generation perspective and removes the influence of environmental variables affecting the expression of certain genotypes. This larger dataset of samples may reveal differences in genetic diversity between mature planted and natural populations and their offspring (collected seeds and naturally occurring saplings) and may reveal whether local provenance sourcing was successful.

2.0 Materials and Methods

2.1 Study system and species

The WNP in total covers an area of 23,312 ha within the north/central western region of NSW located within the western area of the Warrumbungle Range (Figure 2). The region within the National Park is characterised by a mix of dry sclerophyll forests and Box-Gum Grassy Woodlands with a high variety of shrubs and grasses (Tulau *et al*. 2019). In this region, woodland communities were extensively cleared through the 1800s during European settlement for land use and agricultural purposes within this area. This widespread deforestation spared some remnant solitary trees (termed relictual) which are sparsely scattered within the Central Valley area of the WNP.

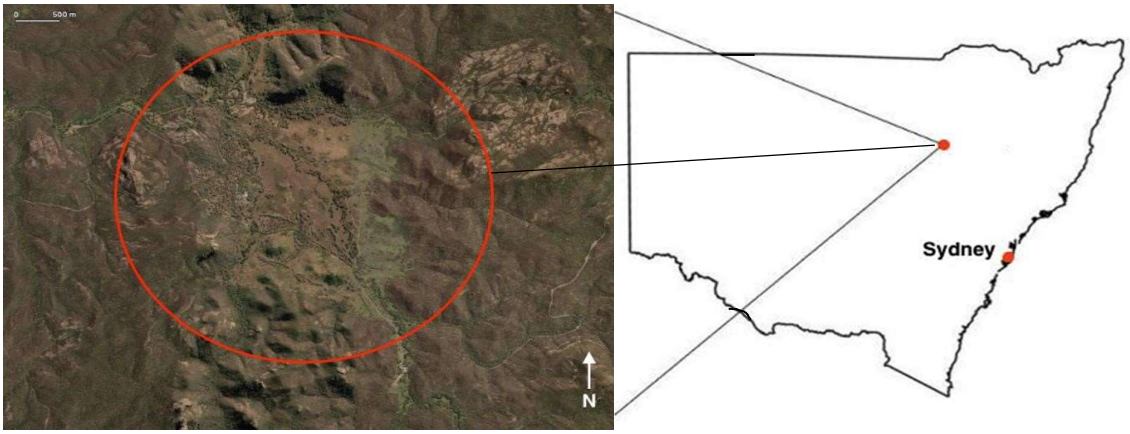


Figure 2. (Left) Satellite image of the Central Valley within Warrumbungle National Park (WNP) (31°17'S, 149°00'E), the valley is circled in red in which the study system is located. (Right) location of the WNP spotted in red within New South Wales (NSW) Australia

A large restoration program occurred throughout the 1980s and 1990s, within the Central Valley (500ha area) and valley walls of the Warrumbungle National Park (WNP; Figure 2) to restore the endangered intact remnants of the once abundant Box-Gum Grassy Woodland. The restoration focused on the prior dominant White Box (*Eucalyptus albens*), Yellow Box (*Eucalyptus melliodora*) and Blakely's Red Gum (*Eucalyptus blakelyi*) species. A series of plantings of these species was conducted by National Parks and Wildlife Services in 1983, 1988, 1992 and 1993 within the Central Valley (Appendix Figure 1; Appendix Figure 2). Historical records were provided by National Parks and Wildlife Services (Appendix Figure 1;

Appendix Figure 2) which show regions and dates of plantings. While records indicate that local provenance sourcing was utilised for this restoration, there is no detail on the collection design. However local sourcing has recently been confirmed in *E. melliodora* (Yellow Box) plantings in the same region (Rosser *et al*. 2023).

This study focused on *E. albens* (White Box) which was once a dominant standing tree in the WNP as a part of Box-Gum Grassy Woodlands. Previously, genetic technologies explored restoration success in *E. melliodora* in the WNP (Rosser *et al*. 2023). Therefore, I focus on this similar species to understand whether these results are broadly applicable across species. *Eucalyptus albens* trees have grey-toned rough fibrous bark on the base of the trunk and smoothed bark above the main branches. Leaves vary in shape, being oval to lance-like and grey to bluish-green on both surfaces. Flowers are creamy, and off-white and may be profuse and conspicuous. Buds are large, glaucous, and spindle-shaped in clusters of 3-7 on the ends of branches in the canopy. Mature trees are up to 25m high. Each tree was identified to be *E. albens* from their juvenile leaves which differ significantly from other common Box-Gum trees such as *E. melliodora* (Yellow Box) and *E. blakelyi* (Blakely's Red Gum) which were common in the plantings and natural populations within the WNP.

2.2 Sampling of mature and juvenile *E. albens* populations

2.2.1 *Description of mature and juvenile populations used for genetic analysis of leaf samples*

The restoration plantings were identified from maps provided by National Parks and Wildlife Services (Appendix Figure 1; Appendix Figure 2). Stands planted in in 1983, 1988, 1992, and 1993 were able to be located in the field while one stand could not be assigned a year of planting (unknown year). Planted stands were generally planted in rows with timber stakes used to mark their location (Figure 3C; Table 2). Natural/remnant populations (termed "natural") were characterised as trees that were not present in the 1956 aerial image and therefore have naturally regenerated since that time (Figure 3A; Table 2). These natural trees were located within the valley walls of the WNP at a distance > 1km from plantings on untouched slopes of the valley or the outskirts of the Central Valley. Relicts were identified

using historical aerial imagery from 1956 to locate trees that pre-date the declaration of the reserve (Appendix Figure 3). Referencing the aerial photography these trees were located in the field and confirmed as *E. albens* and most of the remaining relictual trees (relict/relictual) within the valley were sampled (Table 2; Figure 3A). Many of these were fallen, resprouting trees following the bushfires in 2013. Larger trees were preferred since these were much older and increased the likelihood that they were present before the planted restoration trees. Juvenile plants (termed "saplings") were also collected from under the three categories of mature trees. These were shorter (0.5m – 5m tall) and occurred in scattered areas across the Central Valley or within the canopy of woodland forests (Figure 3B; Table 2). The 116 samples collected in 2023 were combined with 273 *E. albens* samples collected in 2020 and 13 collected in 2021.

2.2.2 *Leaf sampling*

Vegetative (leaf) samples were collected from the Central Valley of the WNP (31°17'S, 149°00'E) in 2020, 2021 and 2023. A total of 402 samples were taken from individual *E. albens* within mature stands of relictual, natural and planted trees in addition to saplings of *E. albens* (Figure 3; Table 2). One to two leaves were collected from each tree and placed in a sealed seed envelope which was later refrigerated to reduce the possibility of mould.

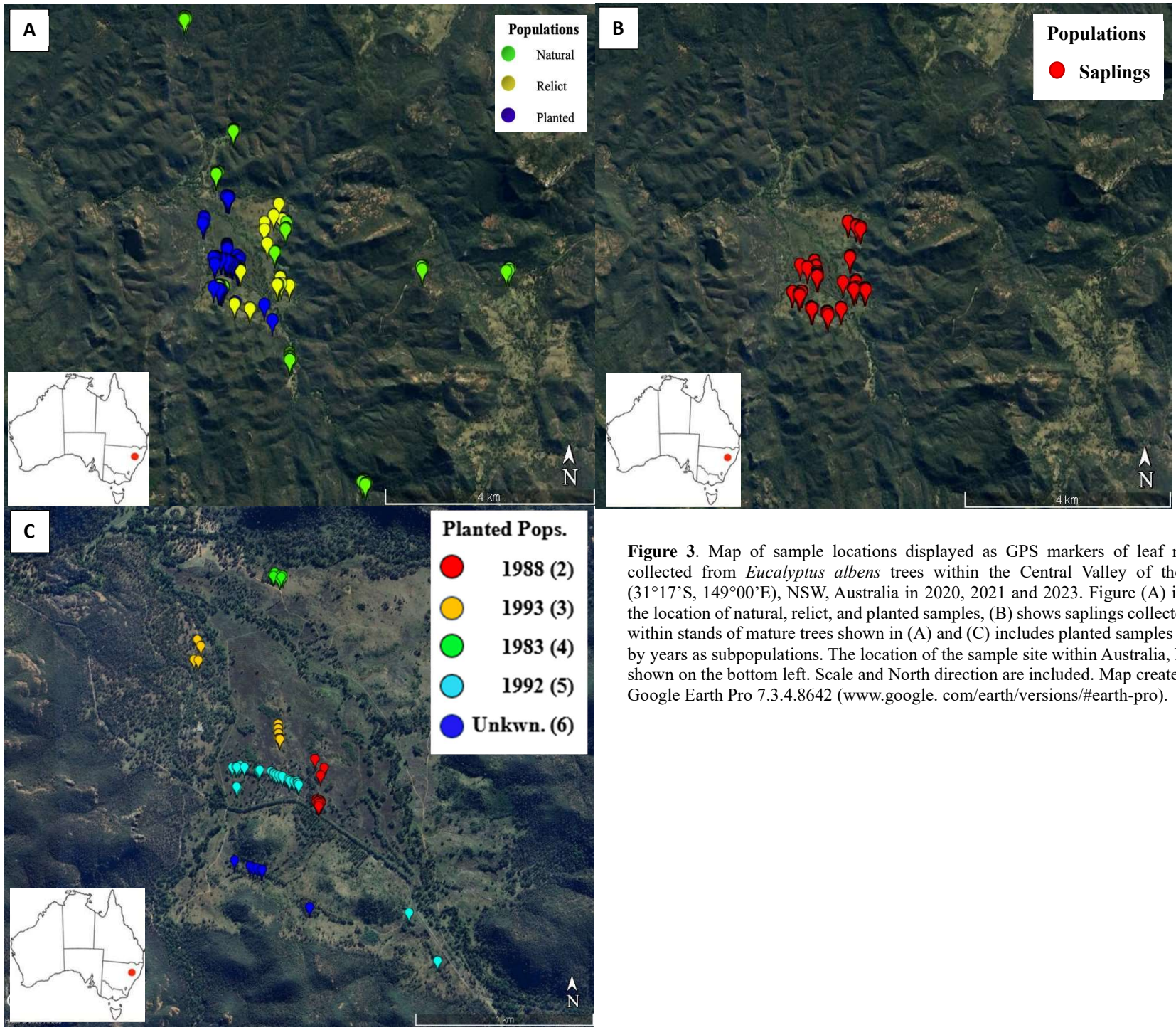


Figure 3. Map of sample locations displayed as GPS markers of leaf material collected from *Eucalyptus albens* trees within the Central Valley of the WNP (31°17'S, 149°00'E), NSW, Australia in 2020, 2021 and 2023. Figure (A) includes the location of natural, relict, and planted samples, (B) shows saplings collected from within stands of mature trees shown in (A) and (C) includes planted samples divided by years as subpopulations. The location of the sample site within Australia, NSW is shown on the bottom left. Scale and North direction are included. Map created using Google Earth Pro 7.3.4.8642 (www.google. com/earth/versions/#earth-pro).

Table 2. Broad population categories of *Eucalyptus albens* targeted for leaf sampling of genetic material within the Central Valley of the WNP (31°17'S, 149°00'E), NSW, Australia. This includes three mature reproductively active categories of trees, from which naturally regenerating juveniles and seeds were also collected. Seeds of known maternal parentage were germinated and sampled after nine weeks of growth. Leaf samples were collected in 2023 and in 2020, 2021 and currently 2023.

Reproductive status	***Population***	***Subpopulation Number***	**Description**	**Definition**	**Age estimate (years)**	**Size range**	**No. of leaf samples sequenced**
Mature	Relictual (see Figure 3A)	Grouped into (1)	Older scattered trees possibly remnants from before the widespread deforestation	Present in aerial imagery in 1956 (Appendix Figure 3).	~ > 70	~ 25 metres tall.	16
	Planted (see Figure 3C)	Grouped by planting year (1988, 1993, 1983, 1992 and unknown years; Appendix Figure 1; Appendix Figure 2)	Planted revegetation plots were assumed to be sourced from local seed but there are no details in the records	Planted in rows	30 – 40 years old. Four stands were planted in 1983, 1988, 1992, and 1993 1 stand is of unknown age (Appendix Figure 1; Appendix Figure 2)	Up to 15 m	100
	Natural (see Figure 3A)	Grouped based on sampling 'stands' separated by distance (about 200m; 7 -14)	Naturally, revegetated trees in scattered stands on the edges of the valley.	Not present in aerial imagery in 1956 (Appendix Figure 3).	Age is estimated to be ~ 40-50 years old.	Up to 20 m	98
Juvenile	Saplings (see Figure 3B)	Assigned to a subpopulation	Naturally establishing	Smaller offspring are found in the field	~ 1-5 years old	50 cm to 5 m	188

		depending on location to mature tree and predicted adult (15-23)	juveniles surrounding mature trees	surrounding adult trees. saplings are from 50cm to 5 metres tall and are around 1-5 years old.			

Juvenile	Collected seeds/seedlings	based on a maternal tree (see Figure 4) (24-28)	Collected seeds of known maternal parentage that were successfully established in controlled greenhouse conditions	Seeds were not able to be collected from all stands of adult fruiting trees in 2023:
				• Relictual (4)
				• Natural (2)
				• Planted
				  ○ 1988 (3)
				  ○ 1992 (4)
				  ○ Unknown (1)
				See Table 3 for more information
					Samples were taken ~ 9 weeks after the seeds were planted	Grown in a greenhouse until 5-10 cm tall.	50 successful

2.2.3 *Subpopulation assignment*

Subpopulations were assigned within the broader categories of mature and offspring populations to maximise the accuracy of the population genetics analysis. This was based on the proximity of mature trees and for saplings based on the nearness to the mature tree. Collected seeds were categorised into the broader categories of mature trees. Firstly, planted trees were assigned to five subpopulations based on the planting year (1983, 1988, 1992, 1993 and unknown; see Appendix Figure 1; Appendix Figure 2). All relictual trees were grouped within a single subpopulation, as they are sparsely distributed (Table 2). Further, natural trees were sampled in 8 distinct stands separated by significant geographical distance throughout the outskirts of the Central Valley (Table 2). Some natural trees were solitary and thus were assigned to a single subpopulation for reasons similar to relictual trees (Table 2).

Juvenile sub-populations were assigned according to the mature trees. Sapling samples were assigned to relictual and planted (1983, 1988, 1992, 1993, unknown) subpopulations depending on nearness to the predicted adult (Table 2). Furthermore, natural saplings were all grouped as they were only sampled within the Central Valley from a few isolated natural trees. The sub-populations for saplings are uncertain as nearness to a mature tree does not guarantee the parentage. However, the maternity of collected seeds was certain and these were assigned to natural (1), relictual (1), and planted (1988, 1992 or unknown) sub-populations (Table 2). Ultimately, the total number of populations was 28.

2.2.4 *Seed sample collection and germination*

Fruits were collected in March 2023 from natural, planted and relictual *E. albens* trees within the Central Valley restoration area for genetic analysis of offspring with known maternal parentage. Fruit collection was limited by both availability and accessibility of fruits with many trees not in fruit or too high for collection. Seeds were unfortunately not able to be collected from the planting stands 1993 and 1983 due to a lack of fruits available within reach for collection. Nevertheless, fruits were identified to be *E. albens* as they were larger than other species within the WNP. Fruits were 8-10mm in diameter and 9-12mm long, glaucous, sessile, or shortly pedicellate with a barrel-like shape. In total, only 33 trees had fruits available to collect, with 23 natural trees, 13 planted trees, 8 relictual trees and no collection from juvenile saplings. (Table 3). A lengthened cutting tool was used to reach higher branches in the canopy to collect approximately 5-10 fruits per tree, depending on how many fruits were available. Fruits were sealed in a seed envelope to allow the fruits to open and release the seeds which occurred after two to three days. Material released within the fruit capsule includes viable seeds (only a few per capsule) which were larger and darker in colour. Fruits also contained 'chaff' (infertile seeds and non-seed material) which existed in greater quantities than seeds and was woody and tan, brown in colour. The diameter and length of fruits (mm) were measured utilising vernier callipers.

Table 3. Seed collection occurred from mature *Eucalyptus albens* populations grouped as relictual, natural and planted trees and the years planted (1983, 1988, 1992, 1993 and unknown; see Appendix figures 1 and 2) within the Central Valley restoration area of the Warrumbungle National Park. The sample numbers are shown for mature trees, fruits gathered, and surviving greenhouse-grown seedlings, (and respective number of trees)

Mature population	No. of trees sampled for fruit collection	No. of fruits gathered	Surviving seedlings grown from seed	From No. of trees
Planted (1983)	1	8	0	0
Planted (1988)	4	38	17	3
Planted (1992)	4	31	17	4
Planted (1993)	1	5	0	0
Planted (Unknown)	3	19	6	1
Relictual	6	42	5	4
Natural	14	109	5	2
Total	**33**	**252**	**50**	**14**

Seeds were germinated in a temperature-controlled greenhouse (15°C/ 25°C). One to four viable seeds were placed in a 50mm square forestry tube pot (volume = 22.8mL) in a 3:1 native potting mix: seed-raising mix. Seeds were automatically watered and set to mist for 10 seconds every 5 minutes to ensure the potting mix would not dry out. Humidity was set to 50% to reduce the likelihood of pots drying out, and yet reduce the ability for mould to grow on and beneath the surface of the potting mix. After 9 weeks, leaf samples were harvested from seedlings. In total, 50 seedlings survived to produce enough tissue for sampling including seeds from 14 *E. albens* trees (2 natural, 8 planted, 2 in 1988, 4 in 1992 and 1 from unknown, 4 relictual; Table 3; see Figure 4 for location of maternal trees). Unfortunately, several seeds from the remaining trees did not germinate or did not grow to a suitable size in time and therefore could not be used for analysis. Harvested leaves were placed in a sealed seed envelope for sample preparation.

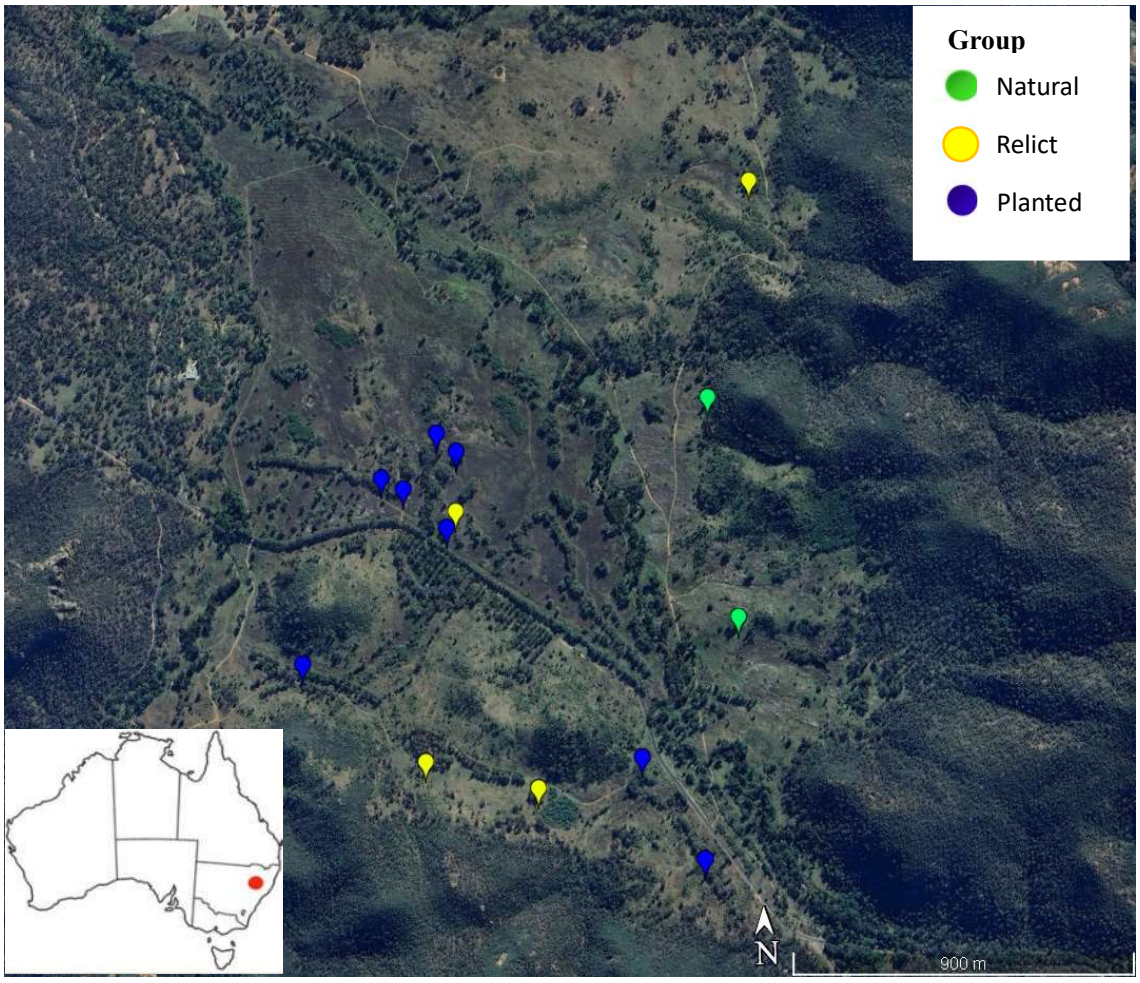


Figure 4. Location of seed collection sites from maternal *Eucalyptus albens* trees showing only seeds that were successfully grown to enable harvesting of genetic material, within the Central Valley of the Warrumbungle

National Park (31°17'S, 149°00'E), NSW, Australia. The location of the sample site within Australia, NSW is shown on the bottom left. Categories of the maternal tree (natural, relict and planted) are classified by colour in the legend. Map created using Google Earth Pro 7.3.4.8642 (www.google. com/earth/versions/#earth-pro).

2.3 Sample preparation for genetic sequencing

Samples were prepared prior to dispatch to Diversity Arrays Technology, Canberra for genetic sequencing, using their guidelines. Leaves were first cut into smaller pieces to insert into the plate for DNA extraction. This involved punching three 5mm diameter discs per leaf and placing them into each tube from the 1.1mL Microtube racked system (Thermo-Fisher catalogue number 15082, 96 tube rack [as per Diversity Arrays Technology's guidelines]) provided by Diversity Arrays Technology, sourced from Thermo-Fisher Scientific. Leaves from each seedling (grown from collected seed) were small, therefore were manually cut into 5-10mm lengths using suture scissors and wiped with 70% ethanol between each sample to avoid contamination as per DArT guidelines. Samples were freeze-dried at the University of Wollongong and were not sent fresh due to time constraints with sending samples. This involved freeze-drying leaf samples in a Martin Christ laboratory Alpha 1-2 LSC basic freeze dryer with an ice condenser temperature of -55°C attached to a vacuum pump. Samples were dried for 18 hours to ensure all moisture was removed from the tissue and were later sealed with a 1.2m microtiter tub 8-cap plug strip (Thermo-Fisher catalogue number QSP847) provided by Diversity Arrays Technology, sourced from Thermo-Fisher Scientific.

2.4 DArT genotyping

DArTseq services (Diversity Arrays Technology, Canberra) for the DNA extraction of collected leaf material and sequencing of SNPs (Single Nucleotide Polymorphisms) were utilised for this study. This process involved using a high-density microarray development for effective *Eucalyptus* DNA sampling (Sansaloni *et al*. 2010, Petroli *et al*. 2012). Furthermore, the DArTseq services extracted genomic DNA to a stringent level in line with a modified CTAB protocol developed by Diversity Array Technologies. Following that procedure, complexity reduction was completed via a *Pst*I/*Taq1*-based method produced by Sansaloni *et al*. (2010). This process was conducted utilising enzymatic breakdown techniques to select highly active genomic DNA regions and specifically remove unwanted repeated sequences. Finally, to detect

SNP polymorphisms within the genome of each sample collected, Next Generation Sequencing (NGS) was conducted against a library developed specifically for *Eucalyptus* (Sansaloni *et al*. 2010). Utilising DArTseq microarray technology at a 'high intensity' run; highly descriptive polymorphic SNP loci were sequenced from all leaf samples throughout the entire genome. Sequencing was implemented on an Illumina HiSeq 2,500 using 75-cycle single-end reads. Raw reads were processed using DArT's proprietary variant calling pipeline known as DArTsof-14. The SNP loci sequenced are bi-allelic and the results produced a database containing the presence and absence of an SNP at specific sites of each genome. This data received from two sample plates sequenced in 2023 was combined with previous data from *E. albens* samples collected previously in the WNP in 2020 and 2021 (Table 2).

2.5 Data Filtering

The primary SNP dataset was rigorously filtered to ensure only the highest quality markers were maintained and the genotypes retained were precise (Table 4). This filtering process was completed using DartR (Gruber *et al*. 2018), and the final dataset only included loci that had a call rate ≥ 0.85, a read depth of ≥ 10, a reproducibility rate of ≥ 0.99 and a minor allele frequency of ≥ 0.01. Maximising the call rate of data for analysis is essential to this study as the proportion of called SNPs can alter the results of the analysis (Sasaki *et al*. 2018). We employed this filtering mechanism as it ensured only high-quality loci were present within each sample within the dataset. This removed any sequencing errors or low-quality loci. Monomorphic loci and individuals with ≥ 15% of missing data were also excluded from the analysis during the filtering process.

There were 5-10 duplicate samples in each sequencing run (5-10 individuals repeated twice) to check the reliability of sequencing results. For each duplicate, the sequencing result was compared at each locus (0, 1, or '–' for no result), and the number of times this result differed between the replicates and divided total by the number of loci.

Table 4. DartR SNP loci dataset filtering descriptions

Call Rate	The percentage of samples in which SNP loci was either a heterozygote (2) or homozygote (1), rather than being not identified (-). Filtering out call rates below a specific threshold (higher call rate = remove more missing/inaccurate data) will remove unidentified SNPs and increase the quality of data.
Read Depth	The number of times an individual base has been sequenced, and the greater the read depth the more confidence in the data.
Reproducibility rate	Filters loci based on average repeatability of alleles at a locus.
Minor allele frequency ('MAF')	Filters loci based on MAF (the frequency at which the second most common allele appears in a dataset)
Monomorphic loci	Filters out monomorphic loci where one allele occurs at a site or locus.

2.6 Data quality

DArTseq high-throughput microarray overall yielded 41,425 polymorphic SNP loci across a total of 452 *E. albens* leaf samples and 50 *E. albens* seedling leaf samples. In total, 1537 loci were isolated under a high stringency filter with a call rate of ≥ 0.85, a reproducibility rate of ≥ 0.99 and a minor allele frequency of ≥ 0.05 (Table 4). This data was of moderate quality, with an average call rate of 54%, and 16% of the loci obtained had a call rate of ≥ 0.85 (including the seedling data). By removing individuals with ≥ 0.15 call rate, (19 in total), this left a remaining total of 383 individuals (1 relictual, 2 planted, 16 saplings removed). Further, this same filtering process was applied to the 50 seedling samples separately, due to the separation between the two genetic differentiation analyses. In total, 2570 loci from seedling samples were isolated under these filtering settings within the seedling dataset. This offspring dataset was of moderate to high quality, with an average call rate of 65% and 36% of the loci obtained had a call rate of ≥ 0.85. No seed individuals were removed from the dataset.

Out of the 10 duplicate samples from one sequencing set, and 5 duplicates from each of the other sets (2), the average similarity was 99.85% and ranged from 94.3% to > 99.99%. There were no instances of loci being genotyped differently between duplicate samples. Additionally, one *E. melliodora* and *E. blakelyi* sample were each sequenced to compare genotypes to *E.*

albens. This process was undertaken to confirm species identification if samples were unknown or not confirmed. This overall validated our dataset and ensured only *E. albens* samples were analysed for this study.

2.7 Analysing connectivity within offspring

2.7.1 *Parentage assignment*

To analyse the level of connectivity and gene flow between restored and remnant populations, a parentage assignment of saplings and collected seeds was conducted (Table; Table 6). This measures the level of connectivity between populations and determines if the restoration was successful with the use of locally sourced seeds. The parentage of saplings collected within the WNP in 2023 (N=50) and collected seeds (N=49; CERVUS program automatically excluded an individual due to loci typing errors) was analysed using the program CERVUS 3.0.7. The paternity assignment analysis was conducted on seeds from known maternal trees which allows us to have increased confidence in parentage assignment. The parent-pair sex unknown analysis was chosen for saplings since neither parent was known prior (Table 5; Table 6). CERVUS utilised allele frequency data to calculate 'LOD' (log odds ratio) and 'Delta,' a derivative of LOD to develop a simulation of parentage to assign unknown paternity and parent pairs (sex unknown). Following the simulation, a parentage assignment was conducted. A model was developed for the most likely parent based on a higher positive LOD score which indicates how closely loci are predicted to be located on a chromosome. For the paternity analysis, the candidate father ID is the individual most likely father found for each seedling. For the parent pair analysis, the first candidate parent ID is the individual most likely, and the second candidate is the second most likely. Significance is calculated by averaging the LOD score and calculating Trio Delta, and fathers/parent pairs with a positive significance indicate that parents are confirmed with a 0.95 confidence interval. Overall, this determines the potential of a genetic relationship by the carrying over of genes to offspring. The genetic origin of saplings and seeds facilitates an understanding of reproductive connectivity between planted stands and natural populations. This is especially advantageous within the paternal analysis (known mothers) since it confirms pollen dispersal distances.

Table 5. Definitions of response variables derived from the SNP dataset including measures of genetic diversity (He: expected heterozygosity and *Ho*: observed heterozygosity), genetic differentiation (F_{ST}) and the Inbreeding co-efficient (F_{IS}), *Bayesian Analysis* (BCA) and *Principal Component Analysis* (PCA). The table includes the interpretation and formula of response variables (Frankham *et al*. 2010)

Response variable	Definition	Interpretation/Formula
Genetic diversity Expected Heterozygosity (*He*)	The fraction of heterozygotes that is expected in the population under the Hardy-Weinberg mnodel; is calculated based on known allele frequencies in a population (Frankham *et al*. 2010).	0-1 Higher values = increased genetic diversity $He = 1 - \sum_{i=l}^{n} Pi^2$ The sum of 1 – homozygote allele frequency. Where *Pi* is the frequency of the i^{th} allele of *n* alleles for a single locus. Or $2pq = 1 - p^2 - q^2$ from the Hardy-Weinberg equilibrium formula. p = dominant allele frequency q = recessive allele frequency
Genetic diversity Observed Heterozygosity (*Ho*)	The fraction of individuals in the population that are heterozygous at a given locus; is calculated based on known genotype frequencies in a population (Frankham *et al*. 2010).	0-1 Higher values = increased genetic diversity $Ho = 1 - \sum_{i=l}^{n} f\ [Ai\ Ai]$ Or, 1 - the sum of the frequency (*f*) of homozygotes at each sequential allele $[Ai\ Ai]$
Inbreeding Coefficient (F_{IS})	The proportion of variance of a subpopulation within an individual (Frankham *et al*. 2010).	-1 to 1 Higher values = more inbreeding $F_{IS} = \frac{H_S - H_I}{H_S}$ H_S = average expected heterozygosity in subpopulations H_I = the average observed heterozygosity in a group of subpopulations

Genetic differentiation *(F_{ST})*	The proportion of total genetic variance in a subpopulation relative to the total genetic variance (Frankham *et al*. 2010).	0-1 Higher values = increased differentiation $$F_{ST} = \frac{H_T - H_S}{H_T}$$ H_T = average expected heterozygosity in the total population
Population Structure ***Principal Component Analysis***	Takes SNP genotypes of individuals producing a diagnostic plot to identify structure in the distribution of genetic variation (Frankham *et al*. 2010).	Each individual is represented as a point on the graph, and further distance apart from each other indicates genetic variation
Population Structure ***Bayesian Cluster Analysis***	Reveals the presence of genetic subdivision by assigning individuals to subpopulations from SNP genotype data and therefore calculating the number of clusters in a dataset (Frankham *et al*. 2010).	Presented as a *Structure plot* in which each bar represents an individual that is comprised of colours each representing a cluster. More clusters = increased gene flow

Table 6. List of comparisons and respected tests conducted for the purposes of aims 1 and 2, the table includes the populations tested, hypotheses and response variable tested.

Aim	Comparison	Populations compared	Hypotheses	Response variable and test
1	Use paternity analysis of collected seeds and parent pair analysis of saplings to assess connectivity between planted and remnant populations	Collected seeds (n=49) Saplings (n=50)	Planted populations lack connectivity to remnant populations	Paternity analysis and parent pair analysis
1	Use genetic differentiation and population structure analyses of collected seeds to assess connectivity between planted and remnant populations	Collected seeds (n=50)	Planted populations lack connectivity to remnant populations	Pairwise F_{ST}, PCA and BCA
2	Use Expected Heterozygosity (*He*) to assess differences in genetic diversity	Relictual (n=15) Natural (n=98) Planted (n=99) Saplings (n=171) Collected seeds (n=50)	Planted populations will lack Expected Heterozygosity to remnant populations	Expected Heterozygosity and 1-way ANOVA to test difference
2	Use Observed Heterozygosity (*Ho*) to assess differences in genetic diversity	Relictual (n=15) Natural (n=98) Planted (n=99) Saplings (n=171) Collected seeds (n=50)	Planted populations will lack Observe Heterozygosity to remnant populations	Observed Heterozygosity and 1-way ANOVA to test the difference
2	Calculate inbreeding levels (F_{IS}) of populations to determine the presence of inbreeding	Relictual (n=15) Natural (n=98) Planted (n=99) Saplings (n=171) Collected seeds (n=50)	Planted populations will have increased inbreeding levels compared to remnant populations	Inbreeding Coefficient and 1-way ANOVA to test difference
2	Use F_{ST} to assess genetic differentiation between planted, remnant and offspring populations	Relictual (n=15) Natural (n=98) Planted (n=99) Collected seeds (n=50)	Planted populations will experience increased genetic differentiation to remnant stands	Pairwise F_{ST}
2	Use PCA and BCA to assess the population structure of planted, remnant and offspring populations	Relictual (n=15) Natural (n=98) Planted (n=99) Saplings (n=174) Collected seeds (n=50)	Planted populations will form a unique differentiated cluster to remnant stands	*Principal Component Analysis* (PCA) and *Bayesian Cluster Analysis* (BCA)

2.7.2 *Genetic differentiation and population structure* E. albens *collected seeds*

Collected seeds of known maternity from 5 populations (1 relictual, natural, planted 1988, planted 1992, and planted unknown) were investigated as a subset of the full data to assess if gene flow is present between populations or if sub-populations are occurring within the juveniles. Pairwise F_{ST} comparisons between seeds collected from 5 seedling populations were explored (N=50; from 14 trees; Table 5). Differences in population structure were investigated in separate *Principal Component Analysis* and *Bayesian Cluster Analysis*, producing another *Structure* plot to examine the structure of seedlings alone (N=50). The population structure and genetic differentiation (pairwise F_{ST}) of seedling populations can tell us if gene flow is present between populations, or if subpopulations are forming between generations.

2.8 Analysing the genetic success of the restoration

2.8.1 *Genetic diversity and inbreeding*

The full data set of all mature and juvenile samples was analysed to detect differences in genetic diversity and population structure between natural, sapling and planted populations of *E. albens*. To analyse genetic diversity and explore the level of inbreeding of *E. albens*, observed heterozygosity (*Ho*), and expected heterozygosity (*He*) and F_{IS} inbreeding coefficient across all 28 populations was calculated under Hardy-Weinberg equilibria assumptions utilising dartR in RStudio 2023.03.1 (Gruber *et al*. 2018; Table 5). Expected heterozygosity for a population takes the expected proportion of heterozygotes, that is expected under Hardy-Weinberg equilibrium for each locus. Following that, it then averages this across the loci for an average estimate of the population. Observed heterozygosity for individuals is calculated as the proportion of loci that are heterozygous for that individual, then averaged as a population (Table 5). Expected heterozygosity is the main response variable for use within this study to measure genetic diversity, as it is less sensitive to sample sizes than observed heterozygosity. A one-way ANOVA was conducted in RStudio 2023.03.1 to determine significant differences in expected heterozygosity, observed heterozygosity and inbreeding levels (F_{IS}) between groups (natural – including relictual, planted stands (all populations were pooled), saplings, and seedlings; Table 5). Specifically, I focused on whether there was a reduction in these variables in planted populations to determine whether genetic diversity decreased in restored populations. Further, the inclusion of collected seeds within this analysis allowed me to

determine whether collected seeds grown under ameliorant greenhouse conditions increased genetic diversity due to the removal of environmental variables. The *Ho*, *He* and F_{IS} datasets met the assumptions of normality ($P > 0.05$) which was tested using the Shapiro-Wilk test. Further, to test the homogeneity of variances, a Bartlett test of equal variances was performed and concluded that the variance of *Ho*, *He* and F_{IS} were equal ($P > 0.05$).

2.8.2 *Genetic differentiation and population structure*

To examine the genetic differentiation of all *E. albens* populations, pairwise F_{ST} was calculated in dartR (Gruber *et al*. 2018) for 19 populations of natural, relictual, planted and collected seed samples (N=262; excluding saplings, due to the substantial number of them; Table 5; Table 6). This is used as a tool to measure population subdivisions and differences to determine the success of local provenance sourcing to produce a well-homogenised and connected population of *E. albens*.

To analyse population structure, a *Principal Component Analysis* of all 28 populations was also conducted in dartR (Gruber *et al*. 2018) and a *Bayesian Cluster Analysis* producing a *Structure* plot was completed within the software *ParallelStructure 2.3.4* on the CIPRES portal (Miller *et al*. 2015; Table 5; Table 6). This allows me to identify the approximate number of genetic clusters within the filtered dataset between all populations. This clustering analysis included an excluded population (N=436; with a total number of 29 populations). This process was completed by conducting the analysis process with a total length of 80,000 burn-ins and 120,000 MCMC reps. This was iterated 5 times for each of K=1 to K=29 (or K=1 to K=5 for *E. albens* seedlings) for each population. Following this, the data was entered into *StructureSelector* (Li and Liu 2018), and the output calculated the number of genetic clusters using the Puechmaille method (Puechmaille 2016). The Puechmaille method (Puechmaille 2016) on *StructureSelector* (Li and Liu 2018) was utilised to determine the number of genetic clusters within the dataset as this process is not heavily affected by uneven sample sizes (which are prominent within this study) and is known to be more accurate and surpass various methods (Puechmaille 2016). This method calculated MedMeaK, MaxMeaK, MedMedK and MaxMedK to determine the number of genetic clusters, and the results were ultimately retained. Finally, to strengthen the analysis of the population structure of all *E. albens* populations, a *Principal Component Analysis* (PCA) was performed utilising dartR (Gruber *et al*. 2018) and graphed in Excel (Table 5; Table 6).

3.0 Results

3.1 Connectivity analysis

3.1.1 *Paternity analysis of* E. albens *collected seeds*

The paternal source of pollen was identified by a genetic match within the dataset for a total of 9 seeds collected from maternal trees with a 95% confidence level. (Figure 5; Figure 6; Table 7; Appendix Table 1). This analysis found 9 candidate parental parents within the genetic dataset which is 18% of the 50 total seedlings (Table 7). Four of the matches were between the same maternal and paternal trees, that is, they were produced from a single parent pair. Within the planted stands 3 maternal trees from the planted stand (unknown) (Figure 5; Figure 6; Table 7) had a mix of planted and natural parents. Here, the contribution of natural parents is due to the close geographical range between the planted stands and adjacent mature natural trees (Figure 5; Figure 6). Other planted stands shared only planted parents or were not matched to a paternal parent within the genetic dataset. However, there was evidence of connectivity between planted populations with a significant geographical distance separating them (Figure 5). There is evidence of connectivity with collected seeds from one maternal relictual tree matching to one natural tree, but there was no evidence of relictual trees contributing pollen to the offspring of planted trees (Appendix Table 1; Figure 5).

Table 7. Collected seeds with assigned candidate fathers with 95% confidence. The table includes seedling ID, known mother ID and group, candidate father ID and group

Seed ID and Pop.	Mother ID (Known)	Group and Pop.	Candidate father ID	Group and Pop.
S8851 (1988)	WB8801	Planted 1988 (2)	P01100	Planted 1988 (2)
S88371 (1988)	WB8801	Planted 1988 (2)	P01100	Planted 1988 (2)
S88372 (1988)	WB8801	Planted 1988 (2)	P01100	Planted 1988 (2)
SUN56 (Unknown)	PLC11	Planted unknown (6)	R100	Natural (14) (2)
SUN69 (Unknown)	PLC11	Planted unknown (6)	R200	Natural (14)
SUN73 (Unknown)	PLC11	Planted unknown (6)	R100	Natural (14)
SUN771 (Unknown)	PLC11	Planted unknown (6)	R100	Natural (14)
SUN772 (Unknown)	PLC11	Planted unknown (6)	R100	Natural (14)
SRE111 (Relictual)	R083	Relictual (1)	R08305	Natural (14)

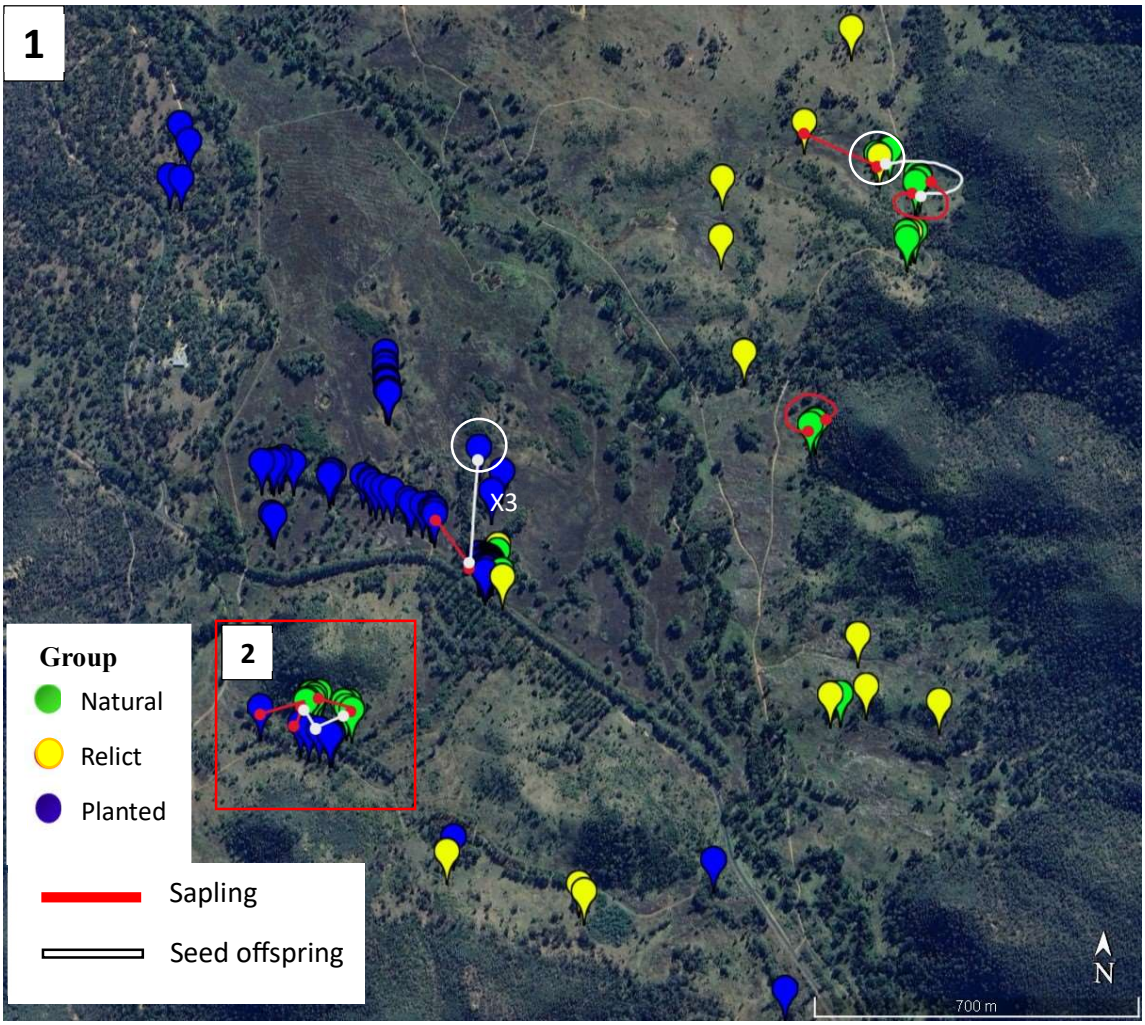


Figure 5. Location of parent pairs (Map No. 1) of *Eucalyptus albens* saplings (red arrows) and seedlings (white arrows) with 95% confidence within the Central Valley of the Warrumbungle National Park (31°17'S, 149°00'E), NSW, Australia. Sample type (natural, relict and planted) is classified by colour in the legend. The red box highlights a zoomed-out area of Figure 6 (Map No. 2). White circles represent the maternal parent of seedlings. Number of offspring produced from single parent pair highlighted between them (x3). Map was created using Google Earth Pro 7.3.4.8642 (www.google. com/earth/versions/#earth-pro).

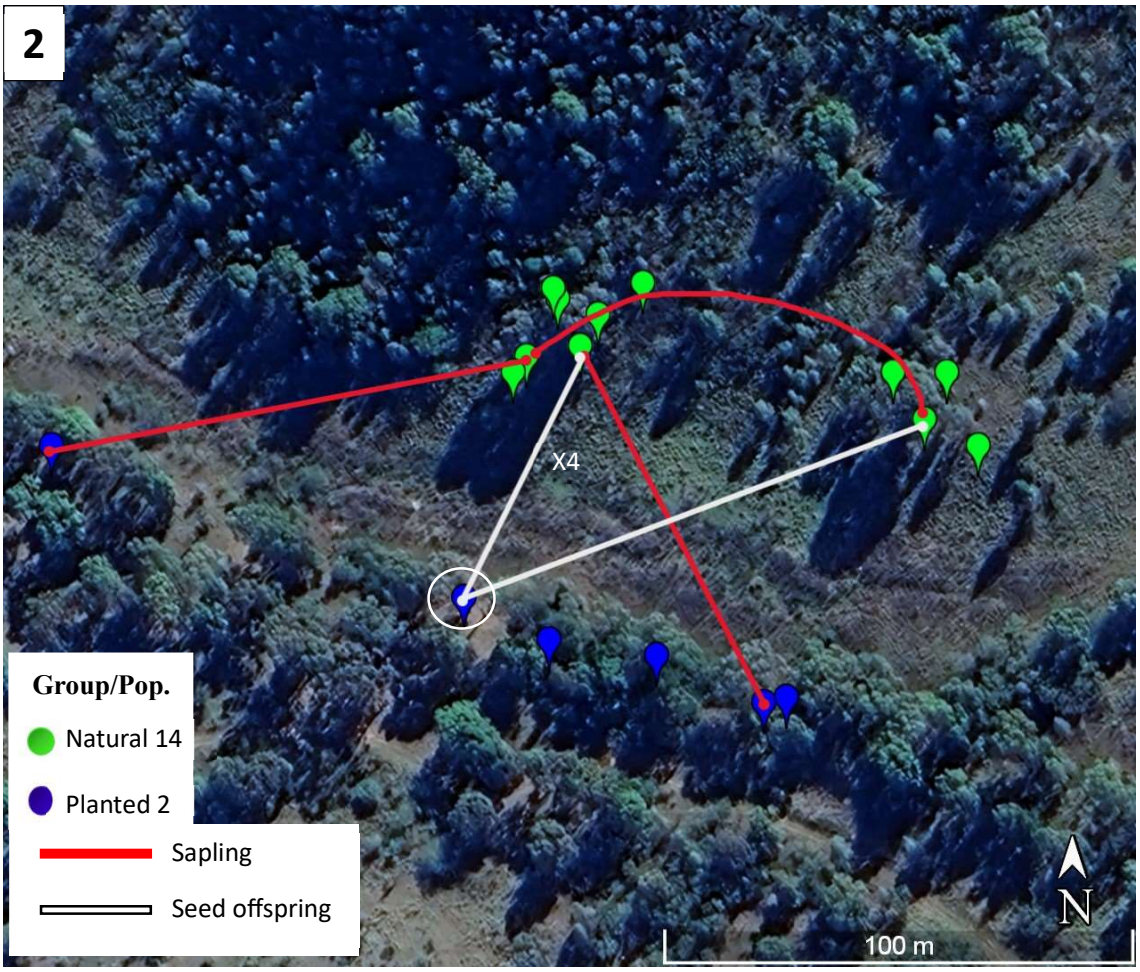


Figure 6. Location of parent pairs (Map No. 2) of *Eucalyptus albens* saplings (red arrows) and seedlings (white arrows) with 95% confidence within the Central Valley of the Warrumbungle National Park (31°17'S, 149°00'E), NSW, Australia. Sample type (natural 14 and planted 2) is classified by colour in the legend. White circles represent the maternal parent of seedlings. Number of offspring produced from single parent pair highlighted between them (x4). Map created using Google Earth Pro 7.3.4.8642 (www.google. com/earth/versions/#earth-pro).

3.1.2 *Parentage pair analysis of* E. albens *saplings with both parents unknown*

In saplings, parentage pairs (sex unknown) were assigned for a total of 8 parent pairs with 95% confidence (Figure 5; Figure 6; Table 8; Appendix Table 2). The results suggest again a contribution from both natural and planted populations to saplings with 95% confidence. Interestingly, a sapling was produced from two relictual trees with a significant distance between trees (~200m; Figure 5) which is an unexpected result. Confidence in allocating parent pairs was however low across the site, with only 8 of the 50 saplings allocated to parents and was similar to seedlings with a known mother (16%) and a single parent pair produced three saplings. Nevertheless, both analyses suggest that planted, saplings and collected seeds have

natural trees contributing to the production of offspring in some populations. Furthermore, the analysis output provided additional matches for saplings (N=28) with one parent confirmed and another 'most likely'; (denoted by '-'). Therefore, we consider the other that there is additional evidence of outcrossing between remnant and planted populations albeit without a confirmed confidence level (Appendix Table 2). Planted populations often were outcrossing with relictual trees, similar to natural and relictual populations. There was also further evidence of connectivity between natural and planted populations similar to the above results (Appendix Table 2).

Table 8. Saplings with assigned candidate parents with 95% confidence. The table includes Sapling ID, sapling predicted maternal tree population, first candidate parent ID and group and second candidate parent ID and group

Sapling ID and Pop.	From predicted maternal tree Pop.	First candidate parent ID	Group and Pop.	Second candidate parent ID	Group and Pop.
PLC06 (19)	Planted unknown (6)	R100	Natural (14)	PLC01	Planted unknown (6)
PLW03 (19)	Planted unknown (6)	R105	Natural (14)	PLW01	Planted unknown (6)
WB880205 (23)	Planted 1988 (2)	wbPA1 26	Planted 1988 (2)	wbplant2 11	Planted 1992 (5)
MB0108 (22)	Natural (14)	MB01	Natural (14)	MB0105	Natural (14)
R213 (22)	Natural (14)	R200	Natural (14)	R105	Natural (14)
R08210 (20)	Relictual (1)	R08203	Natural (14)	R08205	Natural (14)
R08306 (20)	Relictual (1)	R08305	Natural (14)	R083	Relictual (1)
R08310 (20)	Relictual (1)	R083	Relictual (1)	wb37 relict	Relictual (1)

3.1.3 *Genetic differentiation of* E. albens *within collected seeds*

The population differentiation among seeds of planted, relictual and natural stands was moderate (pairwise F_{ST}) ranging from 0.095 to 0.199: Table 9). Differentiation was highest between populations planted in 1992 and planted unknown years F_{ST} = 0.199). The 1992 planted stand was also different to 1988 (pairwise F_{ST} = 0.176), natural trees (pairwise F_{ST} = 0.180) and relicts (Pairwise F_{ST} = 0.133). However, lower F_{ST} values were found between relicts and natural trees (pairwise F_{ST} values = 0.095) and planted unknown (pairwise F_{ST} = 0.124). Planted 1988 seedlings also had lower levels of population differentiation in comparison to natural and relictual seedlings (F_{ST} = 0.108 and 0.123; Table 9).

Table 9. Pairwise F_{ST} matrix comparisons between relictual, planted, natural populations of *Eucalyptus albens* collected seed groups (N=50)

Population	1988	Unknown	1992	Relict	Natural
	1	2	3	4	5
1988	0				
Unknown	0.143	0			
1992	0.176	0.199	0		
Relict	0.108	0.124	0.133	0	
Natural	0.123	0.176	0.180	0.095	0

3.1.4 *Population structure of* E. albens *collected seeds*

The PCA graph reflects results found within the pairwise F_{ST} analysis (Table 9; Figure 7). Relictual, natural and planted unknown populations are all strongly grouped close to the origin indicating a lack of genetic differentiation as seen in the pairwise F_{ST} analysis (Table 9; Figure 7). However, planted populations 1988 and 1992 were significantly separated.

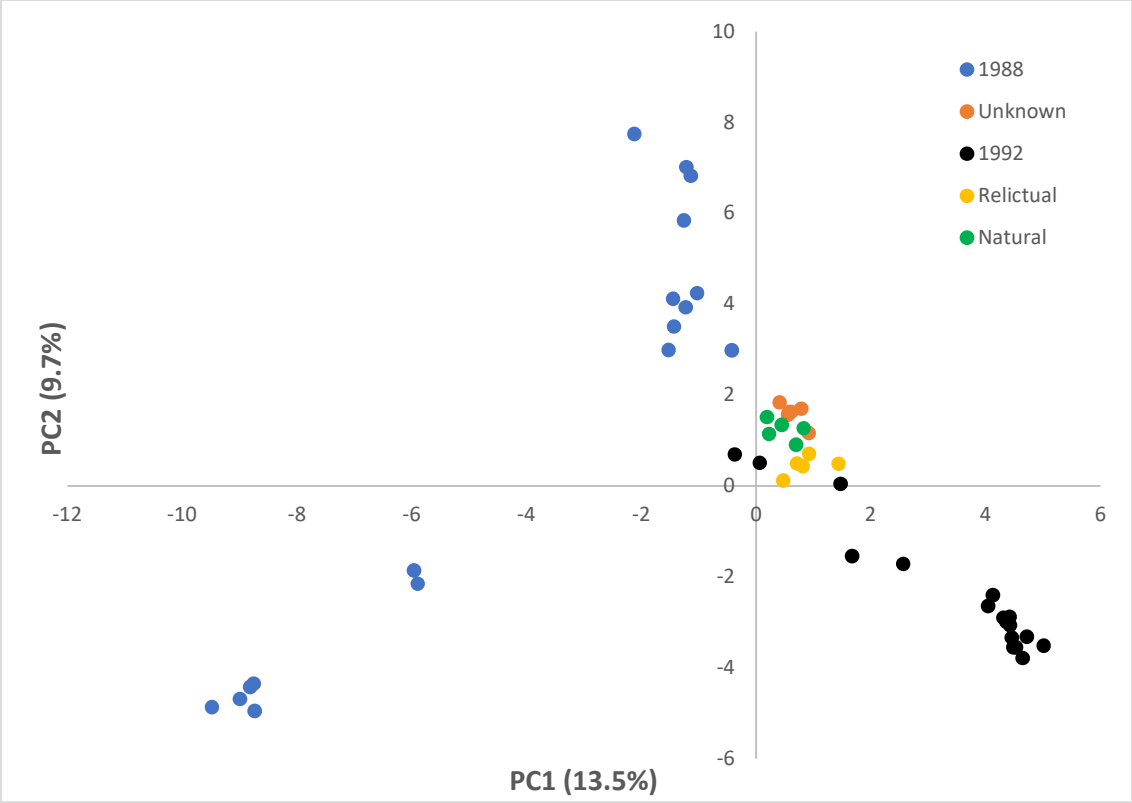


Figure 7. Principal Component Analysis plot (PCA) from all populations of *Eucalyptus albens* seedlings grown ex-situ in a greenhouse; including 1988, unknown, and 1992 planted stands and natural and relictual seedling groups, represented by colour (legend in the top right corner). PC1 and PC2 axes represent a total of 23% of the relationship. (N=50)

The *Structure* analysis discovered 3 distinct clusters within collected seeds (Figure 8; Appendix Figure 4). Overall, there was a subpopulation in population 1988, another in population 1992 and the final subpopulation considering populations unknown, relictual and natural. Seeds collected from trees planted in 1988 are uniquely differentiated, (denoted by purple cluster; Figure 8), which reflects the large spread for this group in the PCA graph (Figure 7). Additionally, seeds collected from relictual, natural and stands planted in an unknown year are similar (denoted by the size of the blue and orange structure; Figure 8). This concurs with the low pairwise F_{ST} and strong clumping in the PCA (Table 9; Figure 7). Population structure is variable within seeds collected from trees planted in 1992 (Figure 8) which concurs with the broad spread of this group within the PCA (Figure 7).

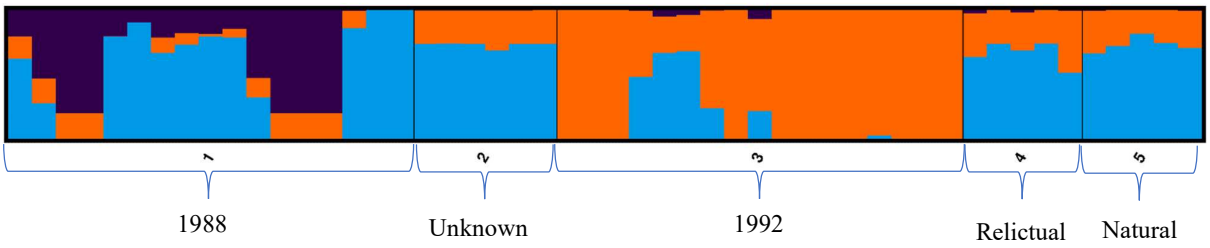


Figure 8. *Structure* plot from all populations (1-5) of *Eucalyptus albens* seedlings from ex-situ in a greenhouse; including 1988, unknown, and 1992 planted stands and relictual and natural seedling groups, showing genetic clustering when K=3 using the Puechmialle method (Puechmialle 2016; N=50)

3.2 Genetic diversity and inbreeding of mature and juvenile *E. albens* trees

The genetic diversity of *E. albens* populations was similar when all categories of mature and juvenile trees were included (mean *He* ranged from 0.090– 0.143 ± 0.004; Table 10). However, there was no significant difference in genetic diversity (*He*) among all groups ($P > 0.05$; $F = 1.041$, $df = 3$). Relictual trees had the highest levels of genetic diversity (*He* = 0.143 ± 0.004) and planted populations had slightly lower levels of genetic diversity than other remnant populations (mean *He* = 0.104 ± 0.004; Table 10).

Interestingly, observed heterozygosity was significantly higher in collected seeds in comparison to other groups ($P < 0.001$, $F = 8.567$, $df = 3$; mean *Ho* = 0.102 ± 0.005). Observed heterozygosity was remarkably similar between natural, relictual, planted and sapling populations (*Ho* ranged from 0.072 ± 0.003 – 0.100 ± 0.004; Table 10). However, this is likely

due to the smaller sample size of collected seeds, skewing results as observed heterozygosity is more sensitive to sample sizes. Therefore, this result should only be taken as a function of both expected and observed heterozygosity together.

Table 10. Genetic diversity measures of *Eucalyptus albens* sub-populations (1-28) including relictual, planted, natural, saplings and collected seed with standard errors and means. *Ho* = observed heterozygosity, *He* = expected heterozygosity, F_{IS} = inbreeding coefficient. (Loci = 1537 for all individuals; N = 433)

Type	Population	N Individuals trees	*Ho* ± SE	*He* ± SE	F_{IS}
Relictual	1	15	0.093 ± 0.003	0.143 ± 0.004	0.374
Planted	2 (1988)	32	0.085 ± 0.003	0.132 ± 0.004	0.368
	3 (1992)	20	0.074 ± 0.004	0.094 ± 0.004	0.232
	4 (1983)	11	0.079 ± 0.004	0.101 ± 0.004	0.252
	5 (1993)	28	0.082 ± 0.003	0.104 ± 0.004	0.230
	6 (Unkwn.)	7	0.091 ± 0.005	0.091 ± 0.004	0.075
	Mean		**0.082 ± 0.004**	**0.104 ± 0.004**	**0.231**
Natural	7	12	0.080± 0.003	0.112 ± 0.004	0.316
	8	10	0.100 ± 0.004	0.122 ± 0.004	0.224
	9	10	0.077 ± 0.003	0.105 ± 0.004	0.305
	10	11	0.083 ± 0.004	0.103 ± 0.004	0.240
	11	9	0.084 ± 0.004	0.105 ± 0.004	0.241
	12	9	0.092 ± 0.004	0.143 ± 0.004	0.393
	13	10	0.072 ± 0.003	0.107 ± 0.004	0.372
	14	28	0.094 ± 0.003	0.117 ± 0.004	0.217
	Mean		**0.085 ± 0.004**	**0.114 ± 0.004**	**0.289**
Saplings	15	27	0.082 ± 0.004	0.102 ± 0.004	0.217
	16	24	0.097 ± 0.004	0.106 ± 0.004	0.107
	17	30	0.092 ± 0.003	0.116 ± 0.004	0.217
	18	39	0.086 ± 0.003	0.121 ± 0.004	0.303
	19	9	0.090 ± 0.004	0.105 ± 0.004	0.195
	20	14	0.090 ± 0.004	0.114 ± 0.004	0.247
	21	10	0.088 ± 0.004	0.103 ± 0.004	0.191
	22	10	0.091 ± 0.004	0.109 ± 0.004	0.207
	23	8	0.096 ± 0.004	0.116 ± 0.005	0.232
	Mean		**0.090 ± 0.004**	**0.108 ± 0.004**	**0.213**
Collected Seeds	24 (P 1988)	17	0.103 ± 0.004	0.140 ± 0.004	0.286
	25 (P Unkwn.)	6	0.099 ± 0.005	0.090 ± 0.004	-0.004
	26 (P 1992)	17	0.098 ± 0.005	0.103 ± 0.004	0.081
	27 (Relictual)	5	0.101 ± 0.005	0.105 ± 0.004	0.163
	28 (Natural)	5	0.111 ± 0.005	0.111 ± 0.004	0.083

	Mean		**0.102 ± 0.005**	**0.110 ± 0.004**	**0.122**

3.2.1 *Genetic diversity of juvenile* E. albens *samples*

Genetic diversity varied between juveniles (*He* ranged from 0.090 to 0.140; Table 10). However, the mean *He* of collected seeds was similar to all groups (mean *He* = 0.110 ± 0.004). Seeds collected from stands planted in 1988 seedlings had significantly higher genetic diversity than other populations (*He* = 0.140 ± 0.004) which is reflected in their structural complexity (Figure 8). Sapling populations also had similar levels of genetic diversity to natural populations (mean *He* = 0.108 ± 0.004) and between all sapling populations, genetic diversity was stable (*He* 0.102 - 0.121 ± 0.004; Table 10).

3.2.2 *Inbreeding levels of* E. albens *samples*

Conversely, the levels of inbreeding between *E. albens* relictual trees were higher in comparison to other populations (F_{IS} = 0.374; Table 10). Similar levels were also found in planted population 1988 (F_{IS} = 0.368), and natural population 12 (F_{IS} = 0.393). Nevertheless, collected seeds had the lowest mean level of inbreeding (mean F_{IS} = 0.122). There was also a high range of inbreeding between seed populations (F_{IS} ranged from -0.004 in seeds collected) from stands in the planted unknown category to the highest levels in stands planted in 1988 (Table 10). Collected seeds had lower levels of inbreeding in comparison to all other groups, due to the negative value in planted (unknown), (mean F_{IS} = 0.096; Table 10). However, the 1988 population also had unexpectedly prominent levels of inbreeding (F_{IS} = 0.286). There was a significant difference in the level of inbreeding between groups ($P < 0.01$, $F = 5.395$, $df = 3$) and post-hoc analysis reveals that inbreeding levels of seedlings were significantly lower than natural stands. There was no statistical difference in inbreeding levels between natural/remnant and planted *E. albens* trees.

3.3 Genetic differentiation of *E. albens* samples

The pairwise F_{ST} comparison between sub-populations within relictual, planted and natural stands reveals that there are differing amounts of genetic differentiation between sub-

populations (Table 11). Populations of relictual trees lacked genetic differentiation in comparison to natural, planted and seedling populations, excluding planted population 1993 (F_{ST} ranged from -0.009 - 0.116; Table 11). The planted populations were unique in terms of the genetic differences they had between all populations (F_{ST} ranged from 0.116 – 0.234; Table 11). Natural stands also lacked differentiation from all populations, excluding the planted population 1988 and some of the relictual and natural populations (F_{ST} ranged from 0.009 – 0.178; Table 11). The variable levels of genetic differentiation, especially from planted population 1993 being the most genetically different population, indicates that seed was sourced externally from the Central Valley.

Table 11. Pairwise F_{ST} comparisons between mature specimens and collected seeds from relictual, planted and natural populations of *Eucalyptus albens*. Saplings were excluded due to the substantial number of individuals. High values ≥ 150 are shown in bold. (N=262)

		Relict	Planted					Natural								Seeds (1988)	Seeds (Unkwn.)	Seeds (1992)	Seeds (R)	Seeds (N)
		1	2 (1988)	3 (1993)	4 (1983)	5 (1992)	6 (Unkwn.)	7	8	9	10	11	12	13	14	24	25	26	27	28
Relict	**1**	0																		
Planted	**2 (1988)**	0.042	0																	
	3 (1993)	0.116	0.145	0																
	4 (1983)	0.058	0.094	**0.193**	0															
	5 (1992)	0.061	0.102	**0.170**	0.140	0														
	6 (Unkwn.)	0.025	0.087	**0.208**	0.135	0.128	0													
Natural	**7**	0.012	0.054	**0.129**	0.081	0.079	0.070	0												
	8	0.038	0.084	**0.176**	0.114	0.128	0.120	0.066	0											
	9	0.010	0.059	0.149	0.089	0.091	0.079	0.031	0.071	0										
	10	0.013	0.057	**0.161**	0.089	0.088	0.086	0.026	0.060	0.034	0									
	11	0.022	0.069	**0.156**	0.115	0.095	0.096	0.044	0.069	0.056	0.042	0								
	12	0.027	0.083	**0.178**	0.111	0.129	0.095	0.060	0.081	0.069	0.061	0.077	0							
	13	0.009	0.054	0.137	0.083	0.077	0.075	0.022	0.056	0.024	0.030	0.037	0.057	0						
	14	0.009	0.057	0.137	0.079	0.068	0.054	0.028	0.064	0.025	0.027	0.040	0.073	0.026	0					
Seeds (1988)	**24**	0.075	0.024	**0.183**	0.128	0.145	0.132	0.096	0.116	0.097	0.102	0.104	0.105	0.087	0.102	0				
Seeds (Unkwn.)	**25**	0.055	0.104	**0.234**	**0.168**	**0.152**	0.064	0.095	0.134	0.115	0.119	0.132	0.114	0.105	0.066	0.138	0			
Seeds (1992)	**26**	0.084	0.109	0.126	**0.152**	0.085	**0.164**	0.092	0.145	0.107	0.113	0.122	0.139	0.100	0.098	0.149	**0.179**	0		
Seeds (R)	**27**	-0.009	0.061	**0.154**	0.107	0.093	0.087	0.038	0.074	0.042	0.055	0.052	0.057	0.040	0.022	0.102	0.122	0.118	0	
Seeds (N)	**28**	0.032	0.079	**0.193**	0.136	0.122	0.134	0.075	0.096	0.068	0.074	0.089	0.095	0.068	0.040	0.116	**0.164**	**0.161**	0.077	0

3.3.1 *Genetic differentiation of* E. albens *collected seeds*

Collected seeds from all groups lacked differentiation from relictual populations. (F_{ST} ranged from -0.009 to 0.084; Table 11). Collected seeds from natural populations (28) were closely related to relictual groups. In comparison to all populations, planted seeds from planted stands had a moderate amount of differentiation, especially from planted population 3, as explored above (F_{ST} ranged from 0.024 – 0.234; Table 11). In comparison to natural populations, planted seeds are moderately differentiated (F_{ST} ranged from 0.022 – 0.145), but there is still evidence of some similarity, particularly in population 14 (F_{ST} ranged from 0.022 – 0.102; Table 11). while we observe some genetic differentiation of collected seeds, we can still see similarities to natural and restored stands, and relictual trees are the main contributor to offspring genotypes.

3.4 Population structure of *E. albens* samples

3.4.1 *Principal Component Analysis (PCA)*

The PCA plot represents little genetic differentiation between relictual, planted, natural, sapling and seedling samples. The plot depicts that relictual trees are moderately differentiated from one another, due to a broad clump of samples on the plot and one significant outlier (denoted by yellow dots; Figure 9). Further, natural trees lack dissimilarity, as most individuals seem to cluster at the origin of the plot. However, saplings, and planted populations are more genetically differentiated with samples spread more along the Y axis in addition to the X axis (Figure 9). Collected seed clusters are mostly at the origin, but some are much further apart, indicating they are the most genetically differentiated group, remarkably similar to saplings. Often, planted individuals are near these clusters, due to having parental relationships. The results from the PCA analysis strengthen the findings from the pairwise F_{ST} matrix (Figure 9; Table 11).

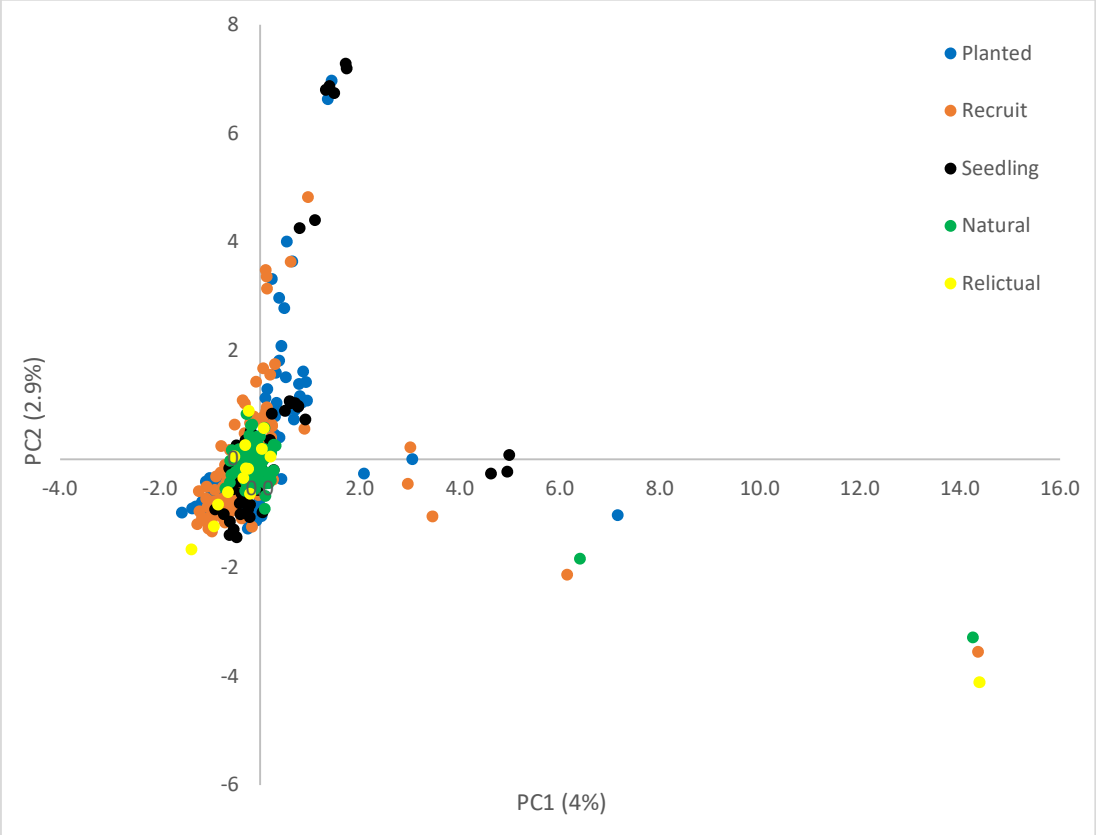


Figure 9. Pearson Principal Component Analysis plot (PCA) from all populations of *Eucalyptus albens* samples (relictual, planted, natural, saplings and collected seeds), represented by a colour (legend in the top right corner). PC1 and PC2 axes represent a total of 6.9% of the relationship. (N=433)

3.4.2 *Bayesian Cluster Analysis (BCA)*

To explore further into population structure, the Puechmaille found a total of 10 clusters (K=10; Appendix Figure 5). The population *Structure* plot using the K=10 result showed many unique genetic subpopulations from the 29 populations (Figure 10). The planted populations showed the highest level of population differentiation in comparison to other groups. Planted populations 1993 and 1992 had a unique genotype (denoted by dark purple) that was not represented within relictual or natural populations, which was confirmed in the pairwise F_{ST} analysis. Planted 1983 and unknown were genetically similar to relictual trees (supported by low F_{ST} values) and planted population 1988 was represented in both relictual and natural populations. Planted 1988 had a substantial number of clusters among individuals, indicating there were high levels of gene flow within this population (denoted by green, yellow, pink, orange etc.; Figure 10).

Furthermore, saplings are derived from relictual trees, planted and natural stands. Sapling populations 18, 20, 21, 23 and 24 are the most unique, consisting of many clusters associated with relictual, planted and natural populations (Figure 10) indicating increased gene flow and reduced subdivision. Saplings from relictual populations (15, 16, 17 and 22) often predominantly consist of one main cluster based on a single relictual tree. Populations 15, 16 and 17 were highly subdivided and lacked gene flow, however, populations 18 – 24 were more genetically complex, consisting of a range of clusters. Collected seed populations often shared genotypes (pink, brown) however were differentiated based on their maternal characteristics (Figure 10). For example, planted population 1988 was remarkably similar to seed population 1988 (denoted by yellow and green clusters; Figure 10).

Natural populations are all genetically similar to one another, but it was evident that gene flow was being shared among all natural populations as there was no subdivision evident. Natural population 14 had a higher level of gene flow based on the increased among of clusters reflected within individuals (Figure 10). Interestingly, natural population 14 were newer samples collected in 2023. This gives us an understanding that there are levels of connectivity between remnant and restored populations, reflected within the population structure of naturally regenerating saplings.

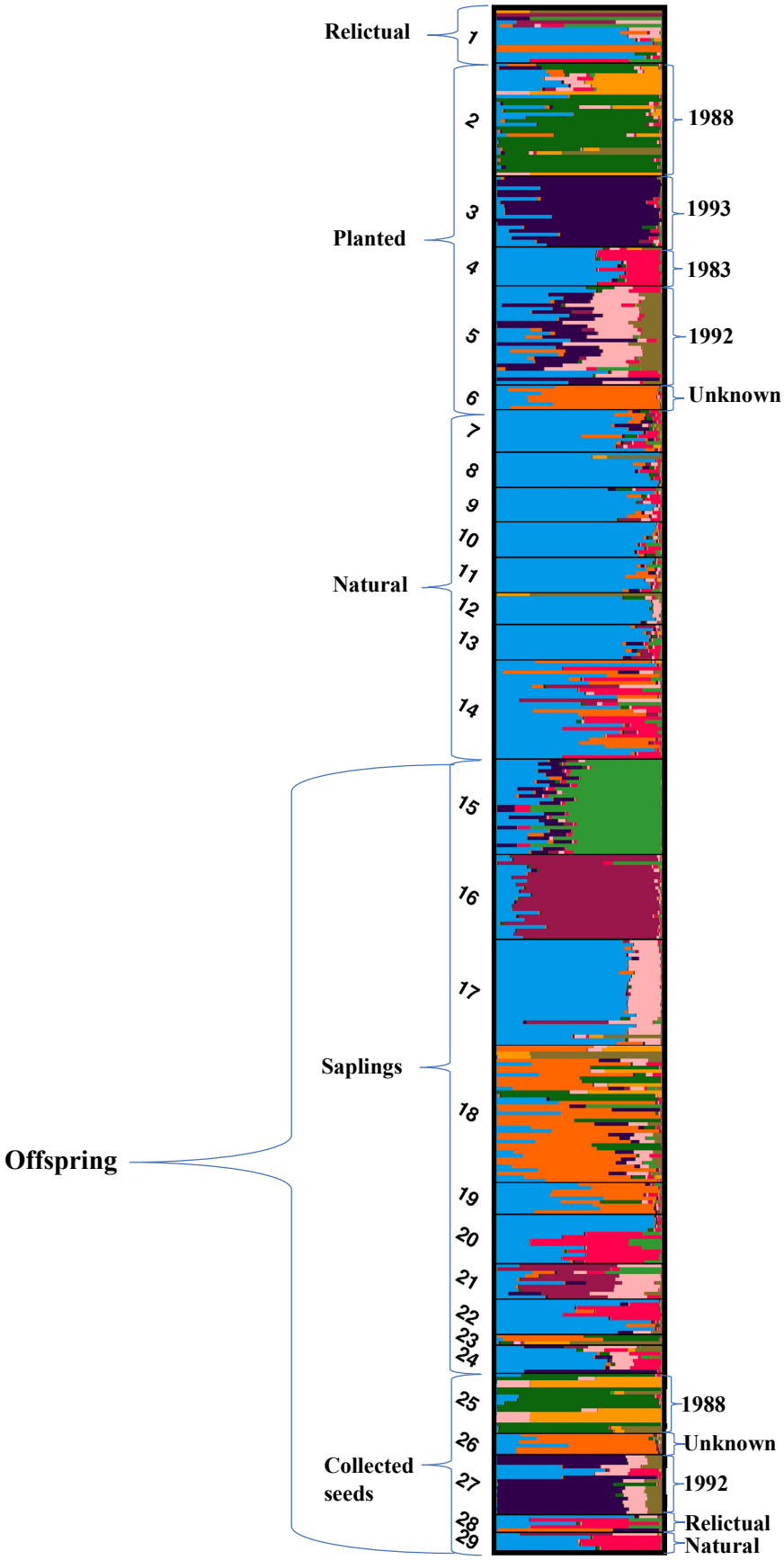


Figure 10. Structure plot from all populations (1-29) of *Eucalyptus albens* samples including relictual, planted, natural, saplings and seedling stands, showing genetic clustering when K=10 using the Puechmialle method (Puechmialle 2016). (N=436; including excluded population)

4.0 Discussion

This study has been effective in determining connectivity and gene flow between planted and natural stands. The genetic homogeneity of planted and remnant stands suggests local provenance but dividing the planted stands into years of planting revealed that 1993 has a distinct population structure and the seeds may have been collected from outside the valley. The parentage analysis for collected seeds with known mothers is particularly insightful in detecting effective pollination between planted stands and natural trees. The inclusion of juvenile saplings and collected seed has allowed me to establish that gene flow and connectivity occurs between the trees planted throughout the 1980s and 1990s and remnant populations. Significantly, I found evidence of planted populations outcrossing with remnant populations suggesting that the restoration of the WNP has successfully re-introduced healthy levels of gene flow and connectivity through pollination services. Indeed, the analysis of the full dataset shows maintenance of elevated levels of genetic diversity and avoidance of further population subdivision, critically protecting important levels of local adaption. The success of this restoration supports the use of local provenance sourcing to promote successful reintroductions of species of trees in endangered woodlands, such as Box-Gum Grassy Woodlands in southeast Australia (Rosser *et al*. 2023).

4.1 Seed provenance in planted populations

4.1.1 *Mixed sourcing or local sourcing?*

My findings highlight the differences in two planted populations (1993 and 1992) that diverge from the relative homogenous structure otherwise found within the WNP and their remnant cohorts (Figure 10; Table 11, F_{ST} ranged from 0.126 – 0.208). Zucchi (2018) and Broadhurst (2006) suggest that the heterogeneous genetic composition of some planted populations may be a consequence of seed collection that is not entirely local and from different distinct areas. Assuming this reasoning, it could be suggested that the seed set used to restore *E. albens* populations in this study may have not been entirely local either, as it is possible that genetic material was collected externally from the valley, and many relictual sources separated with significant geographical distance. Further, Rosser *et al*. (2023) also found a similar result of non-local genotypes within planted populations 12 and 13. Nevertheless, restored trees showed

an adequate amount of genetic diversity, similar in comparison to part of the diverse mosaic found in wider landscapes; and externally from the Central Valley of the WNP. Future analysis into isolation by distance population structure could confirm the reasoning for the similar level of genetic diversity found within restored populations and whether this is affected by distance between populations.

The greatest level of population differentiation was observed within planted populations; however, this may be the result of patterns of seed collection and planting divided by year. Planting years 1983, 1988 and 1992/93 showed levels of subpopulation division, suggesting that plantation year was tightly associated with seed collection patterns, as seen in the *Structure* plot of the *Bayesian Cluster Analysis*. Planted populations 1992 and 1993 had a unique genotype that was not reflected within natural or relictual trees suggesting that these stands may have indeed been sourced externally from the valley in line with mixed provenance sourcing (denoted by dark purple; Figure 10). Population subdivision can increase in restorations if gene flow and pollination are disrupted in a fragmented landscape (Broadhurst *et al*. 2015). Interestingly, these plantation years were also outsourced externally for the restoration of *E. melliodora* in the WNP (Quinton 2019). There is increasing support within the literature to adopt strategies of mixed provenancing to broaden the gene pool and genetic base of revegetated areas, increase the adaptive potential to future environmental changes and reduce inbreeding (Broadhurst *et al*. 2008; Sgro *et al*. 2011; Van Rossum and Le Pajolec 2021). However, we must also consider the risk of genetic contamination and loss of local adaptive potential within the local environment.

The issue of genetic contamination and micro-site genetic variation (as seen in differentiated planted populations 1993 and 1992; Figure 10) may introduce a challenge for mixed sourcing strategies, which can introduce maladapted or invasive non-local genotypes (McKay *et al*. 2005; Hufford and Mazer 2003). A previous study delving into the genetic differentiation of *Eucalyptus obliqua* discovered that micro-site local adaption can occur in as little as a few hundred metres (Wilkinson 2008; measured by seedling survival, height, and frost resistance in situ), which could explain what we saw in planted 1992 and 1993. Although, it is more likely that these populations are simply different as we did not discover any evidence of local adaption. If we exclude these outlier planted populations, the low differentiation (F_{ST} ranged

from 0.009 – 0.061; Table 11) between remnant and restored populations is the result of local sourcing (McKay *et al*. 2005). Similar F_{ST} values in *E. melliodora* (Rosser *et al*. 2023; F_{ST} ranged from 0.029 – 0.203) give us confidence in the clarification of local provenance sourcing. Although historical records of the WNP restoration are not exactly clear on the strict protocol used for genetic sources for each planting, it is possible that crossing with cultivars produced hybrids that were significantly different to remnant populations. Ultimately, the results of this section of the study indicate that planted populations of *E. albens* within the WNP are mostly composed of genetically diverse local provenance trees. The stable levels of genetic diversity and differentiation throughout the system and multiple seed sources found in some planted populations are a testament to the success of this element of ecological restorations of Box-Gum Grassy Woodlands in the WNP. We found no tangible evidence of genetic contamination (introduction of exotic alleles/hybridisation), even with the use of mixed sourcing of some planted populations, indicating that overall, this restoration was successful.

4.1.2 *Genetic diversity and inbreeding*

The results suggest that there was no significant reduction in genetic diversity of the locally sourced planted populations, in comparison to natural and relictual populations in the WNP (mean *He* of planted, natural and relictual populations = 0.104, 0.114 and 0.143). Contrary to the literature (Hufford *et al*. 2021; Breed *et al*. 2012a), both effects were not observed in this study. Additionally, Dunn *et al*. (2023 in review) also found no difference in genetic diversity (mean *He* natural and planted = 0.204 and 0.197) as well as Rosser *et al*. (2023; *He* = 0.141, 0.141 and 0.170). Recently, the use of local provenance sourcing was confirmed in the restoration of *E. melliodora* (Rosser *et al*. 2023) in the WNP. The evidence of gene flow between remnant and restored populations validates the use of local provenancing as a technique for plantings in this restoration project. Based on the results of this study in combination with previous findings (Rosser *et al*. 2023), local provenancing was used successfully due to similarities in genetic diversity and genetic differentiation of planted populations and overall, there was no evidence of a loss in genetic diversity. This study increases in support of the use of locally sourced genetic material to retain historic, local genotypes which is best for effectively adapted offspring in the future.

The results indicate that there was no difference in levels of inbreeding or genetic diversity between planted and remnant populations. A well-known disadvantage of the use of local provenance sourcing within restoration projects is elevated levels of inbreeding (Hufford *et al*. 2012; from shorter crossing distances in *Stylidium hispidum*) and the negligible, or lack of introduction of genetic variation/genetic diversity which limits the adaptive potential of populations (Aitken and Whitlock 2013; Bucharova *et al*. 2019; Broadhurst *et al*. 2008). For example, A study by Breed *et al*. (2012a) found increased inbreeding levels in locally sourced *Swietenia macrophylla* and fitness was increased in genetically diverse, outcrossed individuals. However, similar studies of *E. melliodra* and *E. albens* observed no increase in inbreeding levels of planted cohorts (mean F_{IS} = 0.160 vs 0.203 in naturals; Rosser *et al*. 2023; 0.288 vs 0.292 in naturals; Dunn *et al*. 2023 in review). Consequently, the lack of inbreeding observed within this study may be because self-compatible tree species may be resistant to inbreeding depression as most deleterious alleles have been removed or counter the effects of self-fertilisation by establishing bet-hedging strategies to selectively favour outcrossed seeds (Sampson and Byrne 2008; Kramer *et al*. 2008). Alternatively, the non-significance of results may be the result of lack of time for the genetic effect to take place, as the effect of inbreeding depression and genetic drift can take several decades to appear in long-lived tree species such as *E. albens*, and the overall success based on levels of inbreeding and genetic variation may take hundreds of years to be able to be determined (Kramer *et al*. 2008; Lowe and Allendorf 2010).

4.2 Connectivity between planted and natural populations

4.2.1 *Pollination outcrossing*

The results indicate that saplings and collected seeds exhibited some evidence (total of 17 parents assigned; Table 7; Table 8) of outcrossing between populations of natural/relictual and planted trees, critically demonstrating the re-establishment of connectivity and reproductive functionality within the restored landscape. The reconnection of plants and pollination services is a major challenge associated with ecological restoration (Ritchie and Krauss 2011; Menz *et al*. 2011) but is of extreme importance for the long-term success of self-sustaining, resilient populations (Millar *et al*. 2021; Dixon 2009). Often, limited pollination reduces the capacity to outcross between individuals and can lead to increased inbreeding levels and reduced fitness (Millar *et al.* 2021; Crémieux *et al*. 2010; Keller and Edwards 2000). Long-distance pollination

(facilitated by honeybees and other insects) was observed up to 1km in *Eucalyptus* species (Byrne *et al*. 2007; Ottewell *et al*. 2009; Sampson and Byrne *et al*. 2008) and has a high potential for outcrossing between natural and planted populations. Related results were found for *E. melliodora* (Yellow Box; Quinton 2019; Broadhurst 2013) where there was evidence of relictual trees outcrossing with restored trees; and restorations of several species of *Banksia* (Ritchie and Krauss 2011; Coates *et al*. 2007). The highly successful production of saplings and seeds within planted stands suggests that fitness was not affected, according to a valuable metric of fitness, reproductive success. This research is one of the first studies to investigate the connectivity of a restored landscape via employing a parentage assignment of offspring from a known mother. This produced valuable results indicating pollen dispersal distances with high confidence.

4.2.2 *Short-distance connectivity*

Most of the outcrossing between natural and locally sourced planted population 1988 was observed between nearby natural trees. There was a high amount of gene flow producing both saplings and collected seeds with high confidence in assigning paternal parents and parent pairs in this one location within ~100m (Figure 6; Table 7; Table 8). Interestingly, this population of seedlings (1988) also exhibited unique genotypes (expressed as a dark purple cluster; Figure 8) which indicates that this genotype may be expressed in outcrossed individuals. Altered and reduced gene flow patterns are outcomes of isolated and fragmented species (Lowe *et al*. 2005). Pollen dispersal in eucalypts is idiosyncratic and is influenced by spatial distribution, plant density, and changes in pollinator behaviour (Byrne *et al*. 2008; Sork *et al*. 1999). This can have a major impact on the level of gene flow between planted and remnant populations within a restoration area leading to reduced gene flow and inbreeding depression due to selfing. (Armbruster and Reed 2005; Coates *et al*. 2007). This is evidence that a significant amount of short-distance pollen dispersal is taking place within this restoration, and these distances are well within the typical eucalypt pollen dispersal distances of around 200m (Broadhurst 2013; Byrne *et al*. 2008; Ottewell *et al*. 2009). Typically, these results concur with (Broadhurst 2013; *E. melliodora*) who recommends repopulating fragmented woodlands and reducing isolation distances to assist in facilitating pollination services for eucalypts. Therefore, restorations must consider the spatial arrangement of plantings. Shorter distances between populations receive a greater diversity of pollen due to increased pollinator movements (Breed *et al*. 2012b;

McCallum *et al*. 2019; Yates *et al*. 2007). The production of offspring facilitated by short-distance pollen dispersal is key to re-establishing connectivity to restoration programs, and to do so, isolation between trees must be reduced to encourage pollinator abundance and more reliable pollination services (Ottewell *et al*. 2009).

4.2.3 *Offspring population structure and genetic diversity shows connectivity*

The population structure of *E. albens* collected seeds within the WNP was less homogenised than expected according to the *Bayesian Cluster Analysis* and *Structure* plot. A similar result was found in an earlier study (Rosser *et al*. 2023) where differences in the population structure of offspring were associated with patterns of sampling clusters. The total of 10 subpopulations generated within the *Structure* plot is high compared to other studies of *E. melliodora* (Rosser *et al*. 2023; Quinton 2019) which found a total of K=6 clusters and may be due to the large sample size increase in subpopulations found in comparison to similar studies. However, the PCA plot depicted a more homogenised population structure, alluding to the fact that there is a substantial amount of gene flow being distributed between populations due to a lack of population clustering and subdivision (Figure 9). This is contrary to similar studies (Dunn *et al*. 2023 in review) where the PCA graph showed distinct clustering of sapling and planted populations of *E. albens*. My result is due to the lack of confidence in this analysis, with only 6.9% of the variation explained by the two co-ordinates. Therefore, the PCA analysis should only be inferred as a function of a combination of other analyses. There is convincing evidence to suggest that nearby planted trees have outcrossed with relictual and natural trees, as saplings often shared genotypes with both planted (1993, 1983, 1992) and remnant populations (18-22; Figure 10). This suggests that an admixture of mature planted and remnant individuals are producing outcrossed offspring, revealing re-connectivity within the landscape. Ultimately, this study highlights that both remnant and planted trees actively contribute to recruitment, as there were many juvenile trees observed within the field since the restoration took place and evidence of outcrossing within the *Bayesian Cluster Analysis* (Figure 10).

Population *Structure* plots of juvenile offspring confirmed connectivity between populations due to the sharing of similar genotypes between subpopulations displayed active gene flow. If offspring are homogenous in structure they are well connected by many clusters (Figure 10), while a decrease in gene flow/connectivity would be indicated by further population

subdivision with defined clusters not shared among populations. Fragmented landscapes suffer low levels of connectivity between populations and atypical gene flow due to increased spatial separation between individuals and altered pollinator behaviour (Aguilar *et al*. 2006; Lowe *et al*. 2005; e.g., Zucchi *et al*. 2018). Therefore, it is important to consider the population structure of offspring to denote levels of connectivity and whether pollination services have been re-established (Broadhurst *et al*. 2015). The *Bayesian Cluster Analysis* and *Structure* plot showed some levels of subdivision within collected seeds; however, there were some genotypes shared among all populations (denoted by orange cluster; Figure 8). In comparison to one another (Figure 8), collected seeds from relictual, natural and unknown planted trees were undifferentiated, but seedlings from the 1988 plantation contained a genotype not represented in the other subpopulations (denoted by purple cluster; Figure 8). Although population 1988 was different, this still gives us evidence of a relatively homogenous subset of seed offspring, confirming the parentage assignment (Table 7) and that connectivity and gene flow are present between planted and remnant individuals. This result was also reinforced within the PCA (Figure 7) between seeds as the unknown population was very similar to natural and relictual collected seeds. We can be more confident with this result due to the known maternal relationships of these seedlings, and ex situ germination of these seedlings nullifying local environmental selection pressures. A similar study of *E. albens* showed evidence of gene flow within offspring, sharing genotypes with planted and natural populations (Rosser *et al*. 2023; Dunn *et al*. 2023 in review). These results of the PCA and *Structure* plot reflect the success of the restoration, with re-establishing pollination services and gene flow between populations and restored and remnant individuals, a crucial aspect of maintaining a self-sustaining ecosystem that is likely to be resilient to environmental change (Jordan *et al*. 2019; Ruiz-Jaen and Mitchell Aide 2005; Suding *et al*. 2015).

High genetic diversity was found in seeds collected from planted, natural, relictual and sapling populations, indicating that heterozygosity was not lost throughout generations. Further, the results also demonstrated that controlled greenhouse conditions revealed a broader genetic range of collected seeds that germinated successfully (*He* ranged from 0.090 – 0.140; Table 10). This suggests that the restoration was effective from a genetic perspective and may increase the evolutionary potential of future generations (Sgro *et al*. 2011) due to higher inbreeding levels. It also indicates that there is a possibility that outcross offspring with higher genetic diversity is favoured in the local environment, due to the broader range of genetic

diversity revealed in greenhouse conditions. Further, the significantly reduced levels of inbreeding in collected seeds (F_{IS} as low as -0.004; Table 10) demonstrates a successful admixture between populations, with sufficient levels of pollen dispersal to initiate gene flow, increasing genetic variation and reducing inbreeding levels. Levels of genetic diversity of seeds (*He* ranged from 0.104 - 0.140; Table 10) were comparable to saplings of *E. melliodora* (*He* ranged from 0.158 - 0.170; Rosser *et al*. 2023) and *E. albens* (*He* ranged from 0.195 – 0.211; Dunn *et al*. 2023 in review). Although collected seeds' genetic diversity was slightly lower than those studies, they had significantly prominent levels of observed heterozygosity (*Ho* ranged from 0.098 – 0.111; Table 10) at an individual level. Equivalent results were also observed in other species (Van Rossum and Le Pajolec 2021) that saw higher levels of fitness, phenotypic plasticity, flowering, and reproductive success in *Dianthus deltoides* that had reduced inbreeding levels. Admixed genotypes in collected seeds may increase the adaptive potential and as a result, increase the fitness of offspring throughout generations to come (Prober *et al*. 2016). This indicates that, with confidence, the restoration was successful in retaining connectivity within the landscape, promoting elevated levels of gene flow between populations.

The assessment of collected seeds grown for genetic harvesting is limited within the literature (e.g., Breed *et al.* 2012b; Woods *et al*. 2021), however, often strengthens the measurement of restoration success as these offspring are key to envisioning the future of restoration programs and measurement of outcrossing between populations. Limitations with this technique were surrounding time constraints, as only the largest seedlings could be harvested for DNA extraction and sequencing. This can create a bias with results as the larger seedlings are the fitter offspring due to outcrossing (Broadhurst 2013). Additionally, the externally sourced and subdivided planted population 1993 was not assessed for outcrossing, genetic diversity and differentiation of seedlings due to the lack of seeds available. This is a limitation within this study, as these results could demonstrate whether this population is experiencing gene flow between nearby stands producing effective outcrossed progeny or is it just favouring self-fertilising reproductive methods and further creating an isolated subpopulation. Further, genetic diversity and inbreeding offspring of 1993 could also indicate whether this population is introducing genetic variation to the environment, increasing the gene pool and fitness of trees within the WNP. Nevertheless, this study found no difference in genetic diversity between planted stands and remnant populations, with generally high levels observed in all populations.

This gives us evidence of a successful restoration in being able to maintain a genetically diverse and viable population with similar offspring.

4.2.4 *Connectivity of natural populations*

Natural trees had no distinct structuring or differentiation between populations (except population 14), despite the significant geographical distance separating populations. The PCA plot (Figure 9) depicts no distinct clustering of natural populations, reflected within the *Bayesian Cluster Analysis* (Figure 10) and Pairwise F_{ST} matrix (F_{ST} ranged from 0.0022 – 0.081; Table 11). This suggests that remnant populations are relatively homogenous across the entire region, similar to results found in other studies (F_{ST} ranged from 0.046 – 0.108; Rosser *et al*. 2023). This result is contrary to Broadhurst (2013) where earlier recommendations suggest that the increased variation of planted stands could be due to broader spatial separation of seed sourcing across the Central Valley of the WNP, but there appears to be no difference in structure between natural populations despite the large distance between them. Overall, the historical level of gene flow has been sufficient to maintain strong connections among natural stands. Nevertheless, natural population 14, a newly sampled stand within the Central Valley of the WNP and adjacent to 1988 plantings was more differentiated than other natural stands according to the *Bayesian Cluster Analysis* (Figure 10). This result was due to increased outcrossing, creating more genetically complex individuals with many clusters because of increased gene flow. This natural population was also seen to outcross with the nearby planted stands (Table 7; Figure 6) to produce several outcrossed seeds (Figure 8; Unknown population) and saplings (Figure 10, populations 19, 20, 22). Therefore, it is evident that natural stands provide significant levels of gene flow across significant distances to share a variation of genotypes.

4.3 Importance of relictual trees

Genetic diversity was especially higher in relictual trees than in other natural and planted populations. The elevated levels of genetic diversity within older fragmented trees (*He* = 0.143; Table 10) reflect the historic levels of genetic variation that were once present before the deforestation of the WNP. A similar result was also found in studies of *E. melliodora* (*He* = 0.170; Rosser *et al*. 2023) and *E. albens* (*He* = 0.230; Dunn *et al*. 2023 in review). This indicates

the importance of the remaining historic remnant populations for conservation management and overall ecosystem health (Ottewell *et al*. 2010; Broadhurst 2013) as they provide high genetic variation which can be used for seed for restoration programs (Broadhurst 2013; Rosser *et al*. 2023). Relictual trees must be protected in the future, not only for their prominent levels of historic genetic diversity but failure to protect them will reduce the likelihood of achieving conservation objectives of the maintenance and representation of species in agricultural landscapes (Gibbons and Boak 2008).

An interesting result within the parentage analysis is the identification of two relictual parents in saplings, indicating that these isolated historic trees have established current connectivity and gene flow (Table 8). This contrasts with other studies which have not been able to show reproductive inputs from incredibly old trees (e.g., Rymer *et al*. 2015). There was only one example of this process within this study, within ~200m from one another (Table 8; Figure. 5). However, the connectivity between relictual trees can be attributed to pollination distances increasing between scattered trees in an agricultural landscape to compensate for increased isolation (Ottewell *et al*. 2009; Byrne *et al*. 2008). Overall, this reinforces the capability of relictual trees to produce viable, genetically diverse offspring and contribute to increasing self-sustaining populations by reducing the effect of inbreeding depression in offspring (Broadhurst 2013; Manning *et al*. 2006; Rosser *et al*. 2023). However, the results also indicate that relictual trees had increased levels of inbreeding in comparison to other populations (F_{IS} = 0.374; Table 10), contradicting similar studies (F_{IS} = 0.194; Rosser *et al*. 2023). Although these trees contain significant levels of genetic variation, other studies have found that isolated relictual trees can have higher levels of inbreeding than natural stands due to restricted pollen dispersal and self-fertilisation (outcrossing rate = 0.828, Rymer *et al*. 2015) which is reflected in our results. This indicates that relictual trees are favouring selfing over outcrossing between individuals. Higher inbreeding levels were also observed in seeds collected from relictuals (F_{IS} = 0.160; Table 10) which may be due to lingering effects of fragmentations affecting heterozygosity within next-generation offspring.

Here, we demonstrate that relictual trees are significant sources of genetic variation and contribute valuable genetic material to the many recruited offspring. The *Bayesian Cluster Analysis* (Figure 10) detected many different genetic clusters among relictual trees (population

1; denoted by orange, maroon, green, pink, and red coloured clusters), confirming the fact that these scattered trees are reservoirs of many genotypes favoured within the valley floor. As a group, the high variation in relictual trees reflects their historic importance and shows they harness a variation of alleles that existed before deforestation (reviewed in Ottewell *et al*. 2010; Broadhurst 2013; Rosser *et al*. 2023). This provides genetic diversity for the next-generation offspring to maintain fitness, and adaptive potential and express locally adapted genotypes to mitigate deleterious alleles associated with inbreeding, reducing the likelihood of genetic contamination and genetic drift in the future (Breed *et al*. 2015; Jordan *et al*. 2011; Broadhurst 2013). This information tells us that sourcing genetic material from these differentiated relictual trees is sufficient to produce sufficient quality, locally adapted, diverse offspring (Ottewell *et al*. 2010) but we must still be mindful of the inbreeding rates within these historic trees.

4.4 Recommendations

Restoration projects are yet to fully harness the potential of genomics, and recent reviews have determined that these emerging technologies will assist in bridging the gap of knowledge within the field. This study employed next-generation sequencing (NGS) to harvest genome-wide SNPs to explore genetic issues within and between populations. By using genomic technologies in restoration-based research, the likelihood that a restoration project becomes a resilient population can be improved and success can be efficiently measured. Among new genetic technologies, next-NGS deserve particular attention when applying genetics to restoration ecology (Mijangos *et al*. 2014). This faster and more affordable sequencing method is enabling the analysis of genome-wide samples in population genetic applications, and conservation managers should take advantage of this emerging technology to assist in creating faster and more affordable biodiversity assessments.

4.4.1 *Further samples to reveal more connectivity*

Nevertheless, there is excellent value in parentage analysis of *Eucalyptus* species to determine if connectivity and pollinator services have been re-established as this can give us insight into whether the restoration program can result in a self-sustaining ecosystem. Attracting pollinator

services is a crucial aspect of restoration that is required for population persistence in the short and long term (Dixon 2009; Millar *et al*. 2021). In the future, emphasis is required to determine greater confidence in parentage assignments and allocating parent pairs by additional field sampling (Quinton 2019). Although this study increased confidence by knowing the maternal parents in seedlings, there was still only moderate to low confidence in paternal parent assignment in saplings due to the lack of assigning parent pairs to offspring. There is immense value in understanding pollen dispersal distances, as it is often difficult to measure and quantify gene flow and long-distance pollination (Pasquet *et al*. 2008). This type of study in the future can assist with understanding sampling distances for increased confidence in connectivity analyses. I have only uncovered a small piece of the puzzle; therefore, I recommend sampling natural trees near plantings to reveal further connectivity. A wider sampling of adult parents may be necessary to measure pollination dispersal distances and gene flow to pair parents with offspring.

4.4.2 *Fitness testing*

In general, this study demonstrated that local seed sourcing did not produce any negative genetic outcomes such as loss of adaptive traits through inbreeding, genetic drift, or low genetic variation. However, crucially we must now ask whether offspring are tolerant or resistant enough to be able to withstand harsh environmental conditions in the future. It has been suggested that management actions focus on the restoration of ecological functions and resilience, rather than returning the ecosystem to a historic state to combat predicted environmental changes (Mijangos *et al*. 2014). A more thorough investigation may be necessary to denote whether fitness is reduced in planted offspring perhaps utilising measures such as annual seed set, seed weight and germination rate (Barmentlo *et al.* 2018). Such an investigation can help establish whether offspring are experiencing outbreeding depression or inbreeding depression even with some levels of connectivity. Further, fitness tests of the 2nd generation of seedlings may reveal whether inbreeding depression effects are masked in naturally regenerating saplings. Fitness testing can also help assist in understanding whether reintroduced populations are viable to adapt to the local environment. We recommend that fitness testing of offspring (for example, drought testing) would be an informative future study in the future to denote whether *E. albens* offspring can withstand predicted warmer and more arid climates.

4.4.3 *Seed sourcing protocols*

This study also highlighted the importance of accurate seed-sourcing methods to be recorded in protocols for communication in the future. The results indicated that there was evidence of genetic material being sourced externally from the valley, which can cause issues with local adaption and genetic contamination. Further research tested the fitness of offspring from these external trees to determine whether this population of planted trees are appropriately adapted to the local environment, or whether this population could potentially introduce maladapted genotypes into the Central Valley restoration project of the WNP. Therefore, this study implies that it is especially important to maintain strict local provenance protocols when attempting to restore landscapes, maintain site-specific adaption and reduce genetic contamination. The exact method for sourcing genetic material for the restoration within the WNP was not maintained to a strict protocol, evident by outsourced planted populations discovered within this study.

4.4.4 *Sourcing strategies in the future*

This study provides an alternative outcome to sourcing seeds from fragmented populations, since here large-scale population sourcing was not necessary to retain adequate levels of genetic diversity and reinforces the role of relictual trees as important pools of genetic variation and significant foci for recruitment of highly diverse offspring (Broadhurst 2013; Rosser *et al*. 2023; Quinton 2019). One of the significant arguments against local provenancing is that in landscapes with fragmented habitats (such as this study system), it should not be used as local populations may already be small and, thus lacking genetic variation leading to low-quality seed sets and/or failure of offspring establishment (Leimu *et al*. 2006; Breed *et al*. 2019). It is frequently mentioned within the literature that seeds should not be sourced from small, populations to avoid founder effects and reduction in genetic diversity. Sourcing seed is recommended to be up to 500 individuals (Prober *et al*. 1998) and more than 10-20 individuals as this would be far too small to avoid founder effects (Fischer and Matthies 1998; Vander Mijinsbrugge *et al*. 2010). Therefore, in the future, we can now understand that sourcing from relictual trees, a scattered population, may be adequate to retain levels of genetic diversity within the ecosystem.

Recently, there has been a focus on restoration ecology to maximise the climatic adaptive potential in *Eucalyptus* species by determining the extent of outcrossing to achieve this (Prober *et al*. 2016). This involves developing models for seed sourcing to ensure a range of environmental adaptions are withheld within the genome sources of the restored population (Rossetto *et al*. 2018). Previous research by Prober *et al*. (2016) discovered putative genomic regions of DNA associated with climate adaptability in *Eucalyptus* species which is responsible for expressing climate-related function traits (for example leaf thickness, water use efficiency, growth rate and survival). These regions on the genome can be identified with the use of micro-array technologies and genomic DArTseq sequencing (Sansaloni *et al*. 2010) to increase the ability of restored cohorts to adapt and persist in differentiated arid climates in the future, predicted within Australia. These technologies can be of significant use in the future, as from a conservation perspective, they allow managers to develop a cohort of adaptive individuals. Although it requires more research to measure the adaptive potential of individuals, it is entirely possible for this target study system. It can allow us to facilitate longer-term climate-resilience of restored populations, by selectively choosing genetic sources of restoration plantings and allowing adaptive alleles to be mapped into restored landscapes (Prober *et al*. 2016).

5.0 Conclusion

Ecological genetic approaches to assessing restoration success have been highly recommended for some time (SER 2004), but in application, genetic technologies remain rare. Few studies within the literature have begun using these technologies to their advantage (see Broadhurst 2011; Ritchie and Krauss 2012; Zucchi *et al.* 2018; Millar *et al*. 2012) and very few studies used these technologies to assess levels of connectivity and gene flow (see Broadhurst 2013; Breed *et al*. 2012; McCallum *et al*. 2019; Yates *et al*. 2007). Here we have demonstrated success in restoring populations of *E. albens* in the WNP through comparable levels of genetic diversity, a moderately homogenised population structure, and sufficient evidence of outcrossing between remnant and restored stands. With the analysis of juveniles, we can be more confident in the parentage analysis (by knowing the mother) and that there is no inbreeding or lower genetic diversity levels being hidden in further generations. This study suggests little negative effect of local provenance sourcing, with evidence of mixed seed sourcing. Pollinator services have effectively been re-established to maintain gene flow and reduce inbreeding between restored and source populations. Although there were some concerns raised regarding the genetic parameters surrounding planted populations 3 and 5 (1992-93 plantings), suggesting that local provenance protocols may not have been strictly followed, there were no negative effects observed regarding genetic diversity. Unfortunately, this study was limited in the analysis of these externally sourced planted populations, as 1993 offspring could not be assessed. Importantly, relictual trees were an extremely important reservoir of historical levels of genetic diversity for this restoration project and continue to produce saplings in the revegetated area. However, relictual trees exhibited higher levels of inbreeding which should be considered if seed sourcing was used. There was also evidence of contemporary gene flow between these isolated trees. It was clear in my field observations of the WNP that White Box Gum restoration attempts have been successful in producing many saplings in both planted and remnant populations, ameliorating further population decline. Further testing of fitness might determine the viability of offspring in the face of climatic change in the future.

6.0 References

Abdel-Haleem, H., Ji, P., Boerma, H. R. and Li, Z. (2013). An R package for SNP marker-based *parentoffspring* tests. *Plant Methods* 9(1), 44-44. https://doi.org/10.1186/1746-4811-9-44

Aguilar, R., Ashworth, L., Galetto, L. and Aizen, M. A. (2006). Plant reproductive susceptibility to habitat fragmentation: Review and synthesis through a meta-analysis, *Ecology Letters* **9**(8), 968–980. https://doi.org/10.1111/j.1461-0248.2006.00927.x.

Aitken, S. N. and Bemmels, J. B. (2016). Time to get moving: assisted gene flow of forest trees, *Evolutionary Applications* **9**(1), 271–290. https://doi.org/10.1111/eva.12293.

Aitken, S. N. and Whitlock, M. C. (2013). Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution, and Systematics* **44**(1), 367–388. https://doi.org/10.1146/annurev-ecolsys-110512-135747.

Armbruster, P. and Reed, D. H. (2005). Inbreeding depression in benign and stressful environments. *Heredity* **95**(3), 235–242. https://doi.org/10.1038/sj.hdy.6800721.

ALA (2023a). 'Atlas of Living Australia occurrence records of *Eucalyptus albens'*. Available at: https://biocache.ala.org.au/occurrences/search?q=lsid:https://id.biodiversity.org.au/node/apni/2910848#tab_recordsView

ALA (2023b). 'Atlas of Living Australia occurrence records of *Eucalyptus melliodora'*. Available at: https://biocache.ala.org.au/occurrences/search?q=lsid:https://id.biodiversity.org.au/node/apni/5294597#tab_recordsView

Barmentlo, S., Meirmans, P., Luijten, S., Triest, L., and Oostermeijer, J. G (2018). Outbreeding depression and breeding system evolution in small, remnant populations of *Primula vulgaris*: consequences for genetic rescue. *Conservation Genetics* **19**(3), 545–554. https://doi.org/10.1007/s10592-017-1031-x

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W. and de Bruyn, M. (2014). Erratum to Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution* **29**, 358-367. https://doi.org/10.1016/j.tree.2014.05.012.

Bower, A. D., and Aitken, S. N. (2008). Ecological genetics and seed transfer guidelines for *Pinus albicaulis* (Pinaceae). *American Journal of Botany* **95**(1), 66–76. https://doi.org/10.3732/ajb.95.1.66.

Bradbury, D., Binks, R. M. and Byrne, M. (2021). Genomic data inform conservation of rare tree species: clonality, diversity and hybridity in *Eucalyptus* series in a global biodiversity hotspot. *Biodiversity and Conservation* **30**(3), 619–641. https://doi.org/10.1007/s10531-020-02106-2.

Breed, M. F., Harrison, P.A., Blyth, C., Byrne, M., Gaget, V., Gellie, N. J. C., Groom, S. V. C., Hodgson, R., Mills, J. G., Prowse, T. A. A., Steane, D. A. and Mohr, J. J. (2019). The potential of genomics for restoring ecosystems and biodiversity. *Nature Reviews Genetics* **20**(10), 615–628. https://doi.org/10.1038/s41576-019-0152-0.

Breed, M. F., Gardner, M. G., Ottewell, K. M., Navarro, C. M., and Lowe, A. J. (2012a). Shifts in reproductive assurance strategies and inbreeding costs associated with habitat fragmentation in Central American mahogany. Ecology Letters **15**(5), 444–452. https://doi.org/10.1111/j.1461-0248.2012.01752.x

Breed, M., Marklund, M., Ottewell, K., Gardner, M., Harris, B and Lowe, A. (2012b). Pollen diversity matters: revealing the neglected effect of pollen diversity on fitness in fragmented landscapes. *Molecular Ecology* **21**, 5955–5968.

Breed, M.F., Ottewell, K.M., Gardner, M.G., Marklund, M.H.K., Dormontt, E.E. and Lowe, A.J. (2015). Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. *Heredity* **115**(2), 108–114. https://doi.org/10.1038/hdy.2013.48.

Broadhurst, L. M., Breed, M., Lowe, A., Bragg, J., Catullo, R., Coates, D., Encinas-Viso, F., Gellie, N., James, E., Krauss, S., Potts, B., Rossetto, M., Shepherd, M. and Byrne, M. (2017). Genetic diversity and structure of the Australian flora. *Diversity and Distributions* **23**(1), 41–52. https://doi.org/10.1111/ddi.12505.

Broadhurst, L. M. (2011). Genetic diversity and population genetic structure in fragmented *Allocasuarina verticillata* (Allocasuarinaceae) implications for restoration. *Australian Journal of Botany* **59**(8), 770–780. https://doi.org/10.1071/BT11253.

Broadhurst, L. M. (2013). A genetic analysis of scattered Yellow Box trees (*Eucalyptus melliodora* A.Cunn. ex Schauer, Myrtaceae) and their restored cohorts. *Biological Conservation* **161**, 48–57. https://doi.org/10.1016/j.biocon.2013.02.016.

Broadhurst, L. M., Jones, T. A., Smith, F. S., North, T. and Guja, L. (2015). Maximizing seed resources for restoration in an uncertain future. *BioScience* **66**(1), 73–79. https://doi.org/10.1093/biosci/biv155.

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P.A. and Yates, C. (2008). Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* **1**(4), 587–597. https://doi.org/10.1111/j.1752-4571.2008.00045.x.

Broadhurst, L. M., Mellick, R., Knerr, N., Li, L. and Supple, M. A. (2018). Land availability may be more important than genetic diversity in the range shift response of a widely distributed eucalypt, *Eucalyptus melliodora*. *Forest Ecology and Management* **409**, 38–46. https://doi.org/10.1016/j.foreco.2017.10.024.

Broadhurst, L. M., Young, A. G., Thrall, P. H. and Murray, B. G. (2006). Sourcing seed for *Acacia acinacea*, a key revegetation species in south eastern Australia. *Conservation Genetics* **7**(1), 49–63. https://doi.org/10.1007/s10592-005-7855-9.

Bucharova, A., Bossdorf, O., Hölzel, N., Kollmann, J., Prasse, R. and Durka, W. (2019). Mix and match: regional admixture provenancing strikes a balance among different seed-sourcing strategies for ecological restoration. *Conservation Genetics* **20**(1), 7–17. https://doi.org/10.1007/s10592-018-1067-6.

Byrne, M., Elliott, C. P., Yates, C. J. and Coates, D. J. (2007). Maintenance of high pollen dispersal in *Eucalyptus wandoo*, a dominant tree of the fragmented agricultural region in Western Australia. *Conservation Genetics* **9**(1), 97-105. https://doi.org/10.1007/s10592-007-9311-5

Charlesworth, D. and Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics* **10**(11), 783–796. https://doi.org/10.1038/nrg2664.

Coates, D. J., Sampson, J. F. and Yates, C. J. (2007). Plant mating systems and assessing population persistence in fragmented landscapes. *Australian Journal of Botany* **55**(3), 239–249. https://doi.org/10.1071/BT06142.

Cork, S. and Catling, P. (1996). Modelling distributions of arboreal and ground-dwelling mammals in relation to climate, nutrients, plant chemical defences and vegetation structure in the eucalypt forests of southeastern Australia. *Ecology and Management* **85**(1), 163-175.

Corlett, R. T. and Westcott, D. A. (2013). Will plant movements keep up with climate change? *Trends in Ecology and Evolution* **28**(8), 482–488. Available at: https://doi.org/10.1016/j.tree.2013.04.003.

Crémieux, L., Bischoff, A., Müller-Schärer, H., and Steinger, T. (2010). Gene flow from foreign provenances into local plant populations: Fitness consequences and implications for biodiversity restoration. *American Journal of Botany* **97**(1), 94–100. https://doi.org/10.3732/ajb.0900103

Cunningham, S. A., Floyd, R. B., Griffiths, M. W., and Wylie, F. R. (2005). Patterns of host use by the shoot-borer *Hypsipyla robusta* (Pyralidae: Lepidoptera) comparing five *Meliaceae* tree species in Asia and Australia. *Forest Ecology and Management* **205**(1–3), 351–357. https://doi.org/10.1016/j.foreco.2004.10.042.

Dick, C. W., Hardy, O. J., Jones, F. A. and Petit, R. J. (2008). Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Tropical Plant Biology* **1**(1), 20-23. https://doi.org/10.1007/s12042-007-9006-6.

Dixon, K. (2009). Pollination and restoration. *Science* **325**, 571-573

Dolan, R. W., Marr, D. L. and Schnabel, A. (2008). Capturing genetic variation during ecological restorations: an example from Kankakee sands in Indiana. *Restoration Ecology* **16**(3), 386–396. https://doi.org/10.1111/j.1526-100X.2007.00318.x.

Dunn, E. R., Rosser, N. J., Denham, A. J. and Ayre, D. J. (2023, in review). Local provenance seed-sourcing: a successful strategy in the restoration of an endangered Box-Gum Eucalypt woodland. *Restoration Ecology*, (in review)

Edmands, S. (2007). Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* **16**(3), 463–475. https://doi.org/10.1111/j.1365-294X.2006.03148.x.

Etterson, J. R. (2004). Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. II. Genetic architecture of three populations reciprocally planted along an environmental gradient in the great plains. *Evolution* **58**(7), 1459–1471

EUCLID Eucalypts of Australia (3rd ed.). (2006). (CSIRO Publishing, Collingwood, Vic.)

Field, D. L., Ayre, D. J., Whelan, R. J. and Young, A. G. (2008). Relative frequency of sympatric species influences rates of interspecific hybridization, seed production and seedling performance in the uncommon *Eucalyptus aggregata*. *Journal of Ecology* **96**(6), 1198–1210. https://doi.org/10.1111/j.1365-2745.2008.01434.x.

Field, D. L., Ayre, D. J., Whelan, R. J. and Young, A. G. (2011). Patterns of hybridization and asymmetrical gene flow in hybrid zones of the rare *Eucalyptus aggregata* and common *E. rubida*. *Heredity* **106**(5), 841–853. https://doi.org/10.1038/hdy.2010.127.

Fischer, M., and Matthies, D. (1998). Effects of population size on performance in the rare plant *Gentianella germanica*. *The Journal of Ecology* **86**(2), 195–204. https://doi.org/10.1046/j.1365-2745.1998.00246.x

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2010). 'Introduction to conservation genetics (2nd edition).' (Cambridge University Press, Macquarie University, Sydney.)

Frankham, R., Ballou, J. D., Eldridge, M. D. B., Lacy, R. C., Ralls, K., Dudash, M. R. and Fenster, C.B. (2011). Predicting the probability of outbreeding depression. *Conservation Biology* **25**(3), 465–475. https://doi.org/10.1111/j.1523-1739.2011.01662.x.

Fremout, T., Thomas, E., Bocanegra-González, K. T., Aguirre-Morales, C. A., Morillo-Paz, A. T., Atkinson, R., Kettle, C., González-M., R., Alcázar-Caicedo, C., González, M. A., Gil-Tobón, C., Gutiérrez, J. P., Gonzalo Moscoso-Higuita, L., López-Lavalle, L. A. B., de Carvalho, D., and Muys, B. (2021). Dynamic seed zones to guide climate-smart seed sourcing for tropical dry forest restoration in Colombia. *Forest Ecology and Management* **490**, 119127. https://doi.org/10.1016/j.foreco.2021.119127

Funk, W. C., McKay, J. K., Hohenlohe, P. A. and Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology and Evolution* **27**(9), 489–496. https://doi.org/10.1016/j.tree.2012.05.012.

Galloway, L. F. and Fenster, C. B. (2000). Population differentiation in an annual legume: local adaptation. *Evolution* **54**(4), 1173-1181. https://academic.oup.com/evolut/article/54/4/1173/6757506.

Gellie, N. J. C., Breed, M. F., Mortimer, P. E., Harrison, R. D., Xu, J. and Lowe, A.J. (2018). Networked and embedded scientific experiments will improve restoration outcomes. *Frontiers in Ecology and the Environment* **16**(5), 288–294. https://doi.org/10.1002/fee.1810.

Gibbons, P. and Boak, M. (2002). The value of paddock trees for regional conservation in an agricultural landscape. *Ecological Management and Restoration* **3**(3), 205–210. https://doi.org/10.1046/j.1442-8903.2002.00114.x.

Gibbons, P., Lindenmayer, D.B., Fischer, J., Manning, A. D., Weinberg, A., Seddon, J., Ryan, P. and Barrett, G. (2008). The future of scattered trees in agricultural landscapes. *Conservation Biology* **22**(5), 1309–1319. https://doi.org/10.1111/j.1523-1739.2008.00997.x.

Goto, S., Iijima, H., Ogawa, H. and Ohya, K. (2011). Outbreeding depression caused by intraspecific hybridization between local and nonlocal genotypes in *Abies sachalinensis*. *Restoration Ecology* **19**(2), 243–250. https://doi.org/10.1111/j.1526-100X.2009.00568.x.

Gruber, B., Unmack, P. J., Berry, O. F., and Georges, A. (2018). dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources* **18**(3), 691–699. https://doi.org/10.1111/1755-0998.12745

Hardy, O. J., Maggia, L., Bandou, E., Breyne, P., Caron, H., Chevallier, M. H., Doligez, A., Dutech, C., Kremer, A., Latouche-Hallé, C., Troispoux, V., Veron, V. and Degen, B. (2006). Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology* **15**(2), 559–571. https://doi.org/10.1111/j.1365-294X.2005.02785.x.

Harrison, P. A., Vaillancourt, R. E., Harris, R. M. B. and Potts, B. M. (2017). Integrating climate change and habitat fragmentation to identify candidate seed sources for ecological restoration. *Restoration Ecology* **25**(4), 524–531. https://doi.org/10.1111/rec.12488.

Hobbs, R. J. and Norton, D. A. (1996). Towards a conceptual framework for restoration ecology. *Restoration Ecology* **4**(2), 93–110. https://doi.org/10.1111/j.1526-100X.1996.tb00112.x.

Hufford, K. M., Krauss, S. L. and Veneklaas, E.J. (2012). Inbreeding and outbreeding depression in *Stylidium hispidum*: implications for mixing seed sources for ecological restoration. *Ecology and Evolution* **2**(9), 2262–2273. https://doi.org/10.1002/ece3.302.

Hufford, K. M. and Mazer, S. J. (2003). Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution* **18**(3), 147–155. https://doi.org/10.1016/S0169-5347(03)00002-8.

Hughes, L., Dean, A., Steffen, W., Rice, M. (2019). 'This is what climate change looks like'. *Climate Council of Australia.* Available at https://www.climatecouncil.org.au/wp-content/uploads/2019/09/This-is-What-Climate-Change-Looks-Like.pdf

IPCC (2014). Climate change 2013 Part A: Global and sectoral aspects in Climate Change 2014, *Impacts, adaptation and vulnerability. Part A: Global and sectoral aspects. Contributions of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change*. Available at: https://www.ipcc.ch/site/assets/uploads/2018/02/WGIIAR5-PartA_FINAL.pdf

IUCN (2019a). IUCN Red List of Threatened species: *Eucalyptus albens*, *IUCN Red List of Threatened* Species, Available at: https://www.iucnredlist.org/species/133377688/133377690

IUCN (2019b). IUCN Red List of Threatened species: *Eucalyptus melliodora*, *IUCN Red List of Threatened Species*, Available at: https://www.iucnredlist.org/species/61913766/61913768

IUCN (2022). IUCN Red List of Threatened species: *Eucalyptus*, *IUCN Red List of Threatened Species*, Available at: https://www.iucnredlist.org/search?query=yellow%20box&searchType=species

Jones, A. T., Hayes, M. J. and Sackville Hamilton, N. R. (2001). The effect of provenance on the performance of *Crataegus monogyna* in hedges. *Journal of Applied Ecology* **38**(5), 952–962. https://doi.org/10.1046/j.1365-2664.2001.00650.x.

Jordan, R., Breed, M. F., Prober, S. M., Miller, A. D. and Hoffmann, A. A. (2019). How well do revegetation plantings capture genetic diversity? *Biology Letters* **15**(10). https://doi.org/10.1098/rsbl.2019.0460.

Jordan, R., Dillon, S. K., Prober, S. M. and Hoffmann, A. A. (2016). Landscape genomics reveals altered genome wide diversity within revegetated stands of *Eucalyptus microcarpa* (Grey Box). *New Phytologist* **212**(4), 992–1006. https://doi.org/10.1111/nph.14084.

Jordan, R., Hoffmann, A. A., Dillon, S. K. and Prober, S. M. (2017). Evidence of genomic adaptation to climate in *Eucalyptus microcarpa*: Implications for adaptive potential to projected climate change. *Molecular Ecology* **26**(21), 6002–6020. https://doi.org/10.1111/mec.14341.

Kaulfuß, F. and Reisch, C. (2021). Restoration of species-rich grasslands by transfer of local plant material and its impact on species diversity and genetic variation—findings of a practical restoration project in southeastern Germany. *Ecology and Evolution* **11**(18), 12816–12833. https://doi.org/10.1002/ece3.8029.

Keller, M., Kollmann, J. and Edwards, P. J. (2000). Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology* **37**(4), 647–659. https://doi.org/10.1046/j.1365-2664.2000.00517.x.

Kramer, A. T., Ison, J. L., Ashley, M. V. and Howe, H. F. (2008). The paradox of forest fragmentation genetics. *Conservation Biology* **22**, 878-885. https://doi.org/10.1111/j.1523-1739.2008.00944.x

Leimu, R., Mutikainen, P. I. A., Koricheva, J. and Fischer, M. (2006). How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* **94**, 942-952. https://doi.org/10.1111/j.1365-2745.2006.01150.x

Li, Y., & Liu, J. (2018). *StructureSelector*: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular Ecology Resources* **18**(1), 176–177. https://doi.org/10.1111/1755- 0998.12719

Liu, M. H., Chen, X. Y., Zhang, X. and Shen, D. W. (2008). A population genetic evaluation of ecological restoration with the case study on *Cyclobalanopsis myrsinaefolia* (Fagaceae). *Plant Ecology* **197**(1), 31–41. https://doi.org/10.1007/s11258-007-9357-y.

Liu, S., Palti, Y., Gao, G. and Rexroad, I. I. I. C. E. (2016). Development and validation of a SNP panel for parentage assignment in rainbow trout. *Aquaculture* **452**, 178-82. https://doi.org/10.1016/j.aquaculture.2015.11.001

Lowe, A. J., Boshier, D., Ward, M., Bacles, C. F. E. and Navarro, C. (2005). Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* **95**(4), 255–273. https://doi.org/10.1038/sj.hdy.6800725.

Lowe, W. H. and Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology* **19**(15), 3038–3051. https://doi.org/10.1111/j.1365-294X.2010.04688.x.

Luikart, G., England, P. R., Tallmon, D., Jordan, S. and Taberlet, P. (2003). The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics* **4**(12), 981–994. https://doi.org/10.1038/nrg1226.

Manning, A. D., Fischer, J. and Lindenmayer, D. B. (2006). Scattered trees are keystone structures - Implications for conservation. *Biological Conservation* **132**(3), 311–321. https://doi.org/10.1016/j.biocon.2006.04.023.

Mappin, B., Ward, A., Hughes, L., Watson, J. E. M., Cosier, P. and Possingham, H. P. (2022). The costs and benefits of restoring a continent's terrestrial ecosystems. *Journal of Applied Ecology* **59**(2), 408–419. https://doi.org/10.1111/1365-2664.14008.

McCallum, K. P., Breed, M. F., Paton, D. C., and Lowe, A. J. (2019). Clumped planting arrangements improve seed production in a revegetated eucalypt woodland. *Restoration Ecology* **27**(3), 638–646. https://doi.org/10.1111/rec.12905

McGregor, D. C., Kerr, S. E. and Krockenberger, A. K. (2013). The distribution and abundance of an island population of koalas (*Phascolarctos cinereus*) in the far north of their geographic range. *PLoS ONE* **8**(3). https://doi.org/10.1371/journal.pone.0059713.

McKay, J. K., Christian, C. E., Harrison, S. and Rice, K. J. (2005). "How local is local?" – a review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* **13**(3), 432–440. https://doi.org/10.1111/j.1526-100X.2005.00058.x.

Menz, M. H. M., Phillips, R. D., Winfree, R., Kremen, C., Aizen, M. A., Johnson, S. D., and Dixon., K. W. (2011). Reconnecting plants and pollinators: challenges in the restoration of pollination mutualisms. *Trends in Plant Science* **16**, 4–12.

Mijangos, J. L., Pacioni, C., Spencer, P. B. S. and Craig, M. D. (2015). Contribution of genetics to ecological restoration. *Molecular Ecology*, **24**(1), 22–37. https://doi.org/10.1111/mec.12995.

Millar, M. A., Byrne, M., Nuberg, I. K. and Sedgley, M. (2012). High levels of genetic contamination in remnant populations of *Acacia saligna* from a genetically divergent planted stand. *Restoration Ecology* **20**(2), 260–267. https://doi.org/10.1111/j.1526-100X.2010.00758.x

Millar, M. A., Coates, D. J., Byrne, M., Krauss, S. L., Jonson, J. and Hopper, S. D. (2021). Evaluating restoration outcomes through assessment of pollen dispersal, mating system, and genetic diversity. *Restoration Ecology* **29**(3). https://doi.org/10.1111/rec.13335.

Miller, M. A., Schwartz, T., Pickett, B. E., He, S., Klem, E. B., Scheuermann, R. H., Passarotti, M., Kaufman, S. and O'Leary, M. A. (2015). A RESTful API for access to phylogenetic tools via the CIPRES science gateway. *Evolutionary Bioinformatics Online* **2015**, 43-48. https://doi.org/10.4137/EBO.S21501

Mimura, M., Barbour, R. C., Potts, B. M., Vaillancourt, R. E. and Watanabe, K. N. (2009). Comparison of contemporary mating patterns in continuous and fragmented *Eucalyptus globulus* native forests *Molecular Ecology* **18**(20), 4180–4192. https://doi.org/10.1111/j.1365-294X.2009.04350.x.

Neckles, H. A., Dionne, M., Burdick, D. M., Roman, C. T., Buchsbaum, R. and Hutchins, E. (2002). A monitoring protocol to assess tidal restoration of salt marshes on local and regional scales *Restoration Ecology* **10**(3), 556–563. https://doi.org/10.1046/j.1526-100X.2002.02033.x.

Quinton, A. L (2019). Genetic assessment of population restoration within the Warrumbungle National Park for populations of Yellow Box Gum (*Eucalyptus melliodora*): A high-throughput sequencing examination of local provenance sourcing. Honours thesis, University of Wollongong

Reynolds, L. K., Waycott, M., McGlathery, K. J., Orth, R. J., and Zieman, J. C. (2012). Eelgrass restoration by seed maintains genetic diversity: case study from a coastal bay system. *Marine ecology. Progress series (Halstenbek)* **448**, 223–234. https://doi.org/10.3354/meps09386

Ottewell, K. M., Donnellan, S. C., Lowe, A. J. and Paton, D. C. (2009). Predicting reproductive success of insect- versus bird-pollinated scattered trees in agricultural landscapes. *Biological Conservation* **142**(4), 888–898. https://doi.org/10.1016/j.biocon.2008.12.019.

Ottewell, K. M., Donnellan, S. C. and Paton, D. C. (2010). Evaluating the demographic, reproductive, and genetic value of eucalypt paddock trees for woodland restoration in agricultural landscapes. *Restoration Ecology* **18**, 263–272. Available at: https://doi.org/10.1111/j.1526-100X.2010.00659.x.

Passell, H. D. (2000). Recovery of bird species in minimally restored Indonesian tin strip mines. *Restoration Ecology* **8**(2), 112–118. https://doi.org/10.1046/j.1526-100X.2000.80017.x.

Pasquet, R. S., Peltier, A., Hufford, M. B., Oudin, E., Saulnier, J., Paul, L., Knudsen, J. T., Herren, H. R., and Gepts, P. (2008). Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proceedings of the National Academy of Sciences - PNAS* **105**(36), 13456–13461. https://doi.org/10.1073/pnas.0806040105

Peres, C. A., Baider, C., Zuidema, P. A., Wadt, L. H. O., Kainer, K. A., Gomes-Silva, D. A. P., Salomão, R. P., Simões, L. L., Franciosi, E. R. N., Cornejo Valverde, F., Gribel, R., Shepard, G. H., Kanashiro, M., Coventry, P., Yu, D. W., Watkinson, A. R. and Freckleton, R. P. (2003). Demographic threats to the sustainability of Brazil nut exploitation. *Science (American Association for the Advancement of Science)* **302**(5653), 2112-2114. https://doi.org/10.1126/science.1091698

Perring, M. P., Standish, R. J., Price, J. N., Craig, M. D., Erickson, T. E., Ruthrof, K. X., Whiteley, A. S., Valentine, L. E. and Hobbs, R. J. (2015). Advances in restoration ecology: rising to the challenges of the coming decades. *Ecosphere* **6**(8). https://doi.org/10.1890/ES15-00121.1.

Petroli, C. D., Sansaloni, C. P., Carling, J., Steane, D. A., Vaillancourt, R. E., Myburg, A. A., da Silva Jr, O. B., Pappas Jr, G. J., Kilian, A., and Grattapaglia, D. (2012). Genomic characterization of DArT

markers based on high-density linkage analysis and physical mapping to the *Eucalyptus* genome. *PloS One* **7**(9), e44684–e44684. https://doi.org/10.1371/journal.pone.0044684

Prasse, R., Kunzmann, D., and Schröder, R. (2010). Development and practical implementation of minimal requirements for the verification of origin of native seeds of herbaceous plants. *Cooperation with Verband Deutscher Wildsamen-und Wildpflanzenproduzenten. DBU, reference*, (23931).

Prober, S. M., Potts, B. M., Bailey, T., Byrne, M., Dillon, S., Harrison, P. A., Hoffmann, A. A., Jordan, R., Mclean, E. H., Steane, D. A., Stock, W. D. and Vaillancourt, R. E. (2016). Climate adaptation and ecological restoration in eucalypts. *Proceedings of the Royal Society of Victoria* **128**(1), 40–53. https://doi.org/10.1071/RS16004.

Prober, S. M. and Thiele, K. R. (1995). Conservation of the grassy white box woodlands: relative contributions of size and disturbance to floristic composition and diversity of remnants. *Australian Journal of Botany* **43**(4), 349-366. https://doi.org/10.1071/BT9950349

Prober, S. M., Thiele, K. R. and Lunt, I. D. (2002). Identifying ecological barriers to restoration in temperate grassy woodlands: soil changes associated with different degradation states. *Australian Journal of Botany* **50**(6), 699–712. https://doi.org/10.1071/BT02052.

Prober, S. M., Spindler, L. H. and Brown, A. H. D. (1998). Conservation of the grassy white box woodlands: effects of remnant population size on genetic diversity in the allotetraploid herb *Microseris lanceolata*. *Conservation Biology* 1**2**, 1279-1290. https://doi.org/10.1111/j.1523-1739.1998.97100.x

Proft, K. M., Jones, M. E., Johnson, C. N. and Burridge, C. P. (2018). Making the connection: expanding the role of restoration genetics in restoring and evaluating connectivity. *Restoration Ecology* **26**(3), 411–418. https://doi.org/10.1111/rec.12692.

Puechmaille S.J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* **16**(3), 608-727. https://doi.org/10.1111/1755-0998.12512

Raabová, J., Münzbergová, Z. and Fischer, M. (2007). Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus. Biological Conservation* **139**(3–4), 348–357. https://doi.org/10.1016/j.biocon.2007.07.007.

Raeymaekers, J. A. M., Maes, G. E., Geldof, S., Hontis, I., Nackaerts, K. and Volckaert, F. A. M. (2008). Modeling genetic connectivity in sticklebacks as a guideline for river restoration. *Evolutionary Applications* **1**(3), 475-488. https://doi.org/10.1111/j.1752-4571.2008.00019.x

Reusch, T. B. H., Ehlers, A., Hä, A. and Worm, B. (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences* **102**(8), 2826-2831. www.pnas.orgcgidoi10.1073pnas.0500008102.

Rhoades, C. C., Eckert, G. E. and Coleman, D. C. (1998). Effect of pasture trees on soil nitrogen and organic matter: implications for tropical montane forest restoration. *Restoration Ecology* **6**(3), 262–270. https://doi.org/10.1046/j.1526-100X.1998.00639.x.

Rice, K. J. and Emery, N. C. (2003). Managing microevolution: restoration in the face of global change. *Frontiers in Ecology and the Environment* **1**(9), 469–478. https://doi.org/10.1890/1540-9295(2003)001[0469:MMRITF]2.0.CO;2.

Ritchie, A. L. and Krauss, S. L. (2012). A Genetic assessment of ecological restoration success in *Banksia attenuata*. *Restoration Ecology* **20**(4), 441–449. https://doi.org/10.1111/j.1526-100X.2011.00791.x.

Rosser, N. L., Quinton, A., Davey, H., Ayre, D. J., Denham, A. J. (2023). Genetic assessment of the value of restoration planting within an endangered eucalypt woodland. *Scientific Reports* **13**(1), 6583. https://doi.org/10.1038/s41598-023-33720-z

Rossetto, M., Bragg, J., Kilian, A., McPherson, H., van der Merwe, M. and Wilson, P. D. (2019). Restore and renew: a genomics-era framework for species provenance delimitation. *Restoration Ecology* **27**(3), 538–548. https://doi.org/10.1111/rec.12898.

Ruiz-Jaen, M. C. and Aide, T. M. (2005). Restoration success: how is it being measured? *Restoration Ecology* **13**(3), 569–577. https://doi.org/10.1111/j.1526-100X.2005.00072.x.

Rymer, P. D., Sandiford, M., Harris, S. A., Billingham, M. R. and Boshier, D. H. (2015). Remnant *Pachira quinata* pasture trees have greater opportunities to self and suffer reduced reproductive success due to inbreeding depression. *Heredity* **115**, 115-124. https://doi.org/10.1038/hdy.2013.73

Sampson, J. F. and Byrne, M. (2008). Outcrossing between an agroforestry plantation and remnant native populations of *Eucalyptus loxophleba*. *Molecular Ecology* **17**(11), 2769–2781.

Sansaloni, C. P., Petroli, C. D., Carling, J., Hudson, C. J., Steane, D. A., Myburg, A. A., Grattapaglia, D., Vaillancourt, R. E., and Kilian, A. (2010). A high-density Diversity Arrays Technology (DArT) microarray for genome-wide genotyping in *Eucalyptus*. *Plant Methods* **6**(1), 16–16. https://doi.org/10.1186/1746-4811-6-16

SER (Society for Ecological Restoration International Science and Policy Working Group), (2004). The SER International Primer on Ecological Restoration. Available at: https://www.ctahr.hawaii.edu/littonc/PDFs/682_SERPrimer.pdf

Sgrò, C. M., Lowe, A. J. and Hoffmann, A. A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications* **4**, 326-337. https://doi-org.ezproxy.uow.edu.au/10.1111/j.1752-4571.2010.00157.x

Smith, J. and Lill, A. (2008). Importance of eucalypts in exploitation of urban parks by Rainbow and Musk Lorikeets. *Emu* **108**(2), 187–195. https://doi.org/10.1071/MU07062.

Sork, V. L., Nason, J., Campbell, D. R., and Fernandez, J. F. (1999). Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology & Evolution* **14**(6) 219–224. https://doi.org/10.1016/S0169-5347(98)01585-7

Steane, D. A., Potts, B. M., McLean, E., Prober, S. M., Stock, W. D., Vaillancourt, R. E. and Byrne, M. (2014). Genome-wide scans detect adaptation to aridity in a widespread forest tree species. *Molecular Ecology* **23**(10), 2500–2513. https://doi.org/10.1111/mec.12751.

Steane, D. A., Potts, B. M., McLean, E. H., Collins, L., Holland, B. R., Prober, S. M., Stock, W. D., Vaillancourt, R. E. and Byrne, M. (2017). Genomic scans across three eucalypts suggest that adaptation to aridity is a genome-wide phenomenon. *Genome Biology and Evolution* **9**(2), 253–265. https://doi.org/10.1093/gbe/evw290.

Suding, K., Higgs, E., Palmer, M., Callicott, J. B., Anderson, C. B., Baker, M., Gutrich, J. J., Hondula, K. L., LaFevor, M. C., Larson, B. M. H., Randall, A., Ruhl, J. B. and Schwartz, K. Z. S. (2015). Committing to ecological restoration: efforts around the globe need legal and policy clarification. *Science*. *(American Association for the Advancement of Science*) **348**(6235), 638–640. https://doi.org/10.1126/science.aaa4216.

Supple, M. A., Bragg, J. G., Broadhurst, L. M., Nicotra, A. B., Byrne, M., Andrew, R. L., Widdup, A., Aitken, N. C. and Borevitz, J. O. (2018). Landscape genomic prediction for restoration of a *Eucalyptus* foundation species under climate change. *eLife*, **7**. https://doi.org/10.7554/eLife.31835.001.

Tambarussi, E. V., Boshier, D., Vencovsky, R., Freitas, M. L. M., and Sebbenn, A. M. (2017). Inbreeding depression from selfing and mating between relatives in the Neotropical tree *Cariniana legalis* Mart. Kuntze. *Conservation Genetics* **18**(1), 225–234. https://doi.org/10.1007/s10592-016-0896-4

Tulau, M. J., McInnes-Clarke, S. K., Yang, X., McAlpine, R. A., Karunaratne, S. B., Zhu, Q., and Morand, D. T. (2019). The Warrumbungle post-fire recovery project; raising the profile of soils. *Soil Use and Management* **35**(1), 63–74. https://doi.org/10.1111/sum.12467

Van Rossum, F., and Le Pajolec, S. (2021). Mixing gene pools to prevent inbreeding issues in translocated populations of clonal species. *Molecular Ecology* **30**(12), 2756–2771. https://doi.org/10.1111/mec.15930

Vander Mijnsbrugge, K., Bischoff, A. and Smith, B. (2010). A question of origin: where and how to collect seed for ecological restoration. *Basic and Applied Ecology* **11**(4), 300–311. https://doi.org/10.1016/j.baae.2009.09.002.

von Takach Dukai, B., Jack, C., Borevitz, J., Lindenmayer, D. B. and Banks, S. C. (2019). Pervasive admixture between eucalypt species has consequences for conservation and assisted migration. *Evolutionary Applications* **12**(4), 845–860. https://doi.org/10.1111/eva.12761.

Walters, B. B. (2000). Local mangrove planting in the Philippines: are fisherfolk and fishpond owners effective restorationists? *Restoration Ecology* **8**(3), 237–246. https://doi.org/10.1046/j.1526-100X.2000.80035.x.

Wheeler, N. and Sederoff, R. (2008). Role of genomics in the potential restoration of the American chestnut. *Tree Genetics and Genomes* **5**(1), 181. https://doi.org/10.1007/s11295-008-0180-y

Wilkins, S., Keith, D. A. and Adam, P. (2003). Measuring success: Evaluating the restoration of a grassy eucalypt woodland on the Cumberland plain, Sydney, Australia. *Restoration Ecology* **11**(4), 489–503. https://doi.org/10.1046/j.1526-100X.2003.rec0244.x.

Wilkinson, G. R. (2008). Population differentiation within *Eucalyptus obliqua*: implications for regeneration success and genetic conservation in production forests. *Australian Forestry* **71**(1), 4–15. https://doi.org/10.1080/00049158.2008.10676266.

Woinarski, J. C. Z., Braby, M. F., Burbidge, A. A., Coates, D., Garnett, S. T., Fensham, R. J., Legge, S. M., McKenzie, N. L., Silcock, J. L. and Murphy, B. P. (2019). Reading the black book: the number, timing, distribution and causes of listed extinctions in Australia. *Biological Conservation* **239**. https://doi.org/10.1016/j.biocon.2019.108261.

Woods, M. J., Frankenberg, S. J., Juodvalkis, J. R., Lloyd, M. C., Cobb, M., & McEwan, R. W. (2022). Oak seedling performance and soil development across a forest restoration chronosequence following agriculture in the American Midwest—a greenhouse experiment. *Restoration Ecology* **30**(5). https://doi.org/10.1111/rec.13587

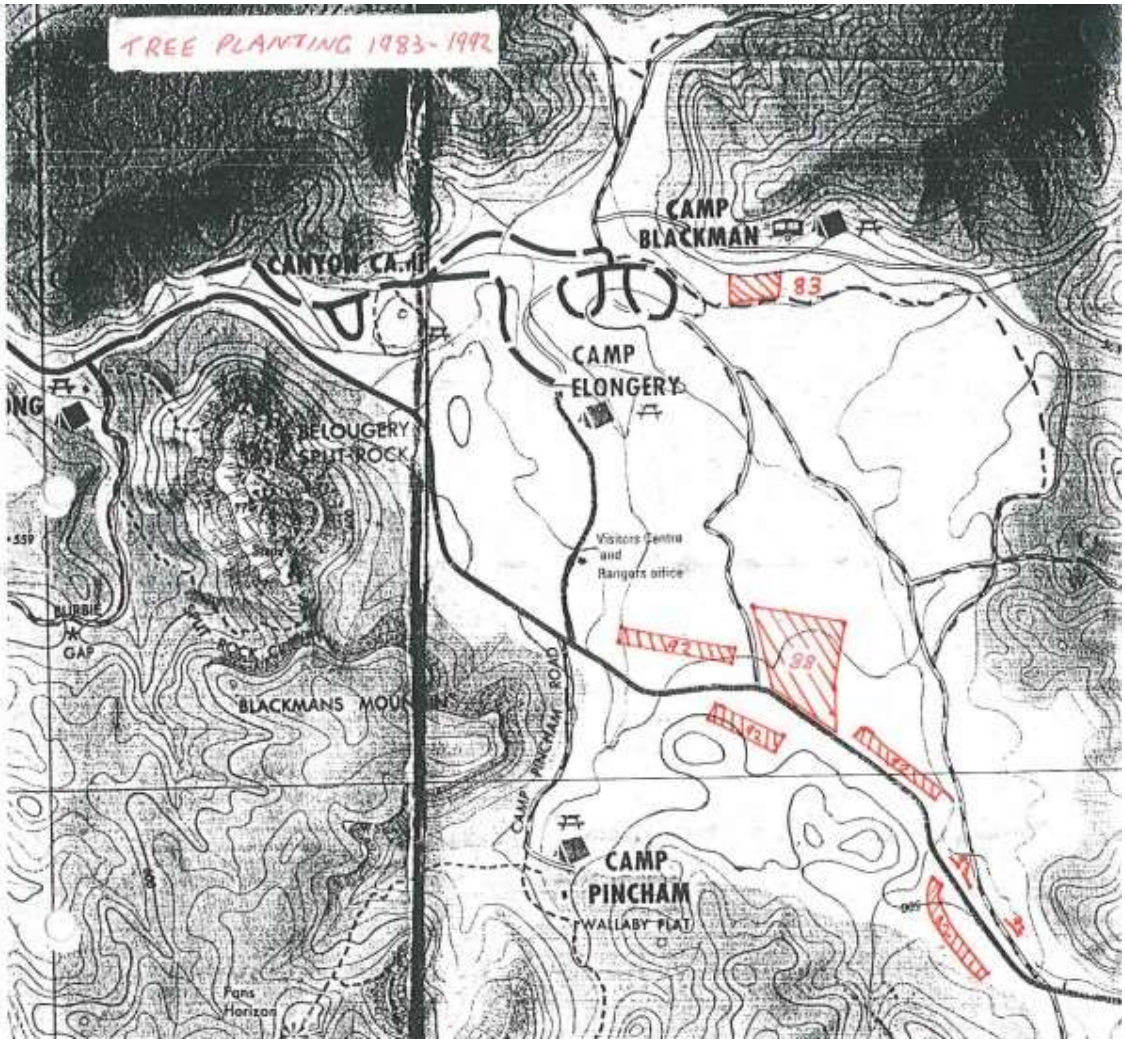
Wortley, L., Hero, J. M. and Howes, M. (2013). Evaluating ecological restoration success: a review of the literature. *Restoration Ecology* **21**(5), 537–543. https://doi.org/10.1111/rec.12028.

WWF (2015). *WWF Living Forest Report: Chapter 5: saving forests at risk*. Available at https://wwf.panda.org/discover/our_focus/forests_practice/forest_publications_news_and_reports/living_forests_report/

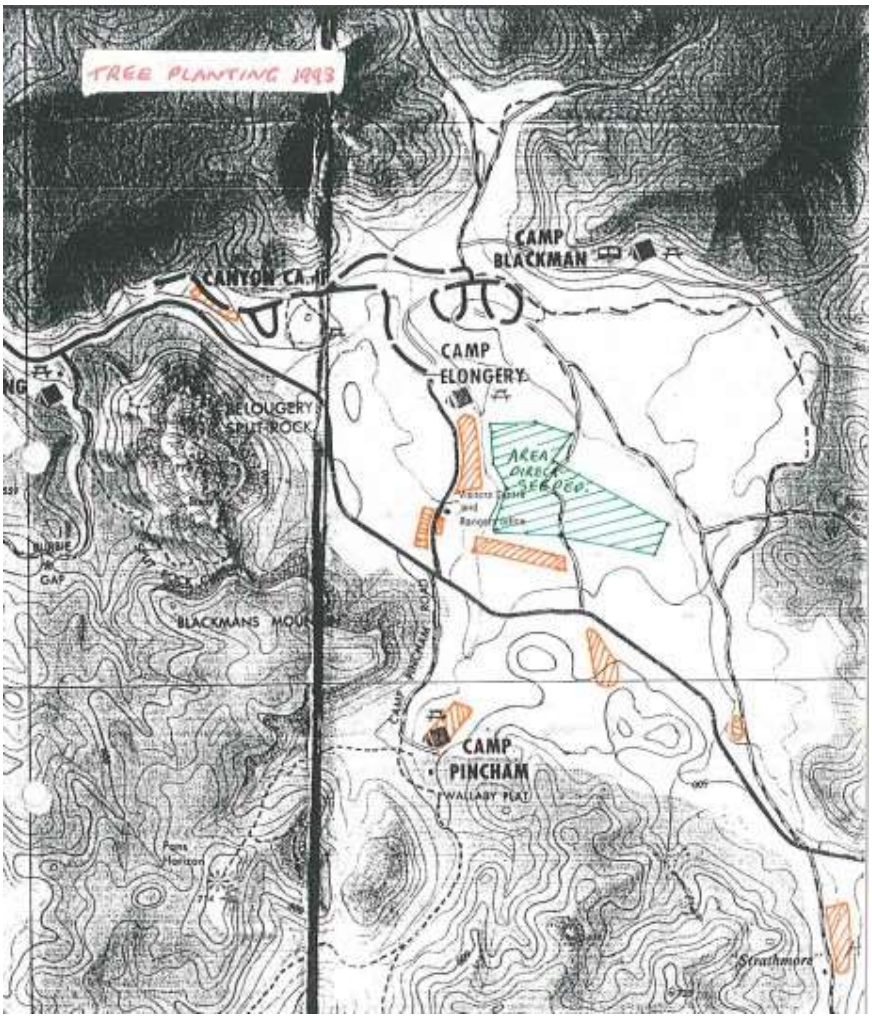
Yates, C., Coates, D., Elliott, C. and Byrne, M. (2007). Composition of the pollinator community, pollination and the mating system for a shrub in fragments of species rich Kwongan in South-West Western Australia. *Biodiversity and Conservation* **16**, 1379–1395

Zucchi, M. I., Sujii, P. S., Mori, G. M., Viana, J. P. G., Grando, C., Silvestre, E. de A., Schwarcz, K. D., Macrini, C. M., Bajay, M. M., Araújo, F. L., Siqueira, M. V. B. M., Alves-Pereira, A., de Souza, A. P., Pinheiro, J.B ., Rodrigues, R. R. and Brancalion, P. H. S. (2018). Genetic diversity of reintroduced tree populations in restoration plantations of the Brazilian Atlantic Forest *Restoration Ecology* **26**(4), 694–701. https://doi.org/10.1111/rec.12620.

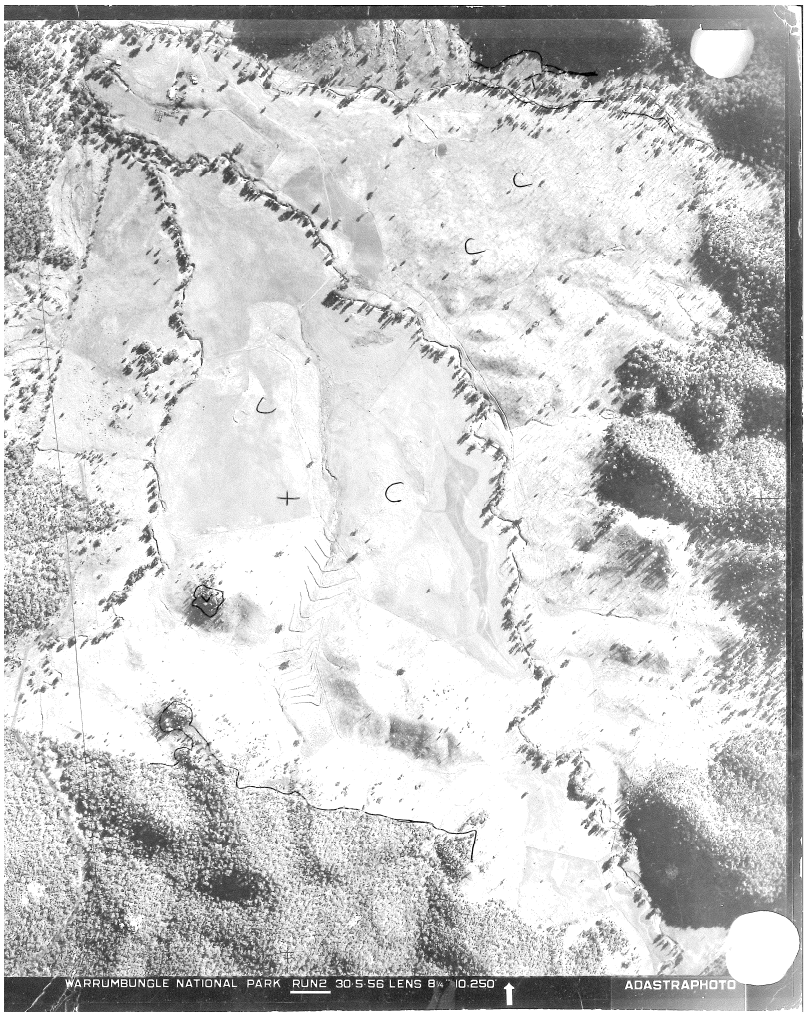
7.0 Appendix



Appendix Figure 1. Proposed *Eucalyptus albens*, *Eucalyptus melliodora* and *Eucalyptus blakelyi* planting areas in 1983, 1988, and 1992 in the Central Valley restoration area of the Warrumbungle National Park, as shown in red shaded annotations. The sketch is reproduced with permission of NSW National Parks and Wildlife Service



Appendix Figure 2. Proposed *Eucalyptus albens*, *Eucalyptus melliodora* and *Eucalyptus blakelyi* planting areas in 1993 in the Central Valley restoration area of the Warrumbungle National Park, as shown in the historical annotation of red shaded areas. Provided by NSW National Parks and Wildlife Service. The sketch is reproduced with permission of NSW National Parks and Wildlife Service



Appendix Figure 3. Aerial photograph from 1956 utilised to locate scattered relictual *Eucalyptus albens* trees within the Central Valley area of the Warrumbungle National Park before the restoration took place. Annotations are historical and the photograph is reproduced with permission of NSW National Parks and Wildlife Service

Appendix Table 1. *Eucalyptus albens* seedling offspring parentage results of a paternity assignment based on the highest LOD score and Trio Delta of the estimated father using CERVUS 3.0.7. (Trio confidence '*' = 95%)

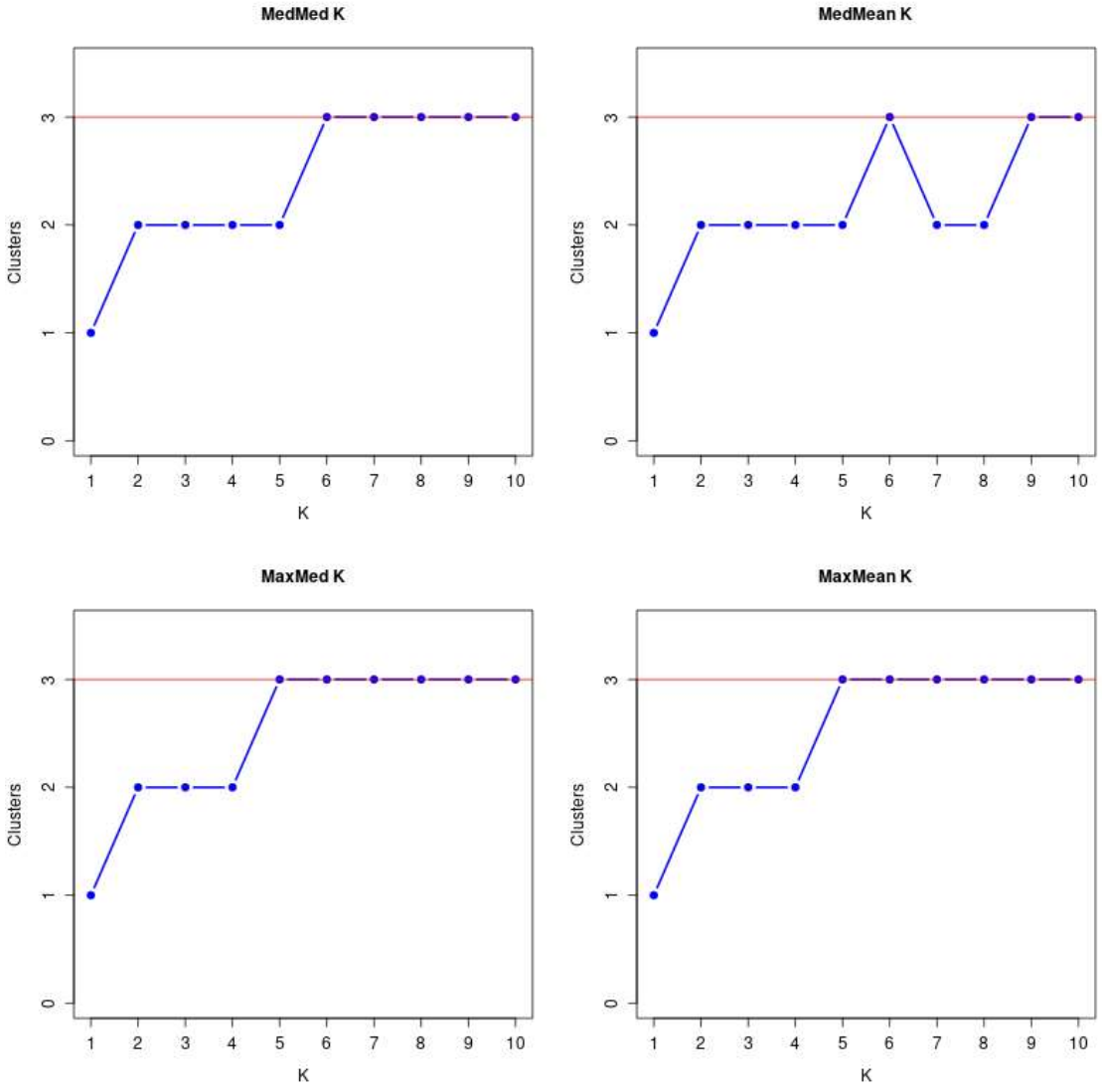
Seedling ID	Mother ID (Known)	Group	Candidate father ID	Group	Trio LOD score	Trio Delta	Trio confidence
S8856	WB8801	Planted	MB0102	Relictual	-232.32	0	
S8867	WB8801	Planted	P05100	Planted	-289.38	0	
S8868	WB8801	Planted	R088	Relictual	-549.65	0	
S8865	WB8801	Planted	R08303	Relictual	-252.23	0	
S8873	WB8801	Planted	R08103	Relictual	-292.83	0	
S8874	WB8801	Planted	R102	Natural	-278.82	0	
S8878	WB8801	Planted	WB8802	Planted	-242.42	0	
S8816	P01100	Planted	R08305	Natural	-293.23	0	
S8817	P01100	Planted	B1556	Planted	-344.75	0	
S8827	P01100	Planted	MB0104	Natural	-259.85	0	
S8828	P01100	Planted	R100	Natural	-229.04	0	
S8851	WB8801	Planted	P01100	Planted	252.4	252.4	*
S88371	WB8801	Planted	P01100	Planted	274.57	274.57	*
S88372	WB8801	Planted	P01100	Planted	264	264	*
S8897	WB8803	Planted	WB8802	Planted	-94.17	0	
S88111	WB8803	Planted	R088	Relictual	-485.41	0	
S88113	WB8803	Planted	R088	Relictual	-515.84	0	
SUN56	PLC11	Planted	R100	Natural	88.8	88.21	*
SUN69	PLC11	Planted	R200	Natural	133.7	133.7	*
SUN73	PLC11	Planted	R100	Natural	42.11	42.11	*
SUN771	PLC11	Planted	R100	Natural	96.22	96.22	*
SUN772	PLC11	Planted	R100	Natural	50.9	50.9	*
SUN775	PLC11	Planted	R101	Natural	-229.51	0	
SR0481	R048	Relictual	R08204	Natural	0	0	
SR0601	R0601	Natural	R0603	Natural	-150.12	0	
SP021071	P02107	Planted	R08101	Natural	-234.44	0	
S9222	B1556	Planted	PL01	Planted	-197.05	0	
S9247	PL01	Planted	OFF002	Natural	-128.89	0	
S9282	P02101	Planted	WB9302	Planted	-233.52	0	
S9285	P02101	Planted	P02107	Planted	-257.01	0	
S9291	P02101	Planted	WB9305	Planted	-246.8	0	
S9294	P02101	Planted	P02107	Planted	-245.68	0	
S9297	P02101	Planted	P02107	Planted	-225.43	0	
S92101	P02101	Planted	R08102	Natural	-243.8	0	
S921012	P02101	Planted	R08201	Natural	-228.42	0	
S92102	P02101	Planted	R08201	Natural	-243.74	0	
S92104	P02101	Planted	P02107	Planted	-259.57	0	
S92105	P02101	Planted	R207	Natural	-249.18	0	
S92110	P02101	Planted	P02107	Planted	-277.35	0	
S921101	P02101	Planted	-		-	-	
SRE1102	R083	Relictual	R08303	Natural	-16.09	0	

SRE57	R052	Relictual	OFF001	Natural	0	0	
SNA03	MB01	Natural	MB0105	Natural	-193.69	0	
SNA05	MB01	Natural	MB0105	Natural	-154.32	0	
SNA08	MB01	Natural	MB0105	Natural	-202.72	0	
SNA33	MB01	Natural	MB0105	Natural	-250.23	0	
SRE111	R083	Relictual	R08305	Natural	10.87	10.87	*
SRE81	R083	Relictual	R08203	Natural	0	0	
S9286	P02101	Planted	P02107	Planted	-238.98	0	

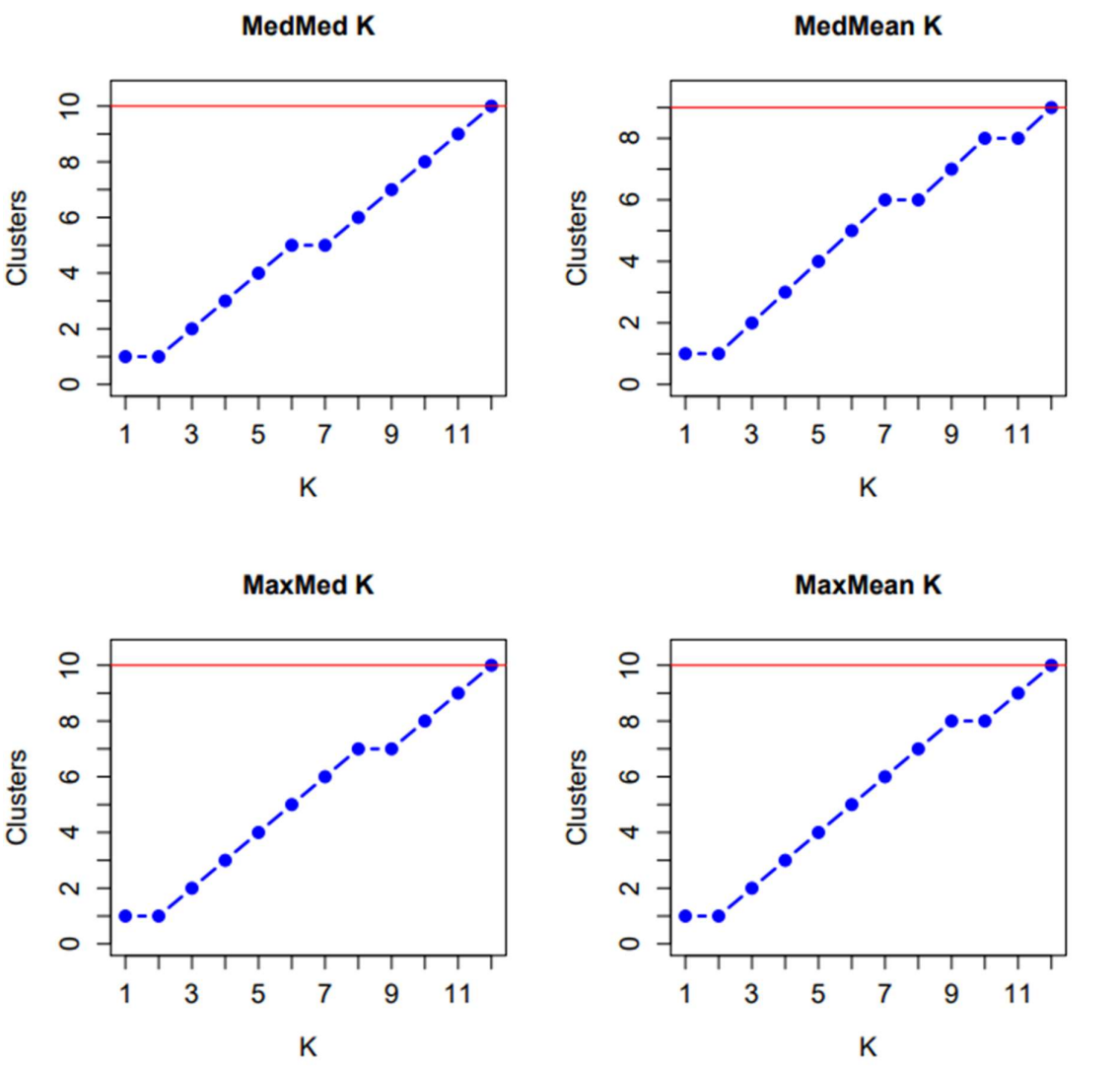
Appendix Table 2. *Eucalyptus albens* sapling parentage results of a parent-pair sex unknown assignment based on the highest LOD score and Trio Delta of the estimated parent pair using CERVUS 3.0.7. (Trio confidence '*' = 95% and '-' = 'most likely parent pair'/one candidate parent has 95% confidence)

Sapling ID	First candidate ID	Group	Pair LOD score	Second candidate ID	Group	Pair LOD score	Trio LOD score	Trio Delta	Trio confidence
PLC03	wbnat6 5	Natural	-123.23	PLC01	Planted	23.78	-155.67	0	-
PLC04	wbnat6 4	Natural	-120.46	PLC01	Planted	56.42	-127.18	0	-
PLC05	wbnat6 4	Natural	-159.22	PLC07	Planted	-17.95	-236.87	0	
PLC06	R100	Natural	1.73	PLC01	Planted	21.82	24.15	24.15	*
PLC08	wbnat6 5	Natural	-117.51	PLC07	Planted	36.45	-106.40	0	-
PLC09	wbnat6 5	Natural	-129.97	PLC07	Planted	13.01	-151.30	0	-
PLW02	OFF001	Natural	-121.34	PLW01	Planted	3.75	-137.73	0	-
PLW03	R105	Natural	63.19	PLW01	Planted	69.27	164.81	147.02	*
PLW06	PLC10	Planted	-11.63	wbplant2 11	Planted	-134.88	-207.12	0	
WB880202	wbPA1 17	Planted	-91.74	wb51 relict	Relictual	5.37	-100.45	0	-
WB880203	wbnat4 10	Natural	-152.79	wb51 relict	Relictual	-8.6	-220.81	0	
WB880205	wbPA1 26	Planted	345.44	wbplant2 11	Planted	-218.92	105.14	15.82	*
P02102	wbnat6 6	Natural	-87.47	wbplant2 5	Planted	-22.75	-117.50	0	
P02106	wbplant2 1	Planted	-18.92	wbplant2 20	Planted	50.15	-105.06	0	-
PL02	wbnat4 10	Natural	-187.2	PL01	Planted	-12.54	-319.83	0	
PL03	wbnat4 10	Natural	-127.15	PL01	Planted	65.62	-91.58	0	-
PL04	PL01	Planted	76.47	wbplant2 11	Planted	-145.55	-130.83	0	-
PL05	wbnat6 5	Natural	-128.23	PL01	Planted	12.12	-139.02	0	-
PL06	wbnat1 7	Natural	-131.4	PL01	Planted	57.81	-125.82	0	-
MB0106	MB01	Natural	61.13	MB0104	Natural	-35.49	-106.64	0	-
MB0107	MB01	Natural	13.94	wbnat4 10	Natural	-90.37	-130.04	0	-
MB0108	MB01	Natural	261.43	MB0105	Natural	46.92	237.30	62.23	*
MB0109	MB0105	Natural	-82.86	wbplant2 12	Planted	-155.96	-262.76	0	
MB0110	MB01	Natural	52.98	wbnat6 6	Natural	-99.38	-134.11	0	-
R209	R200	Natural	28.9	R207	Natural	-16.05	-99.24	0	-
R210	R200	Natural	70.66	wbnat6 5	Natural	-98.51	-87.20	0	-
R211	R200	Natural	67.08	wbnat6 5	Natural	-107.66	-91.34	0	-
R212	R200	Natural	37.63	wbnat6 6	Natural	-130.37	-154.53	0	-
R213	R200	Natural	50.1	R105	Natural	74.16	131.70	112.02	*
R046101	wbnat6 4	Natural	-141.37	wbplant2 11	Planted	-140.96	-294.48	0	
R046102	wb46 relict	Relictual	72.96	wb47 relict	Relictual	-71.31	-32.47	0	-
R046104	R0601	Natural	-39.56	wb6 relict	Relictual	-16.25	-174.63	0	
R046105	wbnat6 4	Natural	-165.32	wbplant2 11	Planted	-149.45	-330.21	0	
R048103	wbnat6 5	Natural	-144.27	wb48 relict	Relictual	44.38	-136.86	0	-
R048104	wbnat4 10	Natural	-146.8	wb48 relict	Relictual	35.71	-148.19	0	-
R048105	wbnat6 5	Natural	-187.7	R088	Relictual	73.09	-153.43	0	-
R08207	R08204	Natural	-27.46	R08205	Natural	32.9	-97.27	0	-
R08208	R08204	Natural	-36.51	R08205	Natural	-0.38	-205.59	0	
R08209	R08203	Natural	31.36	R08204	Natural	9.41	-59.12	0	-

R08210	R08203	Natural	66.94	R08205	Natural	69.94	33.28	12.1	*
R08306	R08305	Natural	36.88	R083	Relictual	239.15	211.58	5.54	*
R08307	wbnat4 10	Natural	-80.76	R083	Relictual	67.53	-65.62	0	-
R08308	wbnat4 10	Natural	-86.29	R083	Relictual	74.6	-77.05	0	-
R08310	R083	Relictual	74.69	wb37 relict	Relictual	69.46	174.62	174.62	*
R06100	wbplant2 11	Planted	-104.24	wb6 relict	Relictual	-42.78	-236.41	0	
R06101	wbplant2 14	Planted	19.03	wb6 relict	Relictual	8.25	-11.99	0	-
R06102	B1556	Planted	-95.98	wb6 relict	Relictual	13.03	-154.26	0	-
R06103	R0601	Natural	91.79	wb6 relict	Relictual	16.3	-30.49	0	-
R06104	R0601	Natural	-96.26	wb6 relict	Relictual	-24.73	-125.17	0	



Appendix Figure 4. The number of genetic clusters (K) among the *Eucalyptus albens* seedling dataset based on 2570 loci estimated utilising the Puechmialle method (Puechmialle 2016)



Appendix Figure 5. The number of genetic clusters (K) among the *Eucalyptus albens* relictual, planted, natural, seedling and sapling dataset based on 1537 loci estimated utilising the Puechmialle method (Puechmialle 2016)